SOLID-PHASE SYNTHESIS OF GLICOSAMINOGLYCANS

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RESUMEN TESIS

Los **glicosaminoglicanos (GAGs)** también conocidos como mucopolisacáridos, son polisacáridos lineares que se encuentran formando parte de la matriz extracelular (MEC). Los GAGs se pueden encontrar unidos mediante el aminoácido serina a la estructura central de diversas proteínas formando complejos de gran tamaño conocidos como proteoglicanos. Los GAGs están compuestos de unidades de repetición de distintos disacáridos, y se clasifican en función de su composición de monosacáridos y en función del enlace glicosídico entre ellos. En general están compuestos por un monosacárido de tipo ácido urónico (D-glucurónico o L-idurónico) y una hexosamina (D -glucosamina o D-galactosamina). La mayoría de los GAGs poseen una importante carga neta negativa debido a la presencia de diversos grupos sulfato en las posiciones 2, 4 y 6 y en los grupos amina no acetilados de su estructura.

La expresión de GAGs es específica de cada tipo de célula o tejido, y puede verse alterada por su estado de desarrollo o por la aparición de ciertas patologías como enfermedades vasculares, enfermedades neurodegenerativas como el Alzheimer y el cáncer. La gran variabilidad estructural de los GAGs se refleja en el gran número de funciones biológicas en las que están involucrados. Los GAGs poseen una función estructural muy importante, mediante la unión a proteínas fibrosas como el colágeno y la laminina, son los principales componentes de la matriz extracelular, que mantiene unidas las células individuales proporcionando protección, forma y soporte a células, tejidos y órganos. La interacción entre GAGs con distintas proteínas tiene un importante efecto en la regulación de procesos como la hemostasia, el transporte de lípidos, el crecimiento celular, la migración y el desarrollo celular. La gran variedad de actividades biológicas asociadas a los GAGs se deriva por la habilidad de regular la actividad de un gran número de proteínas. La unión con GAGs regula la actividad de diversos enzimas, el reconocimiento de ligandos mediante sus receptores y la protección de las proteínas a la degradación. Sin embargo, en muchos casos los requerimientos estructurales responsables de la interacción GAG-proteína no se encuentran bien definidos debido a la enorme complejidad que presentan estos polisacáridos.

La **heparina** tiene una gran importancia en medicina ya que impide la coagulación de la sangre. Una de las interacciones GAG-proteína mejor estudiadas es la interacción de un pentasacárido de heparina presentando un patrón de sulfatación específico con la proteína del plasma antitrombina III (AT-III). Esta unión produce un cambio conformacional que bloquea la cascada de coagulación de la sangre. Aunque existe algún derivado comercial completamente sintético, la mayor parte de los medicamentos antitrombóticos se producen mediante la degradación química o enzimática de heparina proveniente de fuentes animales. En 2007, se produjo una emergencia sanitaria tras la distribución de unos lotes de heparina de origen animal contaminada con sulfato de condroitino, que produjo un centenar de muertes. Por lo tanto, el desarrollo de nuevas estrategias que permitan la preparación de GAGs sintéticos con completo control en su estructura y grado de sulfatación son campos de investigación de amplio interés.

Conceptualmente la **síntesis en fase sólida de carbohidratos** puede ofrecer importantes ventajas a la síntesis de oligosacáridos en disolución y en particular para el ensamblaje de oligo- y polisacáridos compuestos por unidades repetitivas como es el caso de los GAGs. En la síntesis en fase sólida, uno de los bloques de unión sacarídicos se encuentra anclado a un soporte sólido mediante un espaciador, y las transformaciones químicas se llevan a cabo con el resto de los reactivos y bloques de unión en disolución. Un punto clave en el diseño de una síntesis de carbohidratos en fase sólida es la preparación de bloques de unión convenientemente protegidos, sobre los que se puedan llevar transformaciones químicas sin afectar al resto de las funcionalidades y se puedan liberar de manera ortogonal las posiciones que se encuentran involucradas en la elongación del polisacárido. La síntesis en fase sólida permite emplear un exceso de reactivos que da lugar a transformaciones químicas más eficientes sin perjudicar la purificación posterior. El exceso de reactivos y ciertos subproductos de la reacción se pueden eliminar de manera sencilla mediante el exhaustivo lavado del soporte sólido.

En esta tesis se describe el desarrollo y la optimización de la **síntesis en fase sólida de precursores de heparán sulfato y de dermatán sulfato**. Estas familias de GAGs se encuentran ampliamente distribuidas en diferentes tejidos, y se encuentran

involucrados en diversos procesos biológicos. El heparán sulfato se encuentra presente en todos los tejidos animales unido a proteínas, en forma de proteoglicanos y participa en interacciones con diversas proteínas como factores de crecimiento, quimioquinas, componentes de la matriz extracelular y enzimas, modulando su actividad biológica. Por otro lado el dermatán sulfato (condroitín sulfato B) es el principal GAG presente en la piel, aunque también aparece en vasos sanguíneos, en el corazón y en los pulmones. El dermatán sulfato se encuentra implicado en enfermedades cardiovasculares, en la infección bacteriana y en la reparación de heridas.

En general, la síntesis química de este tipo de oligosacáridos se lleva a cabo mediante el ensamblaje de unidades disacarídicas protegidas, que representan los principales bloques estructurales del GAG objetivo. En nuestro caso, se plantea una estrategia más flexible basada en la combinación de bloques de unión de tipo monosacárido. Debido a que la síntesis en fase sólida requiere un exceso de reactivos para la obtención de altas conversiones, el uso de bloques de unión de tipo monosacárido puede resultar más eficaz frente al uso de disacáridos más elaborados. Adicionalmente, la combinación de unos bloques de unión de distinta naturaleza permitiría la preparación de diversas familias de GAGs siguiendo la misma estrategia sintética.

En la presente tesis doctoral, se lleva a cabo el diseño y preparación de un nuevo espaciador de tipo carbamato que se puede anclar a una resina Merrifield mediante un enlace de tipo éster. Este tipo de anclaje permite llevar a cabo las subsecuentes reacciones de glicosidación, que generalmente son catalizadas por reactivos ácidos, sobre el soporte sólido manteniéndose inalterado. Una vez el oligosacárido anclado posee la longitud deseada, es desanclado de la resina bajo condiciones básicas. Los oligosacáridos se obtienen unidos a un espaciador de cinco carbonos protegido en forma de carbamato, que en la secuencia de desprotección final liberaría un grupo amino para la futura conjugación de los GAGs sobre diversos soportes sólidos.

Inicialmente, se llevó a cabo la optimización en disolución de la estrategia sintética de unión secuencial de monosacáridos mediante el ensamblaje sobre un espaciador modelo y empleando diferentes donadores de glicosilo. En la presente tesis doctoral, se lleva a cabo una evaluación sistemática de la reactividad de diferentes monosacáridos derivados de L -idosa (Ido) y de ácido L -idurónico (IdoA). En el caso de donadores derivados de L -idosa, se preparó una colección de donadores con distinto patrón de sustitución de grupos protectores y adicionalmente se evaluó la diferente reactividad de distintos grupos salientes en la posición anomérica.

Entre los donadores derivados de ácido L-idurónico (IdoA), se sintetizaron y evaluaron por primera vez donadores de tipo *n*-pentenil ortoester (NPOE). El uso directo de donadores IdoA para el ensamblaje ser oligómeros evita pasos adicionales de desprotección y oxidación para la obtención de los GAGs naturales. Sin embargo, los ácidos urónicos debido a la capacidad electrón-atractora del grupo carboxílico, son menos reactivos que sus análogos no oxidados. En la presente tesis doctoral se muestra que el uso directo de IdoA NPOEs como donadores en fase sólida es viable, llegándose a preparar un trisacárido precursor de heparán sulfato.

Los resultados más prometedores del estudio de reactividad en disolución de diversos donadores de L -idosa fueron posteriormente aplicados a la síntesis en fase sólida, verificando las tendencias de reactividad previamente observadas. La reacción secuencial en fase sólida de dos donadores activados en forma de tricloroacetimidatos, permite la preparación de un hexasacárido precursor de heparán sulfato protegido. La combinación de un donador derivado de L -idosa sustituido con un grupo 6-*O*-PMP y un derivado de D-glucosamina cuyo grupo amino se encuentra enmascarado en forma de un grupo azido, permite la preparación estereoselectiva de enlaces glicosídicos en fase sólida.

En la última parte de la presente tesis doctoral se ha llevado a cabo la preparación de dos precursores protegidos de dermatán sulfato, mediante el ensamblaje secuencial del donador derivado de L-idosa previamente optimizado y un derivado de D-

galactosamina, que fue desarrollado para tal efecto. La síntesis en fase sólida permitió la preparación de un tetrasacárido y un octasacárido de DS con altos rendimientos y en tiempos relativamente cortos comparados con la síntesis convencional en disolución.

La estrategia desarrollada en la presente tesis doctoral para la preparación de GAGs en fase sólida mediante el ensamblaje de bloques de unión de tipo monosacárido podría constituir las bases hacia la preparación automatizada de GAGs. La combinación estratégica de tres donadores de tipo L-idosa, D-glucosamina y D-galactosamina, permitiría la preparación automatizada de una quimioteca de precursores de heparán sulfato, heparina y dermatán sulfato de diversa longitud y con un patrón de sulfatación definido por la estrategia de grupos protectores empleada.

LIST OF ABBREVIATIONS

Ac	Acetyl
AcCl	Acetyl chloride
AcOH	Acetic acid
Ac_2O	Acetic anhydride
AIBN	2,2′-Azobisisobutyronitrile
aq	aqueous
AT III	Antithrombin
BAIB	[bis(acetoxyiodo]benzene
Bn	Benzyl
b	broad
***t*BuOH**	*tert*-Butanol
Bz	Benzoyl
C	Carbon
c	concentration
CAN	Cerium (IV) ammonium nitrate
cat	catalytic
Cbz	Benzyloxycarbonyl
CS	Chondroitin sulphate
CSA	Camphorsulfonic acid
δ	chemical shift
d	doublet
COSY	Correlation spectroscopy
DBU	1,8-Diazabicylco[5.4.0]undec-7-ene
dd	doublet of doublets
DIC	*N,N*′-diisopropylcarbodiimide
DIPEA	*N*,*N*-Diisopropylethylamine
DCC	*N,N*′-Dicyclohexylcarbodiimide
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide

DS	Dermatan sulfate
EDC	Ethyl-3-(3-dimethylaminopropyl)carbodiimide
eq	equivalent
ESI	Electrospray ionization
Et	Ethyl
EtOAc	Ethyl acetate
FGF	Fibroblast growth factor
Fmoc	9-Fluorenylmethoxycarbonyl
GAG	Glycosaminoglycan
Gal	Galactose
GalNAc	N-Acetyl galactosamine
GlcA	Glucuronic acid
GlcN	D-Glucosamine
GlcNS	N-Sulfonato glucosamine
HA	Hyaluronan
HP	Heparin
HS	Heparan sulphate
HSQC	Heteronuclear single quantum correlation
HR	High resolution
Ido	L-Idose
IdoA	L-iduronic acid
KS	Keratan sulphate
LG	Leaving group
NaOMe	Sodium methoxide
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
Hz	Hertz
IR	Infrared spectroscopy
J	Coupling constant
LC	Liquid chromatography
Lev	Levulinoyl
m	multiplet
M	Molar

m/z	Mass to charge ratio
MALDI	Matrix Assisted Laser Desorption/Ionization
min	minutes
MS	Mass spectroscopy
ms	Molecular sieves
na	Not analyzed
NMR	Nuclear magnetic resonance
NPOE	*N*-pentenyl orthoester
NPG	*N*-pentenyl glycoside
p	*para*
PEG	polyethylene glycol
PG	Proteoglycan
Piv	Pivaloyl
PMB	*p*-Methoxybenzyl
PMP	*p*-Methoxyphenyl
ppm	Parts per million
PS	Polystirene
quant	quantitative
rt	room temperature
SAR	Structure activity relationship
t	triplet
TBAI	Tetra-*n*-butylammonium iodide
TBDPS	*tert*-Butyldiphenylsilyl
TDS	Dimethylthexylsilyl
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy free radical
TFA	Trifluoroacetic acid
TfOH	Trifluoromethane sulfonic acid
TLC	Thin layer chromatography
THF	Tetrahydrofuran
TMS	Trimethylsilyl
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TOF	Time of flight
TOM	tri-*iso*-propylsilyloxymethyl

t_r	Retention time
Troc	2,2,2-Trichloroethyl
TsOH	*p*-Toluenesulfonic acid
UPLC	Ultra Performance Liquid Chromatography

TABLE OF CONTENTS

1. INTRODUCTION

The main purpose of this thesis has been to develop suitable methodologies for the solid phase synthesis of glycosaminoglycan (GAG) type oligosaccharides, particularly heparan sulphate (HS) and dermatan sulphate (DS). GAGs are complex and highly heterogeneous linear polysaccharides which are involved in a variety of important biological events and their preparation in pure form for further structure-activity studies is difficult to achieve from natural sources. Therefore, effective chemical or chemo-enzymatic syntheses which could afford sufficient amount of these molecules in pure form are much needed. However, these syntheses constitute one of the most serious challenges in preparative carbohydrate chemistry.

1.1 Biological importance of GAGs

GAGs are the polysaccharide components of proteoglycans which constitute the major class of glycoconjugate present at the cell surface and in the extracellular matrix (ECM) that surrounds all mammalian cells. Proteoglycans are formed by different GAG polysaccharides (GAGs) anchored to proteins in the outside of the cell membrane lipid bilayer trough a specific tetrasaccharide composed by a glucuronic acid (GlcA), two galactose (Gal) and a xylose (Xyl) residues (GlcAβ1-3Galβ1-3Galβ1-4Xylβ1-*O*). This tetrasaccharide is coupled through an *O*-glycosidic bond to a serine residue in the protein (Figure 1).[1]

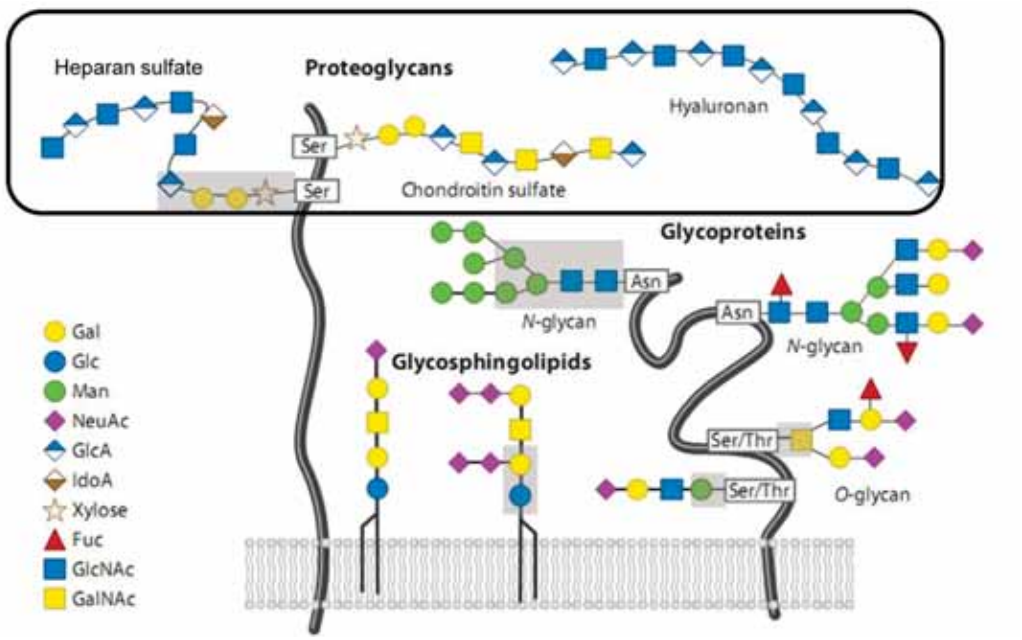


Figure 1.1 Schematic representation of proteoglycans, glycoproteins and glycolipids at the cell surface and in the extracellular matrix (picture taken from *Ann. Rev. BioChem.*, 2011[1])

GAGs are the most abundant heteropolysaccharides in the body. They are historically referred as mucopolysaccharides due to the fact that were originally found in mucus membrane. GAGs constitute a family of linear polisaccharides consisting of repeating disaccharide units with a high density of negative charge and an extended conformation that results in a high viscosity in solution. Along with their high viscosity, GAGs have low compressibility, which makes these molecules ideal as lubricanting fluids in the joints.

Considering their prominent location in the extracellular matrix (ECM), often attached to the cell surface via proteoglycans, and their large structural diversity, it is not surprising that GAGs are involved in many molecular recognition processes including growth factor signaling, cell adhesion, and interactions with other extracellular matrix components, Figure 1.2.[2]

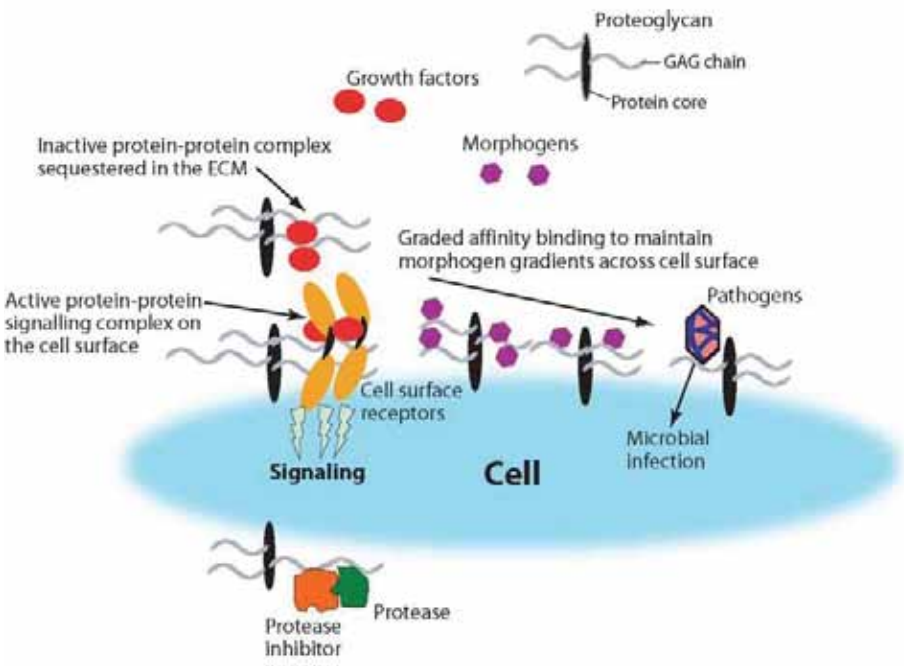


Figure 1.2. Biological interactions of GAGs. Location and distribution of GAGs and their interactions with numerous proteins such as growth factors, morphogens, proteases, and pathogens. The chemical and structure diversity of GAGs enable their specific interactions with these proteins, and affect on their biological role (picture taken from *Ann. Rev. BioChem.*, 2006[2]).

There is growing evidence indicating that GAGs, such as heparin and heparan sulphate (HS), can have significant physiological effects on lipid transport and adsorption, cell growth, migration and development.[3,4] Alterations in GAG expression have been associated with serious diseases as cancer and for example, significant changes in the structure of GAGs have been reported in the stroma surrounding tumours, which is important for tumour growth and invasion.[5,6] In 2014 Nadanaka *et al*[7] reported the inhibition of heparanase activity by synthetic HS-tetrasaccharides containing unsubstituted glucosamine residues and suppression of breast cancer cells invasion *in vitro*. GAGs also have important neurobiological functions and examples include neuroepithelial growth and differentiation, neurite outgrowth, nerve degeneration, axonal guidance and branching, deposition of amyloidotic plaques in Alzheimer´s disease, and astrocyte proliferation.[8] In this context, an improved understanding of the role that GAGs play in cellular communications should facilitate the development of new therapeutic strategies for the treatment of a wide variety of diseases states.

The biosynthesis of GAGs is a complex non-template driven process involving several enzymes that initially assemble the GAG polymer and then introduce sulfate groups at specific positions. The synthesis is initiated by the attachment of the tetrasaccharide anchoring linker (GlcAβ1-3Galβ1-3Galβ1-4Xylβ1-*O*) to the serine by the four specific monosaccharides glycosyltransferase enzymes (Xyl-T, GalT-I, GalT-II y GlcAT-I). After the anchoring of this tetrasaccharide linker, the initiation of the chain by the transfer of GlcNAc or a GalNAc determine the type of GAG to be biosynthesized (except for hyaluronic acid HA, which is an extracellular matrix component). The chain elongation is performed by the action of a multidomain glycosyltransferase, which successfully transfer GlcNAc, GlcA or GalNAc. The GAG polymer is then further processed by tissue-specific deacetylases, sulfuryltransferases and epimerases). These enzymes are responsible of the cleavage of N-deacetylation, the N-, O- sulfation and the C-5 epimerization to convert GlcA into IdoA. Therefore, the large structural diversity of GAG structures results of the substrate specificities and tissue-specific expression of the GAG-chain processing enzymes.

GAGs are classified according to their monosaccharide composition and glicosidic linkage in five groups: hyaluronan (HA), chondroitin sulfate (CS), dermatan sulfate (DS), heparin/heparan sulfate (HS) and keratan sulfate (KS). HA is a non sulfated polysaccharide composed by disaccharide repeating units of *N*-acetyl-D-glucosamine and D-glucuronic acid (Figure 1.3). HA is unique among the GAGs because is not covalently attached to proteins forming proteoglycans, but it is found in non-covalent complexes with them in the extracellular matrix. CS is the most prevalent GAG and it is formed by *N*-acetyl-D-galactosamine and D-glucuronic acid disaccharide units (GalNAc-β-1,4-GlcAβ-1,3). DS is distinguished from CS by the presence also of L-iduronic acid (IdoA) in the repeating disaccharide units (GalNAc-β-1,4-IdoAα-1,3). sulphate HS and heparin (HP) are two related GAGs composed by repeating units of hexosamines typically, *N*-acetyl-D-glucosamine and the uronic acids D-glucuronic acid and L-iduronic acid (GlcNAcα-1,4-GlcAβ-1,4 or GlcNAcα-1,4-IdoAα-1,4). KS lacks uronic acids and instead consists of sulfated galactose and *N*-acetylglucosamine residues (GlcNAc-β-1,3-Galβ-1,4).

Many of the GAGs structures can be further modified by sulfation at different position of both uronic acid and hexosamine, generating a high degree of complexity and making GAGs encode a great number of biological information. For example, HS GAGs could potentially contain up to 48 different disaccharide building blocks based on the different sulfation pattern, which can be contrasted with that of DNA only formed by four building blocks and of proteins, formed by twenty building blocks.

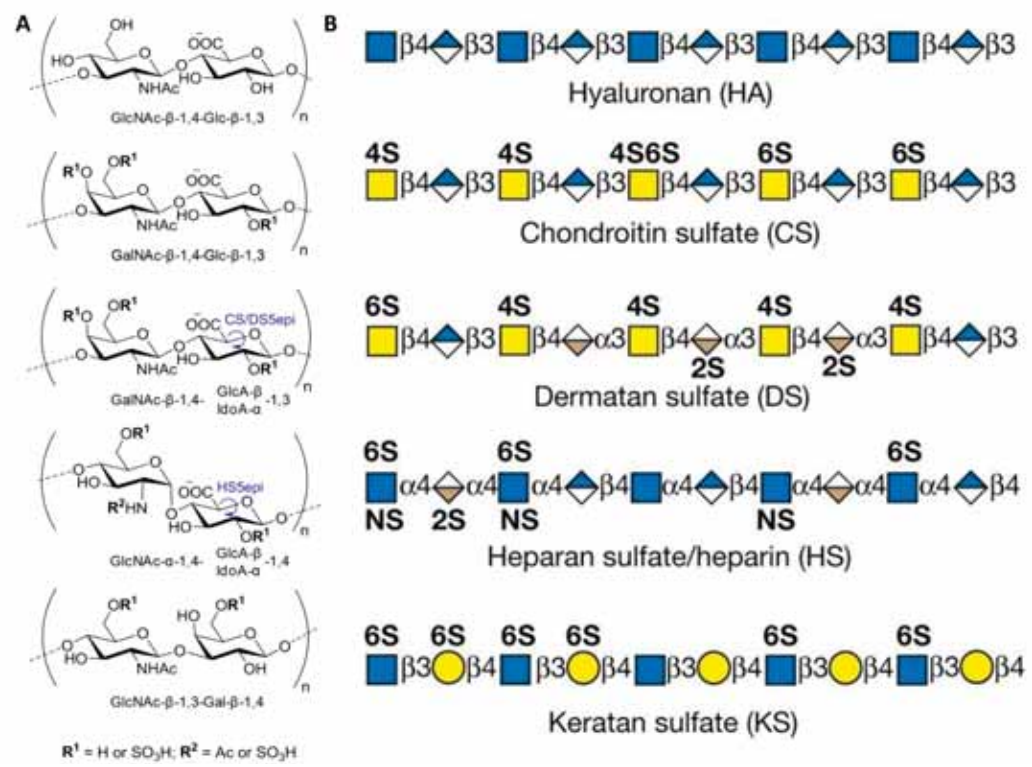


Figure 1.3. A) Disaccharide building blocks for the different types of GAGs: HA, CS, DS, HP/ HS and KS. B) Schematic representation of the five types of GAGs using monosaccharides symbols. Possible sulfation presence and location (2S, 6S, 4S) as well as N-sulfation (NS) are indicated.

1.2 HP/HS and DS: structure, localization and function

1.2.1 Structure and location of HP and HS.

HP and HS are structurally related polisaccharides and for this reason are assigned to one GAG type. HP is the oldest carbohydrate-based drug. It is isolated from porcine mucosal tissue and has been used as an anticoagulant in heart disease for more than 60 years[9]. Its molecular weight ranges from 5 to 40 kDa with an average molecular weight of around 15 kDa. HP is a linear, unbranched, highly sulfated polysaccharide that is composed of disaccharide repeating units consisting of an uronic acid 1,4-linked to a D-glucosamine unit (Figure 1.4). In HP polymers L-iduronic acid accounts for over 90% of uronic acid residues, while in HS α-L-IdoA accounts for 50-90%. With a molecular weight range of 5 to 50 kDa and an average molecular weight of 30 kDa, sulphate HS chains are generally longer than those of HP. Therefore, HS is a far more complex mixture of individual compounds than heparin.

HP has the highest negative charge density of any known biological macromolecule due to the high content of sulfo and carboxyl groups. In addition, HP shows higher degree of sulfation (2.3-2.8 sulfated/disaccharide) when compared to HS (0.6 – 1.5 sulfate/disaccharide). The most common structure in HP is the disaccharide repeating unit sulfated at position 2-OH of uronic acids and at 3-OH and/or 6-OH of the glucosamine unit. The 3-*O*-sulfation into the glucosamine residue occurs infrequently in HS, but is intimately linked to its biological function.[10]

In HS the glucosamine residues can be either *N*-sulfated, unsubstituted, or *N*-acetylated, whereas in HP the occurrence of *N*-acetyl groups corresponds to less than 5%. On this basis, 48 different disaccharide units can theoretically be found in native HP/HS, but due to the restriction on the biosynthesis only 23 disaccharides have been identified to date in HP, sulphate HS or as intermediates in biosynthesis

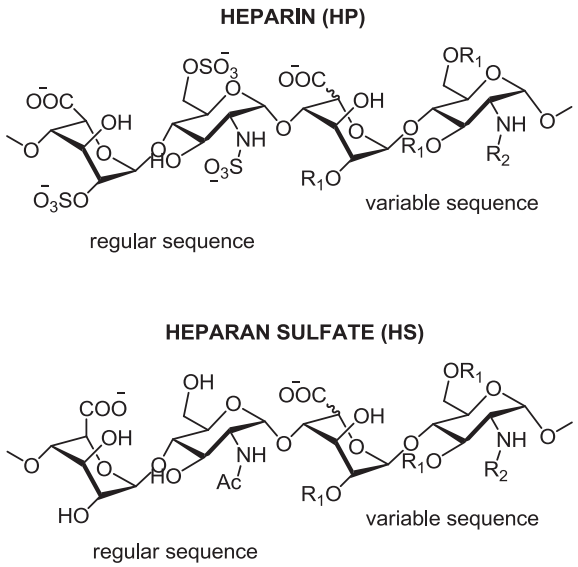


Figure 1.4. Regular and variable disaccharide repeating units in HP and sulphate HS. R^1= H or SO_3^-, R^2= Ac, H or SO_3^-.

HP is expressed in the granules of connective tissue-type mast cells as an intracellular serglycin proteoglycan.[11] Post synthesis, the HP chains are cleaved at random points of the chain to give polydisperse mixtures of smaller HP polysaccharides that are stored in the cytoplasmic secretory granules of mast cells.[12]

HS, in contrast, is expressed and secreted by most if not all, mammalian cells. HS proteoglycans (HSPGs) such as agrin, perlecan and type XVIII collagen are located in the extracellular matrix (ECM) or linked to the cell surface in transmembrane proteins such as syndecans or GPI-anchored proteins (glypicans).[13,14] Therefore, HSPGs could show a dual function: they could be involved in the regulation of different biological processes through the cell membrane[15,16] or be involved in the structural organisation and mechanical support of tissues.[17]

1.2.2 HP/HS-protein interactions

Due to their enormous structural diversity HP and HS are able to bind and interact with a wide variety of proteins, such as growth factors, chemokines, morphogens, extracellular matrix components and enzymes, modulating their biological activity (Figure 1.5)[18,4]. In this way, HP and HS are involved in angiogenesis[19], regulation of cell adhesion[20], tumor development and metastasis[21], brain development[22]and inflammation[23] among others.

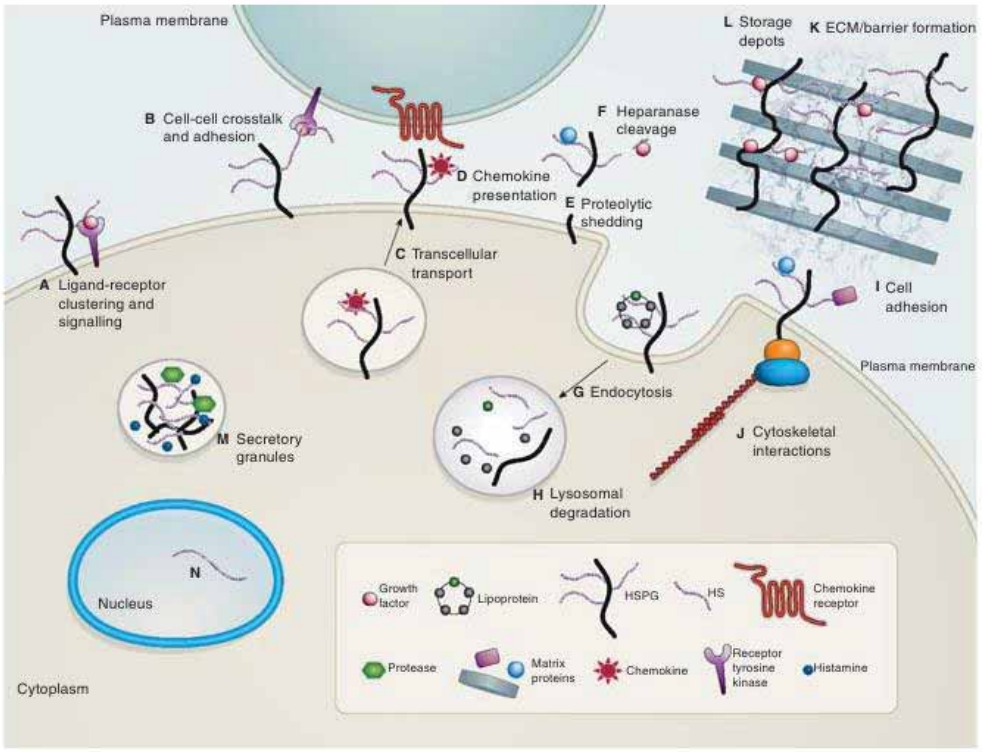


Figure 1.5. HS-protein interactions in various functional settings. (A, B) HSPGs present growth factors to their receptors, on the same or an adjacent cell, and may form part of signalling complexes. Chemokines are bound to HS chains for transcytosis (C) and presentation at cell surfaces (D). Truncation of HSPGs by proteolytic shedding of ectodomains and cleavage of HS chains by heparanase (F). Uptake of cell-surface HSPGs by endocytosis (G) for degradation in lysosomes (H) or recycling back to the surface. HSPGs facilitate cell adhesion by interacting with extracellular-matrix protein through their HS chains (I) and with the cytoskeleton via cytoplasmatic core-protein domains (J). HSPGs in extracellular matrix contribute to physiological barriers (K) and provide storage of growth factors and morphogens (L). Serglycin carrying heparin chains are required for storage of proteases and histamine in secretory granules of mast cells (M). Experiments suggest that HS

chains with special structural deatures may be located in the nucleus, although with so far unknown function (N). Picture taken from *Nature*, 2007.[4]

The different aspects of HP/HS-protein interactions have been studied by a large number of groups and in this section we will summarize the best studied HP/HS protein interactions.

The best understood and most extensively studied GAG-protein interaction is that of HP with the plasma protein antithrombin III (ATIII) in which a specific HP-pentasaccharide structure binds to the protein which upon binding undergoes a conformational change which blocks thrombin and factor Xa in the coagulation cascade.[24] The unique HP pentasaccharide domain recognized by the HP binding region of ATIII was identified in the early 1920 by several authors.[25] As a result of the medical importance of this finding enormous synthetic efforts both by academic and industrial research groups led to the development of de novo synthesis of the pentasaccharide which ultimately was commercialized under the trade name Arixtra (fondaparinux sodium; GlaxoSmithKline) in 2002 (Figure 1.6). This synthetic derivative has a much-improved subcutaneous bioavailability. However, Arixtra does have some clinical disadvantages, like a longer half-life than natural HP and additional adverse side effects limiting altogether its range of applications. Therefore, low molecular weight heparins (LMWHs), prepared through the chemical or enzymatic degradation of porcine unfractionated (UF) heparin, still have the highest market share of all antithrombotic, and the need for additional synthetic heparin molecules with specific activities persists. In a recent publication, Liu *et al*. described the chemoenzymatic synthesis of six 3-*O*-sulfated oligosaccharides at glucosamine residues (including hexasaccharides and octasaccharides) and identified a 3-*O*-sulfated octasaccharide that interacts with antitrombin and display anti-factor Xa activity. Interestingly, the octasaccharide displays a faster clearance rate than fondaparinux, making this octasaccharide a potencial short-acting anticoagulant drug candidate that could reduce bleeding risk.[10]

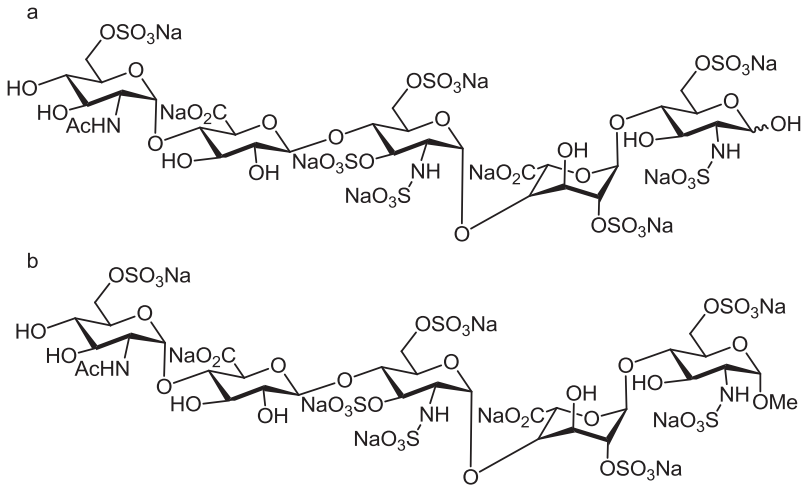


Figure 1.6. Heparin pentasaccharides recognized by AT-III. a) Pentasaccharide sequence of HP responsible for the anticoagulant activitity; **b)** Structure of Arixtra commercialized by GlaxoSmithKline.

The intrinsic problems with complex HP mixtures obtained from animal sources were highlighted in 2007 through the discovery HP batches adulterated with a semi-synthetic over- sulfated sulphate CS, causing hypertension and leading to nearly hundred deaths.[26, 27]

Many growth factors, including the fibroblast growth factors (FGFs), bind to the extracellular matrix of target tissues by the interaction with GAGs such as HP and HS. FGFs are a family of proteins composed by more than 20 members, involved in cell proliferation, differentiation and angiogenesis.[93,28]

Acidic fibroblast growth factor (FGF-1) and basic fibroblast growth factor (FGF-2) are the most widely studied members of the family. FGF signalling implies the binding of FGFs to specific cell surface tyrosine kinase receptors (FGFRs) which results in receptor oligomerization which is followed by phosphorylation of other signalling molecules and initiation of the signalling cascade. HS plays an important role by facilitating the formation of ternary FGF-FGFR complexes stabilizing and enhancing FGF and FGFR oligomerization. X-ray crystal structures and NMR spectroscopy of FGF-1 and FGF-2 complexed with HS oligosaccharide fragments and of HS oligosaccharides-FGF-FGFR ternary complexes have been obtained and

the structural features of the HS fragments for signalling identified. These studies have shown the complexity of the molecular mechanism involved in HP/HS-FGF signalling.[29-31]

Chemokines, a ~45 members family of small cytokine like proteins that facilitate leukocyte migration, angiogenesis, breast cancer metastasis, and leukocyte degranulation, also interact with HP and HS[9,30]. Platelet factor 4 (PF-4), binds to HP and HS with a very high affinity in 1:1 ratio. The interaction of therapeutically administered HP with PF-4 results in HP-induced thrombocytopenia (HIT), a dangerous, immunological loss of platelets. There is a growing interest in understanding the chemokine function at molecular level in order to identify strategies related with their functions. In recent studies it was demonstrated that oligomerization is critical for GAG-chemokine interactions, and in some cases provide specificity.[30]

Surface proteins of many pathogenic microorganism (viruses, bacteria and parasites) interact with cell surface GAGs. Several studies in cell culture demonstrated the role of HSPGs as co-receptors in viral invasion of target cells.[18] In the case of Herpes simplex virus types 1 and 2 (HSV-1, HSV-2), the virus entry is a multi-step process which includes firstly the virus binding to the cell by the interaction of viral glycoproteins gB and gC with sulphate HS chains in cell surface proteoglycan. In the next step, a third viral glycoprotein, gD, interacts with a specific sequence within sulphate HS chains that have been modified by 3-O-sulfation of specific glucosamine residues and this interaction can facilitate fusion pore formation during viral entry.[31,32,33] Sulfation pattern in HS chains are known to play critical role during viral entry, virus trafficking and replication.[34] In addition, strains of *Plasmodium falciparum* associated with development of severe forms of malaria employ heparan sulfate as a host receptor, and a HS-binding protein encoded by the parasite was implicated with the “rosetting” and endothelial binding of infected erythrocytes typical for the disease. The resultant sequestration of malaria parasites *in vivo* was efficiently blocked by administration of a HP derivative lacking anticoagulant activity.[35]

1.2.3 Structure and location of DS

Dermatan sulfate (DS), also known as chondroitin sulfate B (CS-B), is composed of disaccharide units formed by the hexosamine, *N*-acetyl galactosamine (GalNAc) and glucuronic acid (GlcA) joined by β-1,4 or 1,3 linkages respectively (Figure 1.7). DS is defined as CS by the presence of GalNAc. However, the presence of IdoA in DS differentiates it from CS and links it structurally to HP and HS.

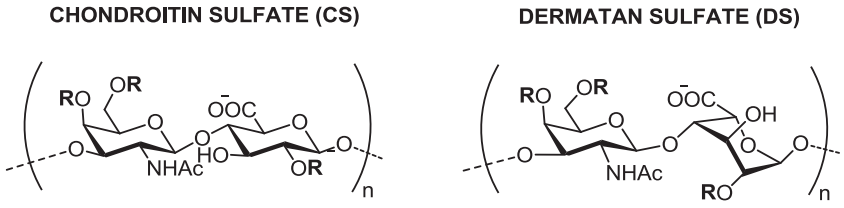


Figure 1.7. Typical Disaccharide Repeating Units in CS and DS. R= H, SO_3^-.

DS can be sulfated at positions C-4 and C-6 of the GalNAc residues (as CS-A and CS-C) and at C-2 of the IdoA (as HS or HP).DS chains can vary in size up to a hundred or more disaccharide repeating units. In the mammalian organisms CS occur either intracellularly in secretory vesicles, membrane-bound, or as secreted PGs in the ECM (decorin, biglycan, syndecan versican, endocan, perlecan, etc.). There is a growing evidence that this variable DS chain length, disaccharide unit, and sulfation pattern determine binding affinity and control functional interactions with proteins.

1.2.4 DS -protein interactions

CSPG/DSPG can bind a diverse range of molecules including matrix molecules, growth factors, protease inhibitors, cytokines, chemokines and pathogen virulence factors.[36,37]

Decorin, a small leucine-rich DSPG, “decorates” the cell surface and is considered to participate in extracellular matrix assembly. The single DS chain of decorin binds to tenascin-X which is an extracellular protein localized in connective tissues. Several studies have been published indicating the important role of GAG chains of decorin in fibrillogenesis and structure [38]. An anti-oncogenic role has been also demonstrated due to the inactivation of ErbB2 (an oncogenic member of the EGF tyrosine kinase receptor family associated with poor prognosis in tumors of the

breast, ovary and prostate. In addition, decorin levels are reduced or even lost by several tumor cells.[14,38]

DS also interacts with **growth factors**. In contrast of HP/HS, interaction between DS and FGF-2 has only been studied with respect to cellular proliferation showing higher capacity to stimulate cell growth *in vitro*.[36] Decorin has also been described to bind specifically and with high affinity the hepatocyte growth factor (HGF) receptor known as Met, which plays a role in morphogenesis, differentiation, motility and angiogenesis in a variety of cell types. An octasaccharide with unsulfated IdoA with a 4-*O*-sulfated GalNAc has been identified as the minimum DS binding epitope.

DS has been shown to be involved in infection. Phatogenesis of *Pseudomonas aeruginosa*, *Enteroccus faecalis*, and *Streptococcus pyogenes* involves release of proteinases that degrade DS-containing PGs. The free DS binds to neutrophil-derived cationic α-defensin and this binding completely neutralized its bactericidal activity.[39]

Several chemokines, as IL-8, MIP-1 α and β (macrophage inflammatory peptides), RANTES (regulated on activation normal T cell expressed and secreted) and MCP-1 (monocyte chemoattractant protein-1) and interferon gamma (IFN-γ) bind to DS. These interactions consist in the formation of gradients along the extracellular matrix and the facilitation of the binding ligands-receptors.[36]

Overall, additional studies on DS are required to expand our understanding of the biological functions of this GAG.

In 2004, Hsieh-Wilson and co-workers published one of the first biological studies using synthetic CS molecules, in which a CS tetrasaccharide was identified as the minimum motif required for neuronal stimulation. In addition, they demonstrated that the sulfation is required to promote the neuronal growth. These studies demonstrated that the CS sulfation pattern was a molecular recognition element for growth factors and the activity was not related to nonspecific electrostatic effects.[40,41]

In addition, they demonstrated the power of GAG oligosaccharide synthesis to provide defined probes of high purity required for deciphering the details of GAG-protein interactions on a molecular scale.

1.3 Chemical Synthesis of oligosaccharides: solution and solid-phase

Carbohydrate organic synthesis is a complicated task due to the polyhydroxylated nature of these molecules. Common challenges in oligosaccharide synthesis include lengthy multiple step routes due to numerous protecting group operations, orthogonal conditions for the introduction and removal of these groups and the conditions for the stereoselective formation of glycosidic bonds as well as efficient access to rare sugars (as IdoA). Therefore, critical aspects of any oligosaccharide synthesis are high yielding chemical transformations and high stereoselectivity in the coupling step. In addition and very relevant for GAG synthesis is an efficient access to protected monosaccharide building blocks, which are often rare sugars.

1.3.1 Protecting group strategy

Considering the intrinsic polyfunctional nature of the GAG´s, their syntheses require extensive use of protecting groups for temporarily masking and differentiating the different hydroxyl, carboxyl and amino groups to achieve high selectivity in the coupling and sulfation reactions. A large number of different protecting groups are available to aid in the differentiation of functions of very similar reactivity.[42] The chosen protecting groups should fulfil a number of conditions to be considered suitable for the lengthy and complex oligossacharide synthesis. Firstly, reagents for the introduction and removal of a protecting group should be easily accessible and ideally commercially available. Secondly, the installation and cleavage reactions themselves should preferably be carried out under mild conditions and not affect any other groups present in the molecule (the ability to add/remove protecting groups in the presence of others is termed orthogonality). Thirdly, the groups should also remain stable throughout the synthesis and during the work-up and purification steps. Usually, the protection of a free chemical function will afford a final product that is more hydrophobic than the starting material, which is easier handled during work-up and chromatographic purification and sometimes could be crystallised for convenient large scale purification. In addition, the reaction should ideally afford a product that is easily separated via chromatography from the starting material during the work-up and purification steps. The sequence of

protecting different hydroxyl groups of similar reactivity in a monosaccharide is determined by their relative reactivities in the required chemical transformation. As a general rule the nucleophilicity of hydroxyl groups decreases in the order anomeric hydroxyl, primary and secondary and equatorial over axial groups. It has been observed that in a first approximation, the reactivity of the hydroxyl groups generally decreases with increasing distance from the anomeric position. It is however to be noted that this rule of thumb is valid in the presence of both axial/equatorial hydroxyl groups (axial hydroxyl groups are far less reactive than equatorial ones).[43]

A typical monosaccharide building block used in oligosaccharide assembly is equipped with permanent and temporary protecting groups. Permanent groups are used to mask those hydroxyl groups that are not going to be modified during the course of the synthesis and will only be exposed at the very end. Permanent benzyl (Bn) protecting groups, which can be cleaved by hydrogenation at the end of the synthesis, are most frequently used but rather stable esters, such as pivaloyl (Piv), acetyl (Ac) or even benzoyl (Bz) esters are also used. Temporary protecting groups are functional groups that are usually less stable and hence more easily cleaved such as fluorenylmethoxycarbonyl (Fmoc), levulinoyl (Lev) and several silyl groups. For the synthesis of HP or HS oligosaccharides numerous orthogonal permanent/temporary groups have to be selected to ensure the flexible access to defined sulfation and acetylation patterns on the GAG chain (Figure 1.9). Most synthetic strategies mask positions for unsubstituted hydroxyl groups as benzyl ether and sulfated positions as ester groups (eg. Ac, Bz and Piv), respectively. However, whether a protecting group is finally employed as permanent or temporary is often dependent on the chosen glycosylation strategy.

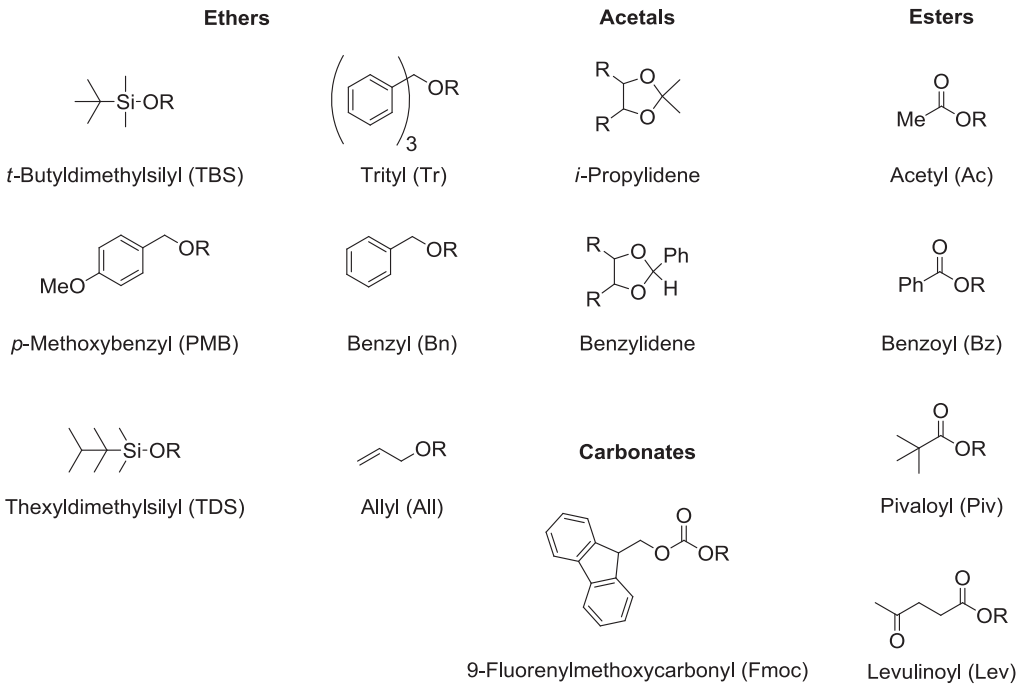
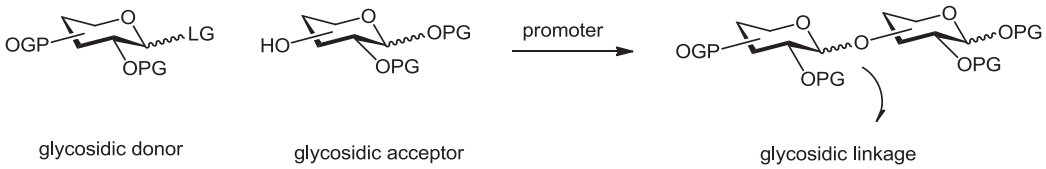


Figure 1.9. Some of the major protecting groups used for HP/HS oligosaccharide synthesis. R = carbohydrate.

1.3.2 Chemical glycosylation

Glycosylation reaction consists in the formation of a covalent bond, known as glycosidic linkage, between two sugar units or between a sugar and an aglycon (Scheme 1.1). This bond can be chemically formed by the displacement of an anomeric leaving group (LG) by an alcohol, or by the OH group of a partially protected sugar moiety. The nucleofilic compound presenting the free hydroxyl group is called glycosyl acceptor, and the sugar residue bearing the leaving group (LG) at its anomeric position is known as glycosyl donor. The reaction generally is performed in the presence of an activator called “promoter” assisting in the departure of the leaving group. The role of the promoter is to assist the departure of the leaving group (LG) and is often used in catalytic amount, although in some instances they could be used in stoichiometric amounts.

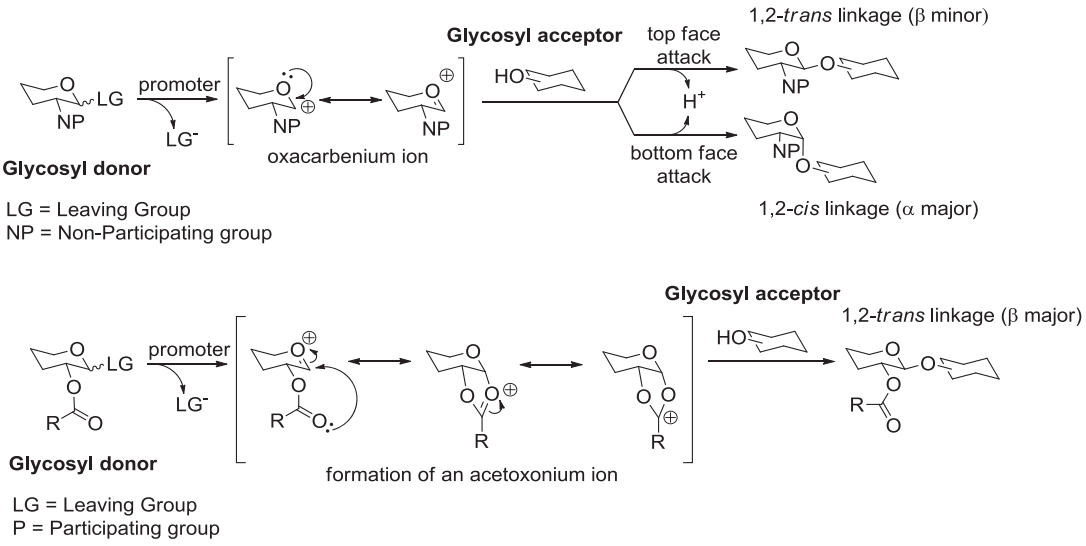


Scheme 1.1. Glycosylation reaction between glycosidic donor and acceptor

The general mechanistic pathways for glycosidic bond formation consists on the activation of the glycosyl donor by the promoter resulting in the departure of the leaving group and the creation of a glycosyl cation intermediate, called oxacarbenium ion (Scheme 1.2). This transient entity can hence be attacked by the free hydroxyl group of the glycosyl acceptor from either the top face or the bottom face of the ring plane, which will finally result in the formation of the new glycosidic bond, with α or β configuration at C-1.

In this context, the stereochemistry at the anomeric carbon must be controlled during the formation of the new glycosidic bond. In theory, a glycosylation reaction could lead to the formation of both 1,2-cis and 1,2-trans glycosides. The resulting stereoselectivity of a glycosylation reaction is governed by many parameters such as the stereochemistry of the donor, the anomeric and remote protecting effects; and the steric hindrance and nucleophilicity of the acceptor. Highly stereoselective glycosylation reactions can be achieved by controlling some of these parameters. The formation of 1,2-trans glycosidic linkages can be achieved exploiting the neighbouring group effect of an acyl protecting group at C-2 of the glycosyl donor (Scheme 1.2). The presence of this electron donating moiety stabilises the oxocarbenium cation intermediate (neighbouring group participation) and leads to the formation of an acetoxonium ion (also called acyloxonium ion). Since one of the faces of the ring plane is sterically more hindered, the nucleophilic attack of the glycosyl acceptor will preferentially occurs on the opposite side of the plane. Ultimately, this leads to the preferential formation of the 1,2-trans glycoside, even though some traces of the 1,2-cis glycoside may be detected. In contrast, non participating C2 protecting group and the anomeric effect [44,45,4546] favour the formation of 1,2-cis glycosides, although it is far more challenging to obtain good stereoselectivity than for 1,2-trans glycosides. Other factors such as conformational strain or solvent effects have also an influence on the stereochemical outcome. Non-polar solvents such as diethyl ether tend to favour 1,2-cis linkage formation whereas

polar solvents, such as acetonitrile stabilize the intermediate oxocarbenium ion, favouring the formation of the 1,2-trans glycosides[44,47]



Scheme 1.2 General mechanistic pathways for glycosidic bond formation. **S_N^1 pathway**: non-participating group –NP at C-2; **S_N^2 pathway**: participating group –OP and the different outcomes ,1,2-*trans or* 1,2-*cis* glycoside

Moreover, even though it is widely agreed that the general mechanism of a chemical glycosylation can be described as depicted in Scheme 1.1, the exact mechanism is still not fully understood. Many mechanistic studies have been carried out during the years to gain a deeper understanding of the underlying parameters that control stereoselectivity of glycosylation reactions[48-49]. Yoshida and co-workers.[50] reported the synthesis of alkoxycarbenium in the absence of nucleophiles and the subsequently reaction with nucleofiles previous to their decomposition. The lifetime of these intermediates is in the order of seconds and is highly dependent on the temperature. In recent studies glycosyl cations derived from 2-deoxy and 2-bromoglucospyranose have been generated in superacid medium and characterized at low temperature by NMR spectroscopy.[49]

On the other hand, protecting groups can affect the reactivity of the donors due to their influence on the conformation and/or through glycosyl oxocarbenium by disarming effects arising from their electron-withdrawing ability[51]. Furthermore, the influence of promoters and additives to the glycosylation reaction has been studied for many years by different groups[52]. A higher knowledge of the reaction mechanism, as well as having a better overview of the impact of all the parameters/components on its outcome, will help in finding appropriate solutions to overcome the difficulties still encountered for the synthesis of complex sugars.

Therefore, the stereochemical outcome of each glycosylation reactions needs to be investigated individually, especially in the formation of large oligosaccharides. In the case of HP/ HS the 1, 2- *trans* linkage between the uronic acid and the glucosamine residues is usually achieved through the use of a participating protecting group at C-2 of the uronic acid glycosyl donor. However, formation of the 1, 2-*cis* linkage the glucosamine and the uronic acid units can be difficult to control. The non participating azido group, is employed to mask the C-2 amino group of the glucosamine glycosyl donor and to permit 1,2-*cis* coupling. High stereoselectivities are generally achieved for L-idosyl acceptors; however, for D-glucuronic acid-based acceptors, an anomeric mixture results from the glycosylation and therefore an extensive protecting group manipulation is required.

The choice of an anomeric leaving group is one of the most important consideration for oligosaccharide assembly. Glycosyl halides[53,54,55], glycosyl trichloroacetimidates[56], glycosyl *N*-phenyltrifluoroacetimidates[57], glycosyl sulphoxides[58], glycosyl phosphates[59], thioglycosides[60], *n*-pentenyl glycosides[61] are major leaving groups that have found entry in the routine synthetic protocols of carbohydrate chemists worldwide (see Figure 1.10). Glycosyl trichloroacetimidates are perhaps the most extensively used glycosylating agents, due to their high reactivity, easy preparation, mild activation under only catalytic amounts of Lewis acid promoters and compatibility with base and acid labile protecting groups.

Also as a general rule, the synthesis of protected monomeric building blocks remains the most time consuming process of oligosaccharide synthesis. Commonly, the differently protected and functionalized monosaccharides are accessed from

naturally occurring sugar starting material through a series of lengthy and often tedious protection-deprotection manipulations.

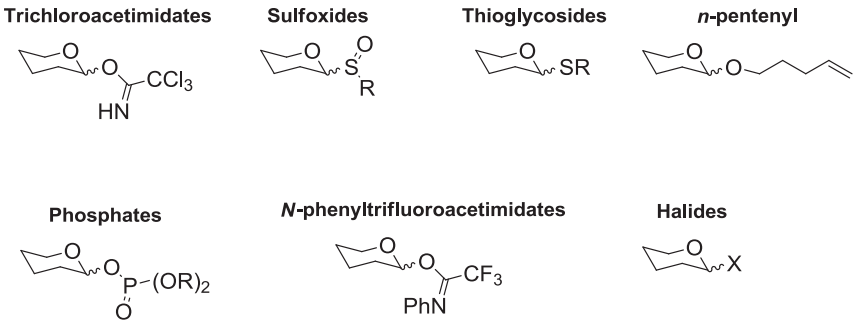


Figure 1.10. Various glycosyl donors used for glycosylation reactions.

In general, there are two major strategies for the assembly of oligosaccharides, and both were applied to GAGs synthesis. The first approach is the linear construction of oligosaccharides, by which each building block (or disaccharide units) is added to the growing oligosaccharide chain. In order to increase the yield of each single step the smaller unit is generally used in excess.

A second strategy is the convergent synthesis involving the assembly of similarly sized units. Despite the fact that this strategy produces shorter synthetic routes with potentially higher overall yields, main disadvantages are the activation of highly elaborated starting material which is lost when used in excess. The decision to use either one of the described strategies usually depends on several factors, including literature precedent, sourcing of starting materials, and individual expertise.

1.3.3 Chemical synthesis of HS/DS oligosaccharides in solution

Due to its significant medical importance and clinical potencial HP/HS oligosaccharides are among the most studied GAGs and considerable efforts have been invested in the development of strategies for their synthesis over the past 30 years.[62]

The synthesis of HS/DS oligosaccharides present several difficulties inherent to the complex structure of the target molecules. First, L-idose and L-iduronic acid

are not readily available from natural sources and affordable prices. Efficient and synthetic routes to produce large amounts of protected monosaccharide building blocks are therefore required. In addition, the development of an appropriate protecting group strategy needs to be considered allowing the implementation of the high degree of functionalization of the HS/DS fragments (see section 1.3.1). A final challenge is the complete stereoselective interglycosidic bond formation between the synthetic building blocks.

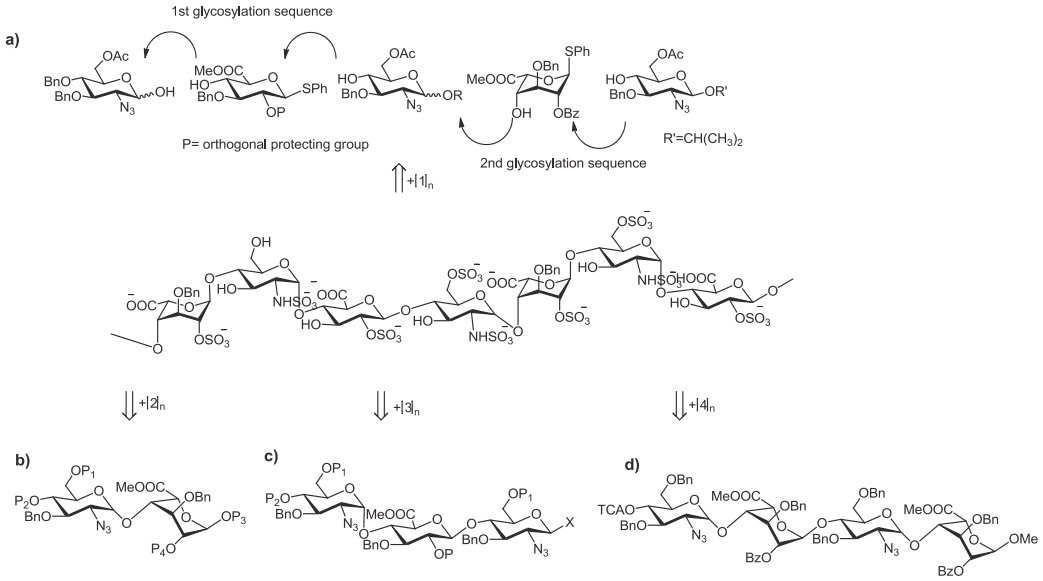
Many procedures and strategies for the synthesis of heparin like oligosaccharides were pioneered by Petitou, Jacquinet and co-workers during the total synthesis of the ATIII binding HP pentasaccharide[63,64,65]. The following years, several studies were published describing the preparation of pentasaccharide analogs to simplify the chemical synthesis and increasing the knowledge of HP structure-activity relationship[66,67,68].

These initial studies inspired the chemical synthesis of a wider range of these types of GAGs and their mimetics. At the end of the 90s and beginning of the 21st century several oligosaccharide sequences (tetra-, hexa- and octasaccharides) of the regular region of HP (IdoA(2S)-GlcNS(6S)) were synthesized by a convergent modular strategy using disaccharide as building blocks.[69,70,71] In order to understand in more detail the interactions of HP with fibroblast growth factors[29], the synthesis of various modified HP sequences replacing L-IdoA residues present in the HP regular sequence by D-GlcA has been reported[72].

Over the last three decades, three main strategies for the synthesis of HP and HS oligosaccharides have emerged. The convergent modular synthesis is based on the sequential assembly of fully differentiated building blocks, until the desired length is reached. This final precursor is then modified to attain the desired functionalised oligosaccharide. This convergent modular synthesis requires extensive and lengthy synthetic pathways and is more suitable for the preparation of structurally homogenous oligosaccharides[70,32,73-74]. On the other hand, the divergent approach requires the synthesis of one or several common precursors which will undergo structural modifications of their backbone prior to further elongation until the desired size is obtained[75-76]. The divergent type of synthesis allows easier preparation of a wider range of structures, unlike the convergent modular one.

Finally, over the last decade, for the aim of developing concise and more efficient synthetic pathways, much effort has been put into the development of versatile syntheses, which can be defined as a combination of both divergent and modular syntheses [77,78,79]. Despite the differences that can be listed between those synthetic strategies, they all rely on diverse glycosylating agents, which thus appear to be one of the keystones of oligosaccharide chemical synthesis.

In general, the synthetic approaches reported in the literature mainly differ in the degree of modulation: monomeric[80], dimeric[81,82], trimeric[83] or even tetrameric residues[84] have been used as elongation blocks in modular glycosylation strategies. They also differ in the type of building blocks used, in the timing of the oxidation of the hydroxyl group at position 6 to obtain the uronic acid and finally in the glycosylation procedures.



Scheme 1.3. Different synthetic approaches for heparin –like oligosaccharides considering the different modulation degree. a) monomers[80]; b) dimers[81]; c) trimers[83] and d) tetramers[84].

In 2006, Lu *et al*. were the first to report the chemical synthesis of the first set of 48 fully and orthogonally protected disaccharide precursors that can be used as building blocks for the modular synthesis of HP/HS oligosaccharides[85]. A multitude of different size building block precursors have been designed throughout the years [86,87]. The differences between the building blocks are essentially based on the type of protecting groups used[88,89].

Although carbohydrate synthesis allows in theory the preparation of any HP/HS structure, many challenges as the exclusive stereoselective formation of α-glycosidic linkages in the absence of a participating neighbouring group or the often low yields in glycosylation reactions including IdoA residues still have to be overcome. In addition, uronic acids are known to be less reactive than their non oxidized analogues[90]. This can be explained by the electron withdrawing effect of the carboxyl group at position 5 that decreases the nucleophilicity of the neighbouring hydroxyl groups. The more reactive idosides [91]and glucosides[73] have therefore been used as glycosylating agents leaving the oxidation to uronic acids to a later stage in the synthesis.

Recently, Boons and coworkers reported the synthesis of modular disaccharide building blocks containing glucuronic acid residues.[92] They demonstrated that glucuronic acids protected at C-2 with a permanent 4-acetoxy-2,2-dimethyl butanoyl- PivOAc or temporary Lev ester can be used as glycosyl donors to prepare dimeric precursors with high yields, avoiding the late-stage oxidation step at the very end of the synthetic process.

The chemical synthesis of long GAGs remains a major challenge. All the work performed in the development of new synthetic routes has allowed the preparation of well defined oligosaccharides of different sizes. Gardiner and coworkers[93] described in 2012 the first gram-scale synthesis of an heparin–related dodecamer and recently[84], the total synthesis of the longest synthetic heparin like oligosaccharide yet described (40 mer).

DS like HS contains the iduronic acid residues in its repeating unit, making the chemical synthesis of these polymers somewhat more complex than that of other related GAGs. In 1987, Jacquinet and Sinaÿ synthesized the first disaccharide fragments of DS employing an unstable L-idopyranose chloride without a participating group at C-2 as donor and azido D-galatopyranose as acceptor which gave rise to an anomeric mixture of formed disaccharide. Comparing the reactivity of different iduronic acid donors (trichloroacetimidates, thioglycosides, *n*-pentenyl and fluorides)[89] the Jacquinet group found trichloroacetimidates and *n*-pentenyl glycosides[94] to be more reactive than thioglycosides and fluorides.

L-Idose instead of IdoA residues were employed as glycosyl acceptors for the synthesis of the DS hexasaccharides. Goto and Ogawa containing IdoA(2S)-GalN(4S) to improve glycosylation yields and the C-6 hydroxyl groups oxidized under Swern conditions followed by treatment with sodium hipochlorite furnishing the final product after esterification in 39% overall yield.[95]

In 2000, Barroca and Jacquinet reported an efficient and stereoselective glycosylation reaction of C4 position of L-IdoA as acceptor employing an 2-deoxy-2-trichloroacetamido-D-galactopyranose as donor. This method was later applied to the synthesis of various sulfoforms of DS trisaccharides.[96] Highlights of this synthesis are the regioselective oxidation of the disaccharides in the Ido 4, 6 diols after treatment with catalytic amount of 2,2,6,6-tetramethylpiperidine 1-oxyl free radical (TEMPO) in the presence of stable calcium hypochlorite and the use of pivaloyl and acetates as temporary protecting groups allowing the preparation of different sulfoforms from a common precursor.

In 2009, Jacquinet *et al*. developed a combinatorial approach to synthesize a library of O-sulfate tetra- and hexa- chondroitin sulfate oligosaccharides from a GlcA-GalN disaccharide precursor which had been prepared in 11 steps from the natural chondroitin sulfate polymer.[97,98] The synthetic route was based on a 2+2 strategy employing this disaccharide with the acceptor position protected as a levulinate. Glycosylations were carried out with a galactosamine trichloroacetimidate donor carrying a 2-deoxy-2-trichloroacetamido group to ensure 1,2-trans selectivity in the glycosylation.

Yamane and co-workers developed synthetic routes towards CS oligosaccharides with the inverse sequence to glycans made by the Jacquinet group. In contrast to previous approaches, disaccharide building block used for the construction of hexa- and octasaccharides was the reverse-type sequence β-GalNAc-GlcA.[99]

In 2013, Maza *et al*. reported the synthesis of sulfated CS/DS tetrasaccharides using *N*-trifluoroacetyl as temporary protecting group in the amine function of the D-galactosamine building block, which can be easily cleaved under mild conditions. Binding studies with FGF-2 by fluorescence polarization assay indicated that the synthesized tetrasaccharides compounds are able to interact with FGF-2.[100]

1.3.4 Enzymatic and chemoenzymatic synthesis

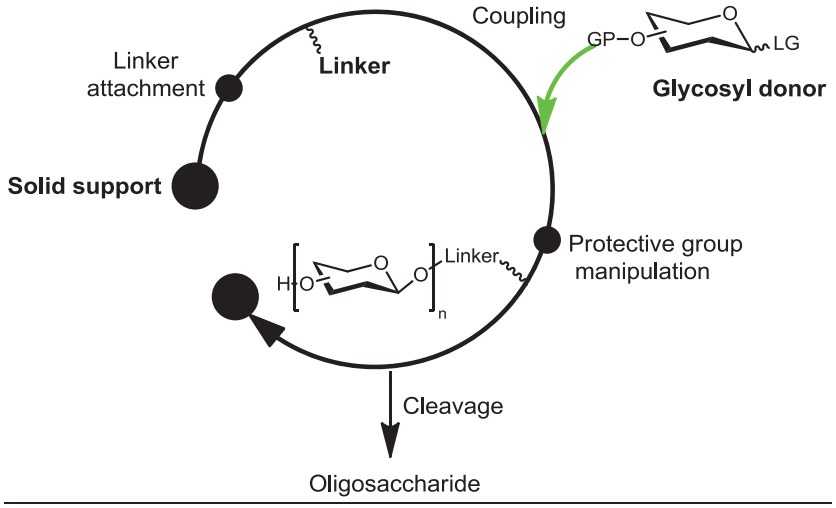
Chemoenzymatic synthesis of GAGs has also been developed.[101,102] Two main methods have been investigated, involving glycosyltransferases and reverse-activity glycosyl hydrolases, and glycosynthases have also been introduced as new efficient tools[103,104]. These approaches can help shorten the synthetic routes, and therefore help save a considerable amount of time when preparing libraries of oligosaccharides[105–108]. The GAG sequences, synthesized using the chemoenzymatic approach include the AT-III binding domain of HP[105] oligosaccharides concurrently displaying several anti-factor activities[109]; Ultra Low Molecular Weight (ULMW) Heparins[110,111] and HS oligosaccharides displaying a range of affinity towards FGF2 and which therefore allow the SAR of these GAGs[112]. The assembly of oligosaccharides by enzymatic methods has the advantages that the oligosaccharides may be prepared without protecting group manipulation. However, several glycosyl transferases are not commercially available and life-time of the enzymes is limited.

1.3.5 Chemical synthesis of oligosaccharides on solid-phase.

Solid-phase synthesis is a methodology in which synthetic transformations are conducted with one of the reactants attached to an insoluble matrix, allowing the removal of other reagents and solvents by simple filtration. This strategy was developed by Merrifield in 1960 for the sequential solid-supported synthesis of polypeptides on a polystyrene resin, now known as Merrifield´s resin. Solid-phase synthesis was conceived under the theory that an excess of reagents could be used to drive reaction on the solid support to completion, and that the excess of reagents and soluble by-products can simply be removed by filtration and washing of the polymeric support. This methodology was efficiently applied for preparation of oligonucleotides and peptides[113], and later adopted for the preparation of complex oligosaccharides[114]. The solid-supported synthesis of oligosaccharides, however, faces additional challenges like the requirement for anhydrous reaction conditions, stereo-selective glycosylation reactions, low temperature coupling procedures, and more complex protecting group regimes that allow for the synthesis of branched structures. The low stability of many of the highly reactive glycosyl donors is particularly a problem for the automation of solid-phase procedures. Therefore,

stereoselective glycosylation and protective group manipulation in high yield are essential for a successful solid-phase synthesis.

In general, solid-supported synthesis of oligosaccharides is carried out from the reducing to the non-reducing end coupling a glycosyl acceptor as a primer via a cleavable linker to the solid support (Scheme 1.4). After attachment, this glycan primer is selectively deprotected to produce a glycosyl acceptor for the subsequent coupling step with a suitable donor. Glycosylations and protective group manipulations are carried out in a repetitive cycle, and the final cleavage from the solid support releases the protected oligosaccharide.



Scheme 1.4. General procedure for solid-phase oligosaccharide synthesis.

A major limitation of the solid-phase synthesis is monitoring the reactions performed on the polymeric support. In general, destructive methods are applied, where a small amount of solid support is cleaved and subjected to ordinary solution-phase analysis, for instance liquid chromatography–mass spectrometry (LC-MS), thin layer chromatography (TLC) and nuclear magnetic resonance (NMR) spectroscopy. The disadvantage with these methods is that valuable product is consumed and that intermediates may decompose during cleavage, which could complicates the analysis. Even though these methods required really small quantities of product, non destructive methods are desirable and several innovative NMR

spectroscopy methods suited for on-bead analysis have therefore been developed. However, considering the automated solid phase synthesis as the new era of oligosaccharide synthesis, a more direct methodology is preferred. Fluorescent monitoring using Fmoc as temporary protective group allowed calculated the efficiency of the glycosylation reaction draining the deprotection solution through a UV-based cell[115,116]. In addition, several innovative NMR spectroscopy methods suited for on-bead analysis have therefore been developed.

The structure and physicochemical properties of the **resin/solid support** may have a major impact on the outcome of a reaction performed on a polymeric support. Therefore, understanding the resin properties is important for an efficient solid-phase glycoconjugate synthesis and the properties of the resin, such as swelling, cross-linking, particle size, stability, functional groups and loading capacity needs to be considered during the synthetic route design. Numerous resins with different synthetic properties are commercially available and the most frequently used are the gel-type resins. These are typically spherical beads of cross-linked polystyrene (PS) or poly(styrene-oxyethylene) graph copolymers. In organic solvents, these resins furnish a solvent-swollen gel, which improves the diffusion of molecules through the solid matrix.

The classical and the most commonly employed resin today is the Merrifield resin (1 or 2% divinylbenzene cross-linked polystyrene). Its exhibits high loading capacity and excellent stability tolerating a wide range of reaction conditions. Polystyrene exhibits excellent swelling in dichloromethane, tetrahydrofurane (THF), dimethylformamide (DMF) and dioxane, but swelling of the beads in polar solvents and also the diffusion of polar reagents into the polystyrene matrix is limited. The compatibility of the polystyrene resin with protic solvents has been improved by grafting polyethylene glycol (PEG) chains onto polystyrene backbone. TentaGel™ or ArgoGel™, is a member of this PEG-family. This resin exhibits good swelling properties even in water. A drawback of the PEG-derived resins is their lower loading capacity and higher price compared to Merrifield's resin.

Insoluble support normally requires additional development to transfer protocols for solution-phase synthesis to the solid-phase. On the other hand, polyethylene glycol soluble polymers (MPEG) combine advantages of the solution-phase synthesis with the easy work-up of solid-phase synthesis. The reactions are carried out in homogeneous solution; however, the polymer is precipitated out after each step in order to remove any excess of reagent by simple filtration. The principal disadvantage of this support is the loss of material during the precipitation step after each coupling, which can decrease severely the overall yield in the synthesis of larger oligosaccharides. In addition the precipitation step is not easily automated for the introduction in automated synthetiser workflows.

Another critical element of solid-phase synthesis is the choice of an appropriate **linker** to attach the first sugar unit to the solid support. Silyl ether linkers[117], acid- and base-labile linkers, thioglycoside linkers, photocleavable linkers[118] and linkers that can be cleaved by oxidation or hydrogenation[119] are commonly used for solid-phase synthesis.[120]

1.3.3.1 Historical perspective of solid-phase synthesis of oligosaccharides-GAGs

The application of solid phase methods toward the preparation of oligosaccharides began in the 70's but interest in this field decreased due to deficiency lack of efficient glycosylation methods available at that time. In the 1980's and 1990's the development of novel glycosylating agents renewed the interest in solid phase carbohydrate synthesis.[121,122,123]

In 1997 Van Boeckel and coworkers demonstrated the successful assembly of several heterogenic HS-like oligosaccharides in a polymer supported solution synthesis using disaccharide building blocks[124]. The same n+2 block synthesis strategy was later employed to synthesize a series of four hexasaccharides and two octasaccharidic heparin-like oligosaccharides with different charge distribution on a soluble polymer support (MPEG) or on a PEG grafted polystyrene resin (ArgoGel^{TM})[125,126]. In these studies, a succinoyl ester linker was used to attach the conveniently protected glycosyl acceptor to the resin. The elongation of the oligosaccharide chain was carried out by repetitive glycosylation of the 4-OH group of the L-iduronate unit of the acceptor with a conveniently protected glycosyl donor

and the trichloroacetimidate method was used in all glycosylations. Several attachment sites of the disaccharide acceptor to the polymer support have been investigated and finally the best results were obtained using MPEG as polymer support and attaching the disaccharide acceptor through the carboxylate group of the L-iduronate unit by mild transesterification in the presence of dibutyltin oxide.

In 2001 Seeberger and coworkers developed the first automated oligosaccharide synthesizer.[114] In the last years, automated solid phase synthesis by sequential assembly of differentially protected monosaccharide as building blocks was successfully applied for the synthesis of partially protected chondroitin sulfate hexasaccharides[116] and conjugated dermatan sulfate oligosaccharides.[127] Nowadays, this improved automated platform, Glyconeer 2.1 synthesizer, is commercially available via GlycoUniverse [115] and Activotec[128].

Therefore, solid phase methodology with appropriate building blocks could be well suited to provide rapid access to defined GAG oligosaccharides such as heparin, heparin sulfate, dermatan sulfate or keratan sulfate of varying length. In this context, new synthetic approaches needs to be developed.

1.4 Microarrays and other possible applications

Solid phase synthesis will produce GAGs functionalized with a linker that could be directly employed in interaction studies.

Several methods have been established to study the interactions of carbohydrate with various glycan binding proteins. Carbohydrates have been immobilized to surfaces for surface plasmon resonance (SPR) and microarray screening. Additionally, carbohydrates could be attached to gold nanoparticles, quantum dots, magnetic beads and chromatographic solid phases for the preparation of affinity columns.

Carbohydrate microarrays have been used in glycomics research to examine the interactions of carbohydrates with glycan binding proteins. The first carbohydrate

microarray was developed in 2002.[129] The use of microarrays offers important advantages, because only minimum amounts of analyte and ligand are required and several binding events can be screened on a single glass slide at the same time. The sugar ligands are immobilized on activated surface of glass slides and screened with solutions of glycan binding proteins (GBPs). After incubation, the array is washed to remove any unbound material and binding events are measured and analyzed. Carbohydrate microarrays have been widely applied for rapid analysis of the carbohydrate-protein binding properties, the detection of specific antibodies, and fast assessment of substrates specificities of glycosyltransferases.[130,129]

In 2006, in order to understand the structure-function relationship of heparin like-glycosaminoglycans and growth factors, J.L de Paz *et al.* published the preparation of microarrays using synthetic heparin oligosaccharides derived by solution and solid phase assembly methods[131]. In recent years, new studies have been published using microarray technology to investigate/understand the molecular mechanism of interactions between GAGs and specific proteins.[132-133]

However, some limitations need to be overcome for wider applications of the carbohydrate array technology.[130] The major limitation is still the preparation and supply of thousands of saccharides that could reflect the heterogeneity of natural GAG populations. Despite the remarkable advantages in the automated assembly of oligosaccharides, the solid-phase synthesis of structures with the complexity and polarity of GAGs is still a difficult goal to achieve and therefore new approaches need to be developed.

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2. OBJECTIVES AND SYNTHETIC STRATEGY

2. OBJECTIVES AND SYNTHETIC STRATEGY

The main objective of this thesis has been to establish a suitable methodology for the solid phase synthesis of glycosaminoglycans (GAGs), with special attention to heparan sulfate (HS) and dermatan sulfate (DS) oligosaccharides. The synthetic strategy is based on the acceptor bound solid phase approach and in performing sequential glycosylations with highly reactive monosaccharide building blocks, avoiding the use of advanced disaccharide units commonly employed in previously published GAGs synthesis.

To achieve this main objective, the following partial objectives have been addressed (Figure 2.1):

1. Selection of a suitable polymer support and the development of a linker that will be stable during all synthetic operations and that could be cleaved efficiently.
2. Identification of appropriately protected building blocks in order to access biologically relevant glycosaminoglycans with different *N*- and *O*- sulfation patterns.
3. Development of rapid, stereoselective and high yielding coupling (glycosylation) reactions. Deprotection conditions for temporary protecting groups in positions where the elongation of the biopolymer will be performed.
4. Study of cleavage of the protected oligosaccharides from the solid support.
5. Sulfation and global deproctection of the final product

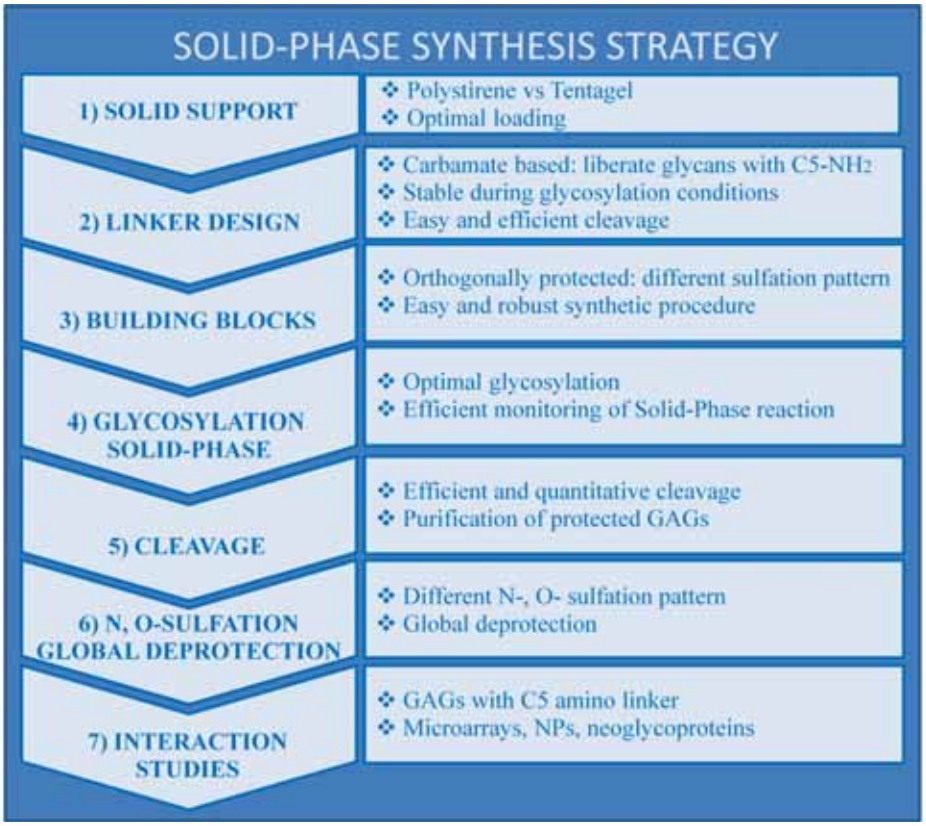


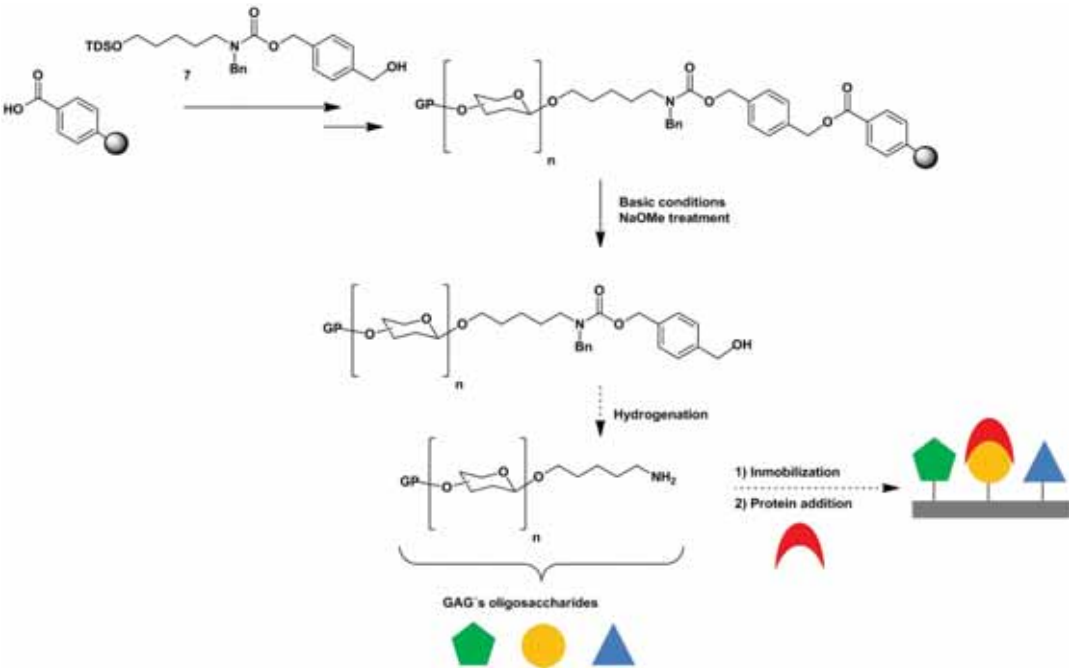
Figure 2.1. Considerations for the development of the solid-phase synthesis strategy.

2.2.1 Solid support

The polymer support has an immense influence on the synthetic strategy and the choice of reagents (refer to section 1.3.3.1). Merrifield´s resin[1] which is composed by polystyrene (PS) backbone cross-linked with 1% divinylbenzene was selected as matrix support based on the previous published work for solid-phase oligosaccharide synthesis[2,3]. Merrifield´s resin is commonly employed in solid phase synthesis due to its high loading capacity, good compatibility with a broad range of reaction conditions, durability and low price. In addition, Merrifield resin only exposes its reactive sites when it is used in a swelling solvent, such as CH_2Cl_2, THF or toluene. On the other hand, Tentagel® resin at different loading capacities was evaluated in the immobilization of linker and in model glycosylation reactions. This optimization process revealed that polystyrene support was the best option to be used for the solid phase synthesis of GAG oligosaccharide precursors.

2.2.2 Linkers for solid phase synthesis

Another critical element of solid phase synthesis is the choice of an appropriate linker to attach the first sugar unit to the solid support. In this thesis, a new linker based on a 4-hydroxymethylbenzyl *N*-(5-hydroxypentyl)-*N*-benzyl carbamate spacer and attached via an ester linkage to the carboxy-functionalized resin has been specifically designed (Scheme 3.1). This linker provides a stable handle under Lewis acid catalysis (TMSOTf) normally employed during glycosylation reactions, under electrophilic conditions necessary for the activation of certain glycosidic donors (NIS) and under mild basic conditions. Once the synthesis of biopolymer has achieved the desired length, it could be easily and efficiently cleaved under basic conditions releasing the amine protected as carbamate. This protecting group is compatible with the azide function employed as N-protecting groups in glucosamine building blocks. Previously described approaches for the immobilisation of amino-functionalized glycoconjugates release highly polar amines[4] which are difficult to purify and are not compatible with the orthogonal functionalization of glycosamines e.g. N-sulfation or acetylation in HS synthesis. Furthermore, the final hydrogenation step would provide the glycoconjugates functionalized with amino C5-linker which can be attached to carrier proteins, nanoparticles or activated surfaces (microarrays) in order to perform interaction studies (Scheme 2.1).



Scheme 2.1. Cleavage and final hydrogenation release the aminopentyl glycoside handle.

2.2.3 Building blocks

The design and synthesis of monosaccharide building blocks used in the solid phase synthesis of GAGs is a laborious and time-consuming task. With a notable exception from the group of van Boom ,[5] the solution-phase synthesis of HS fragments is usually based on the assembly of highly elaborated disaccharides, reflecting the major repeating units of the polymer. Our strategy is based on the sequential assembly of monosaccharide building blocks on a solid support that avoids the multi-step processing of elaborated disaccharides structures and introduces a higher degree of flexibility for the generation of structurally diverse HS/DS libraries. In addition, this approach is more practical from a synthetic point of view; for example, to synthesize all possible different dermatan sulfate oligosaccharides 12 different disaccharides are required, while the same variability can be achieved with only 7 different monosaccharide building blocks.

To improve conversion rates in solid phase synthesis usually an excess of reagents is employed making the preparation of sufficient quantities of fully differentiated building blocks with an appropriate protecting group pattern is a key consideration for the synthesis of complex oligosaccharides. To this end the development of short, high–yielding and convergent synthetic routes to central building blocks are important also to quickly optimize glycosyl donors for stereo-selective and efficient glycosyl bond formation. In our approach benzyl ethers (Bn) were chosen as permanent protection for free hydroxyl groups in the final compound. Benzoyl and acetyl esters (Bz, Ac) were selected to mark hydroxyl groups to be orthogonally functionalized with sulfate groups. Levulinic ester (Lev) was chosen as temporary protecting group, which will be orthogonally cleaved after each coupling reaction to allow the elongation of the growing biopolymer. Furthermore, the protecting groups directly control the stereoselectivity during oligosaccharide synthesis when installed at C-2 by anchimeric assistance. The choice of protecting groups not only influences the stereoselectivity of the glycosylation as described above, but also can have an effect on the overall reactivity of a carbohydrate building block due to the armed-disarmed effect of the protecting group. Carbohydrates substituted with strong electron-withdrawing groups like esters (-OCOR,) are called disarmed donors, they have a destabilizing effect on the oxocarbenium ion and they are less reactive that so called armed-donors. Armed-donors have as substituent electrodonating groups such as benzyl groups (-OBn) that reacts faster than disarmed donors.

In this thesis, different L-idose (compounds **25 – 30, 35 – 38),** L-iduronic (**43, 44, 52 – 54, 57)**, and 2-azido-D-glucopyranosyl (**65, 66**) donors were synthesized to study the effect of different protecting group patterns in the reactivity of the glycosyl donors, and evaluate if Ido or IdoA derived donors are better suited for solid-phase synthesis HS precursors. Under optimized conditions, the preparation on solid phase of trisaccharide and hexasaccharide HS precursors was achieved. Using a similar same strategy, a solid phase approach for the synthesis of tetra and octasaccharide DS precursors was developed. In this case, previously optimized common idose building block donor **30** was used and the reactivity of different D-galactosamine building blocks donors (**81 – 82**) evaluated in solution and on solid support (Figure 2.2).

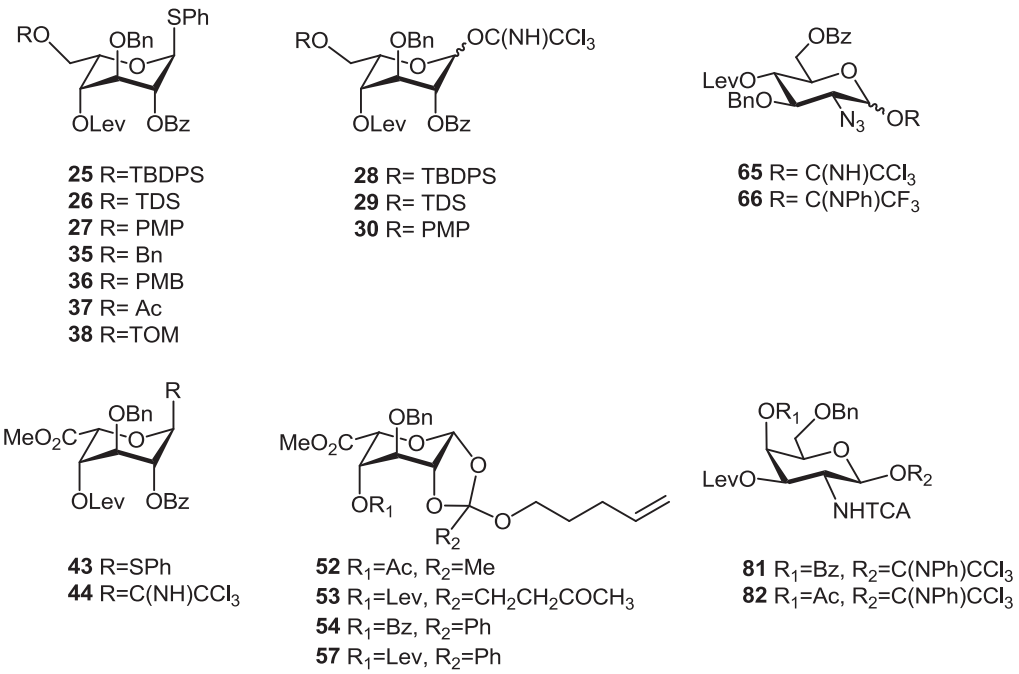


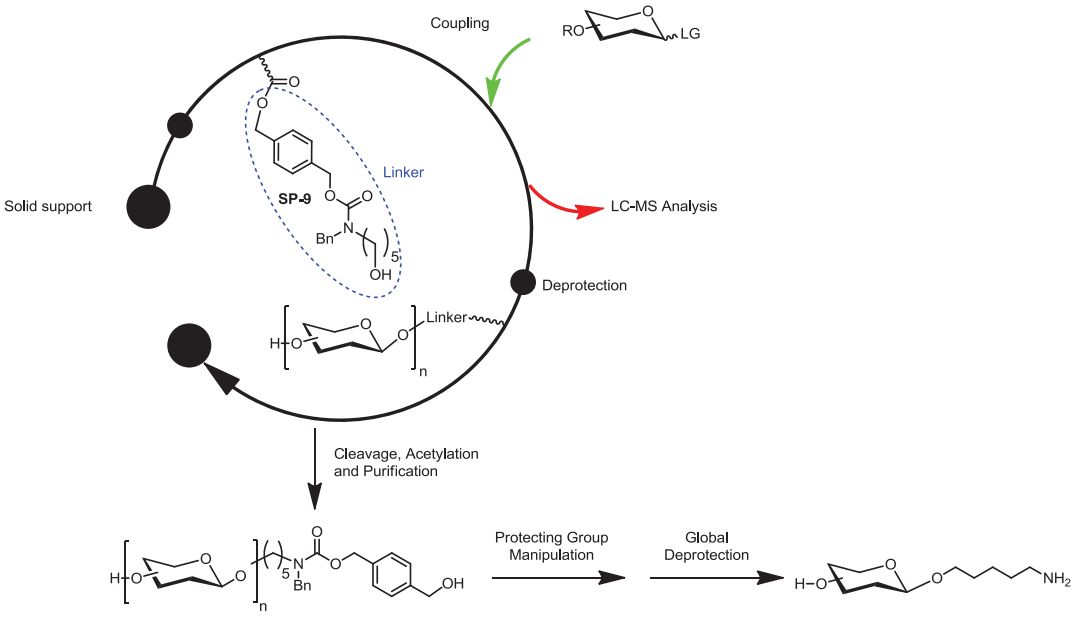
Figure 2.2. a) L-Iduronic acid, L-idose, D-galactosamine and azido-D-glucose donors employed in the solid phase synthesis of HS/DS protected precursors. TBDPS: *tert*-butyldiphenylsilyl, TDS: thexyldimethylsilyl, PMP: *p*-methoxyphenyl, PMB: *p*-methoxybenzyl, TOM: tri-*iso*-propylsilyloxymethyl, Lev:levulinoyl, Bz: benzoate, Ac: acetate, TCA: thrichloroacetimidate.

2.2.4 Synthetic Cycle

The assembly of oligosaccharides in the solid phase involves a synthetic cycle of alternating glycosylation and deprotection (Scheme 2.2) steps. Between these steps the resin needs to be washed thoroughly to remove any excess of reagents or soluble by-products. In the glycosylation step, a resin-bound nucleophile acceptor reacts with the activated glycosidic donor to form a glycosidic linkage on the resin. In the initial glycosylation, the nucleophile is the hydroxyl group of linker **SP-9** that would serve as anchoring point for the growing biopolymer in the solid support. Selective removal of temporary protecting group (PG) in saccharide furnishes a hydroxyl group in saccharides linked to the solid support as nucleophile for the next coupling.

One of the advantages of solid phase synthesis is the possibility of performing each glycosylation step several times and using an excess of building blocks generally, between three to six-fold excess with respect to the solid phase bound nucleophile. These experimental conditions could potentially favor high yielding coupling reactions with the easy removal of excess of reagents by simple washings. The glycosylation reactions would be performed as in solution phase using activating reagents such as *N*-iodosuccinimide (NIS), triflic acid (TfOH) or trimetylsilyltrifluoromethanesulfonate (TMSOTf). Levulinic ester protecting group will be used as temporary protecting group (R in saccharide, Scheme 2.2) for the hydroxyl positions that needed to be liberated to perform glycosylation reactions. This group can be orthogonally deprotected using hydrazine acetate, leaving unaltered the rest of temporary and permanent protecting groups.

One of the challenges in solid phase synthesis is to monitor efficiently the reaction progress. In our case, each step of glycosylation and/or deprotection will be monitored by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) or by Matrix-assisted laser desorption/ionization (MALDI-Tof) mass spectrometry after analytical cleavage of a small aliquot of the resin (2-10 mg).



Scheme 2.2. Coupling cycle of solid phase synthesis, cleavage from the support and global deprotection.

2.2.5 Cleavage from the Resin

Once the protected GAG precursor of the desired length has been assembled on the solid phase, it has to be cleaved for further processing into the final target compound or for reaction monitoring. (Scheme 2.2). The ester linkage chosen for the attachment oligosaccharides on the solid-support is cleaved under strong basic conditions, cleaving all other base-labile functions in the molecule simultaneously.

For complete compound cleavage the resin is transferred to a microwave flask and treated with sodium methoxide under microwave irradiation until thin layer chromatography (TLC) analysis showed no further product being released from the resin. At this point, the purity and composition of the crude product can be assessed by UPLC-MS. For improved purification of the released product mixture, the crude is acetylated by treatment with acetic anhydride in pyridine.

2.2.6 Sulfation and Global deproctetion to the Final Product.

At the end of the synthesis, protecting group manipulations and global deprotection need to be carried out in order to afford the final products. Depending of the nature of the protecting groups one or more transformations would be required. When idose is used as building block, the C-6 position must to be deprotected and oxidized to form the iduronic acid moiety presented in GAGs. *O*- and *N*-sulfation followed by global deprotection would afford the fully unprotected sugars ready to be used in interaction studies.

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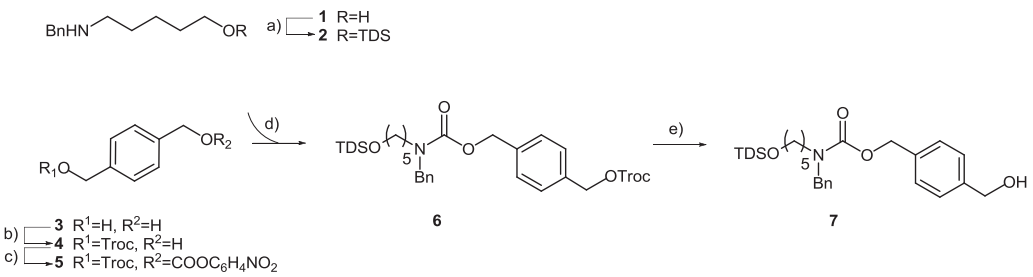
3. RESULTS AND DISCUSSION

3.1 LINKER AND SOLID SUPPORT

3.1.1 Linker synthesis and glycosylation trials on solid support.

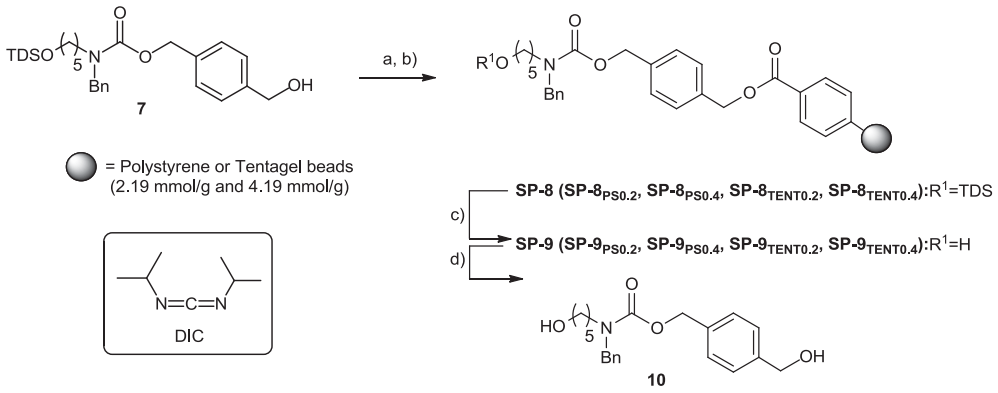
The synthesis of linker **7** was performed as shown in Scheme 3.1. *N*-Benzylaminopentanol **1** was silylated by treatment with thexyldimethylsilylchloride (TDS-Cl) affording benzylamine **2** in 80% yield. On the other hand, 1,4-dihydroxymethylbenzene **3** was mono-protected in 50% yield as trichloroethylcarbonate (Troc) **4**. In order to synthesize carbamate **6**, compound **4** was activated as *p*-nitrophenylcarbonate **5** using *p*-nitrophenyl chloroformate and then reacted with **2** to give the desired carbamate **6** in 80% yield over the two steps. The deprotection of the Troc group in **6** using zinc in acetic acid, afforded **7** in quantitative yield.



Scheme 3.1. Synthesis of linker **7**. Reagents and conditions: a) TDS-Cl, imidazole, DMF, 80%; b) TrocCl, pyridine, CH_3CN, 0ºC to rt, 52%; c) $ClCOOPhNO_2$, pyridine, CH_2Cl_2; d) **2**, DIPEA, DMF, 0ºC to rt, 78% over 2 steps; e) Zn, AcOH/THF (1:1) rt, 95%;

The linker **7** was immobilized onto commercially available carboxy-polystyrene (PS) and Tentagel® (PEG-PS) solid supports using *N,N′*-diisopropylcarbodiimide (DIC) and catalytic 4-dimethylaminopyridine (DMAP) affording resin **SP-8** with different capacity adjusted to 0.2 mmol/g and 0.4 mmol/g (**SP-8$_{PS0.2}$**, **SP-8$_{PS0.4}$**, **SP-8$_{TENT0.2}$** and **SP-8$_{TENT0.4}$**) (Scheme 3.2). Any remaining unreacted carboxylic acid groups were capped as methyl esters by treating the resin overnight with trimethylsilyldiazomethane.[1] The complete capping of the carboxylic acid of the polystyrene resin was confirmed by a rapid and sensitive colour test using a 0.25% solution of malachite green (MG)-oxalate in EtOH in the presence of triethylamine.[2] Cleavage of the TDS-ether in **SP-8** with hydrogen fluoride·pyridine (HF·Py) complex in THF afforded the resin bound alcohol **SP-9** (**SP-9$_{PS0.2}$**, **SP-9$_{PS0.4}$**,

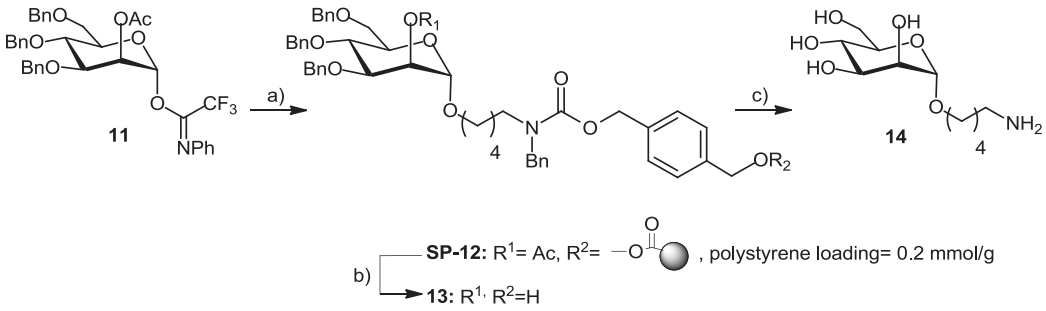
SP-9$_{TENT0.2}$ and **SP-9$_{TENT0.4}$)** in quantitative yield after three hours at room temperature. To monitor the progress of the reaction performed on the solid support, an analytical sample was obtained by treating a resin aliquot with NaOMe in CH_2Cl_2/MeOH for five minutes under microwave irradiation at 55 ºC. This sample was analyzed by Matrix-Assisted Laser Desorption/Ionization (MALDI-Tof) mass spectrometry showing a single and clean product corresponding to compound **10**. Finally, preparative cleavage of the deprotected linker **10** from the resin **SP-9** was performed as reported by Roussel *et al*[3] employing NaOMe in CH_2Cl_2/MeOH and the loading of 0.2 mmol/g and 0.4 mmol/g was confirmed. The different resins prepared were evaluated during the synthesis of HS precursor along current thesis.



Scheme 3.2. Synthesis of solid supported linker **SP-9**. Reagent and conditions: a) carboxypolystyrene 2.19 and 4.19 mmol/g or Tentagel, DIC, DMAP, CH_2Cl_2, quantitative, b) capping: Me_3SiCHN_2, THF, MeOH, rt, overnight; c) HF·pyridine, THF, 0ºC to rt, quantitative; d) MeONa, MeOH, MW, 55ºC, quantitative.

We then tested the general utility of the linker modified resin **SP-9$_{PS0.2}$** for the preparation of C5-aminolinked glycoconjugates (Scheme 3.3). Reaction of 5 equivalents of mannose N-phenyltrifluoracetimidate **11**[4] with **SP-9$_{PS0.2}$** resin under trimethylsilyl trifluoromethanesulfonate (TMSOTf) catalysis afforded resin bound glycoconjugate **SP-12** after two glycosylation cycles. Cleavage from the resin with sodium methoxide at room temperature during 2 hours gave glycoconjugate **13** in 79% yield. This procedure was repeated twice to ensure complete recovery of the

compound from the resin. Finally, compound **13** was completely deprotected to the aminopentyl glycoside **14** by catalytic transfer hydrogenation with palladium black in quantitative yield[5].



Scheme 3.3. First glycosylation trial on solid phase. Reagents and conditions: a) **SP-9**, 10% TMSOTf, CH_2Cl_2; 2 cycles; b) MeONa, MeOH, 79%; c) Pd black, 10% HCOOH in MeOH, H_2, quantitative.

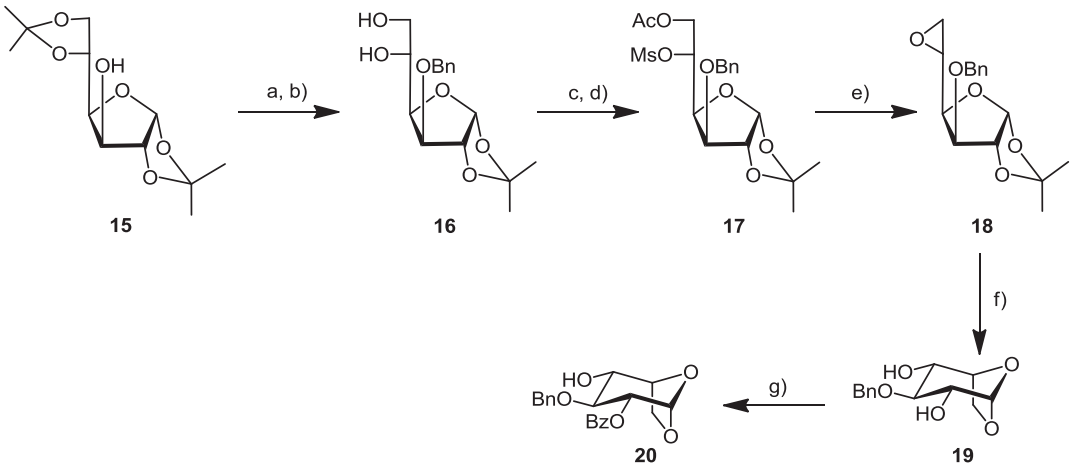
3.2 BUILDING BLOCKS SYNTHESIS

The most time consuming task in the synthesis of GAGs is the preparation of building blocks employed in the assembly of the oligosaccharide. As previously shown, fully differentiated building blocks are required in order to arrive at GAGs of different lengths and/or defined sulfation patterns. The synthesis of these building blocks requires robust and well established synthetic procedures that allow for the preparation of building blocks in large scale from commercially available and inexpensive starting materials like D-glucosamine and D-diacetone glucose.

3.2.1 Synthesis of L-Idose (Ido) Building Block

As L-idose derivatives are not commercially available in large quantities at affordable prices, the synthesis of these building blocks was carried out using previously published synthetic routes which start from D-glucose derivatives and involve C-5 epimerization. Starting from D-diacetone glucose **15**, the OH-3 group was first protected as benzyl ether, using benzyl bromide, sodium hydride and tetra-*n*-butylammonium iodide in DMF and the 5,6-isopropylidene group was then selectively cleaved under mild acidic conditions (Scheme 3.4). [6,7] The resulting diol **16** was converted into L-*ido* epoxide **18** by the method described by van Boeckel,[7] which can be readily performed on a 100 g scale. This method comprises the mesylation of free hydroxyl groups in **16** followed by the selectively substitution of the primary mesylate group by an acetyl group using potassium acetate in the presence of crown-ether (18-crown-6) to afford **17**. Treatment of **17** with potassium *tert*-butoxide yielded the L-*ido* epoxide **18** which underwent epoxide opening, cleavage of the isopropylidene group and intramolecular glycosylation[7,8] to give the 1,6-anhydro-β-idopyranose derivative **19** in a single step after microwave irradiation in 1M H_2SO_4 in dioxane at 120ºC. In compound **19**, the ring is fixed in the pyranose form, and the reaction product was readily isolated by crystallization in 55% yield thus avoiding tedious chromatographic separation of the furanose and pyranose tautomers required in other synthetic routes. Different trials were performed in order to improve the reported yield. Small scale reactions (100 mg) using different acid concentrations (3M, 2M and 1M of H_2SO_4), temperatures (80, 90, 100 and 120 ºC) and different number of cycles under microwave irradiation were tested. Some

improvement (~80% of the desired product by UPLC-MS) was observed when the reaction was performed using 3M H_2SO_4 at 90 ºC in 3 cycles of 10 minutes under microwave irradiation. Unfortunately, when these conditions were applied at one gram scale the reaction yield decreased and the previously described conditions were to be employed for the large scale preparation of **19**. Finally, the anhydro compound **19** was selectively monobenzoylated to give **20** in the presence of pyridine and DMAP at 0 °C via slow addition of benzoyl chloride[8] in order to minimize the formation of dibenzoylated anhydro byproduct.



Scheme 3.4. Synthesis of the anhydro compound **20**. Reagents and conditions: a) BnBr, NaH, TBAI, DMF, 0 °C to rt, 94%; b) AcOH, H_2O, 40 °C, 98%; c) MsCl, pyridine, 0 °C to rt, 87%, d) KOAc, CH_3CN, 18-crown-6, reflux, 83%; e) ^{t}BuOK, ^{t}BuOH, 0 ºC, CH_2Cl_2, 87%; f) 1M H_2SO_4 in dioxane microwave irradiation ,120 °C, 55%; g) BzCl, pyridine, DMAP, CH_2Cl_2, 0 °C, 88%.

The reactivity of a synthetic glycosyl donor is highly dependent on the protecting group pattern and the leaving group at the anomeric position.[9]A series of idose building blocks (**28** – **30** and **35** – **38**) with different protecting groups at OH-6 were prepared and evaluated as glycosyl donors for the solution and solid-phase synthesis of HS precursors. (Scheme 3.5). Thioglycoside **21** was accessible via thiolysis of **20** employing trimethyl(phenylthio)silane in the presence of zinc iodide.[6] Selective protection of primary alcohol in **21** either with *tert*-butyldiphenylsylil chloride (TBDPSCl) or with thexyldimethylsilyl chloride (TDSCl) was achieved to give compounds **22** and **23** in high yields. The protection with *para*-methoxy phenol

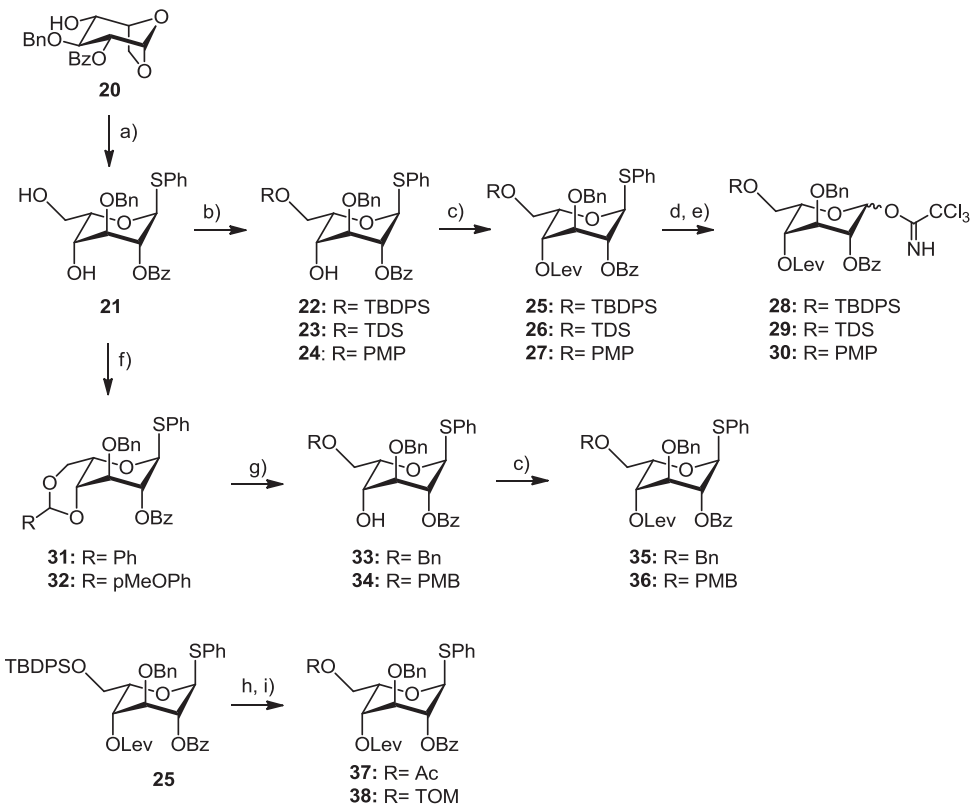
was performed using the Mitsunobu reaction[10] to afford compound **24** in 70 % yield. Levulination of the free OH-4 under Steglich esterification conditions employing levulinic acid in combination with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) and 4-dimethylaminopyridine (DMAP), afforded thioglycosides **25**, **26** and **27,** which could be used directly in glycosylation reactions or could be converted in more reactive trichloroacetimidate donors, after hydrolysis of the thiophenyl group and activation of the resulting hemiacetals.

The reaction of thioglycosides **25-27** with *N*-iodosuccinimide in the presence of triflic acid produced the corresponding hemiacetals. These intermediates were treated with trichloroacetonitrile in the presence of DBU as a base to afford imidates **28**, **29** and **30**. The procedure for the hydrolysis of thioglycoside **27** needed to be optimized in order to avoid the reported NIS-promoted iodination of the *p*-methoxybenzyl group[11]. The formation of an iodinated byproduct was detected by UPLC-MS resulting in a peak of [M+127] corresponding to the correponding hemiacetal plus one iodine atom. The formation of this halogenated byproduct could be completely eliminated when the reaction was performed using *N*-bromosuccinimide in a mixture of acetone and water (9/1). This is related with the amount of the free electrophilic halide (X^+) available, which decreases as bond energies increase (N-Cl > N-Br > N-I).

In order to prepare 6-*O*-*p*-methoxybenzyl and 6-*O*-benzyl protected idoyranosyl thioglycosides **33** and **34**, positions OH-6 and OH-4 of diol **21** were simultaneously protected by *p*-toluenesulfonic acid catalysed acetalisation with benzaldehyde dimethyl acetal or *p*-methoxy benzaldehyde to form respectively thioglycosides **31** and **32** in good yields (79% for **31** and 84% for **32**). Regioselective reductive ring opening of **31** to form 6-*O*-benzyl protected donor **33** was achieved employing triethylsilane in the presence of trifluoracetic acid.[12] Nevertheless, when these conditions were applied to the acid labile *p*-methoxy benzylidene acetal **32**, diol **21** was obtained as the major reaction product. The use of sodium borohydride in combination with different acids[13] such as HCl in dioxane[14] and triflic acid did not improve the outcome of the ring opening reaction. Hence, the reductive ring opening of **32** was performed under neutral conditions with sodium cyanoborohydride and iodine[15] at -20 °C affording the desired 6-*O*-*p*-methoxybenzyl protected donor **34** in

61% of yield. Acetals **33** and **34** were then protected as 4-*O*-levulinic esters to obtain thioglycosides **37** and **38**, respectively.

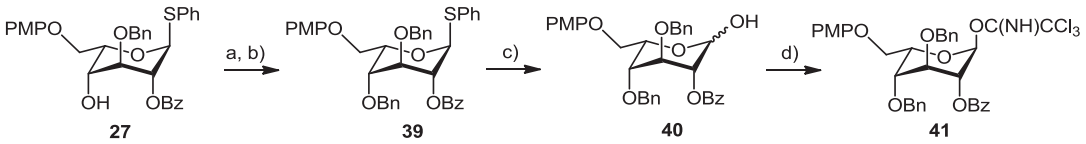
Finally, from fully protected compound **25**, the 6-*O*-TBDPS ether was selectively removed using HF·pyridine and the 6-*O*-acetate protected donor **37** was prepared in quantitative yield by treatment of the resulting compound with acetic anhydride in the presence of pyridine. Similarly, the 6-*O*-TOM protected thioglycoside **38** was prepared in 64% from **25** by alkylation of the 6-O- deprotected derivative employing tri-*iso*-propylsilyloxymethyl chloride (TOMCl).



Scheme 3.5. Synthesis of the thiophenyl idopyranoside donors **28 – 30**, **35 – 38**. Reagents and conditions: a) Me_3SiSPh, ZnI_2, rt, o/n, 77% b) for **22**: TBDPSCl, pyridine, o/n, for **23**: TDSCl, cat. DMAP, pyridine, rt, o/n, 85%; for **24**: PMPOH, PPh_3, DIAD, THF, 2h, 80 °C, 70%; c) LevOH, EDC·HCl, cat. DMAP, CH_2Cl_2, 5h, 91% (**25** over two steps), 65% (**26**), 90% (**27**), 94% (**35**), 81% (**36**); d) for **28**: NIS, TfOH, THF, H_2O, 75%, for **29**: NIS, TfOH, THF, H_2O, rt, 75%, for **30**: NIS, TfOH, THF, H_2O, rt, 56%; e) trichloroacetonitrile, DBU, CH_2Cl_2, 0 ºC, 2h, 90% (**28**), 72% (**29**) , 56% (**30**, over two steps); f) $PhCH(OMe)_2$ (for **31**), *para*-methoxybenzaldehyde (for **32**), CSA, DMF, 80 °C, 5h, 79% (**31**), 84% (**32**); e) Triethylsilane, TFA, 0 °C to rt, 2h, 70% (**33**), I_2, $NaCNBH_3$, CH_2Cl_2, -20 °C, 61% (**34**). h, i) HF·pyridine, THF, 0 °C to rt, 50%; for **37**: Ac_2O, pyridine, CH_2Cl_2, quantitative; for **38**: TOMCl, DIPEA, CH_2Cl_2, 0 °C to rt, 64%.

3.2.1.1 Synthesis of the Ido non-reducing end building block

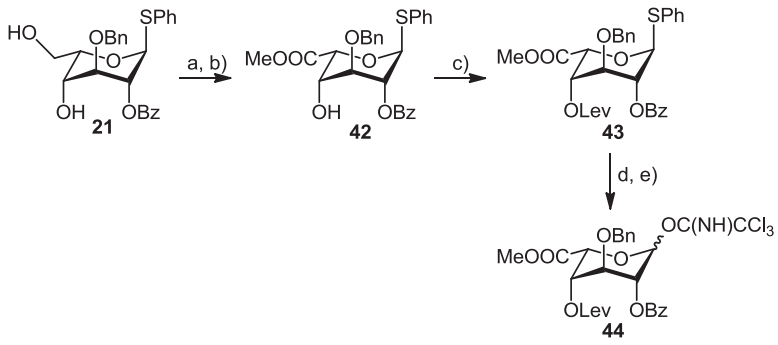
For the synthesis of capping building block **41**, (Scheme 3.6), that contains a permanent benzyl protecting group at OH-4 to avoid sulfation of this position during the final steps, several attempts of benzylation of **27** using benzyl bromide-silver oxide, benzyl bromide-sodium hydride or acid catalyzed (benzyl trichloroacetimidate) benzylation failed. On the other hand, regioselective ring opening of the benzylidene acetal **31** to form the corresponding 4-*O*-benzylated derivative using 1M borane in THF and cupper triflate, followed by Mitsunobu reaction with *p*-methoxyphenol in combination with triphenylphosphine and diisopropyl azodicarboxylate (DIAD) gave the desired compound **39** but in moderate 40% yield. Finally, a benzyl ether was installed in OH-4 of **27** in a two-step process which afforded **39** in 70% yield employing an excess of trimethylsilyl chloride (TMSCl) followed by treatment with benzaldehyde, triethylsilane and a catalytic amount of acid (TMSOTf). In this reaction the order of reagents was essential as the addition of acid prior to the addition of the aldehyde and triethylsilane resulted in the loss of the temporary trimethylsilyl group. Finally, the hydrolysis of the thioglycoside using NBS in acetone/water produced hemiacetal **40** that was activated as trichloroacetimide **41** by treatment with trichloroacetonitrile and DBU (Scheme 3.6).



Scheme 3.6. Synthesis of the non reducing end building block **41**. Reagents and conditions: a) TMSCl, pyridine; b) BnCHO, TES, TMSOTf (cat.), 70% over two steps; c) NBS, acetone/water, 72%; d) CCl_3CN, DBU, CH_2Cl_2, 76%.

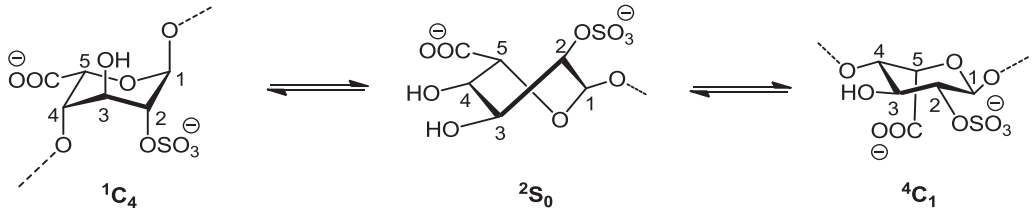
3.2.2 Synthesis of L-iduronic acid (IdoA) building blocks

Since the synthesis of IdoA glycosyl donors from commercial iduronic acid is unpractical and expensive IdoA building blocks were developed from a common intermediate of the idose building blocks synthesis. Selective oxidation of the OH-6 in diol **21** using TEMPO/BAIB[16,17] produced the desired iduronic acid derivative whose free carboxylic group was protected as methyl ester using the Steglich esterification conditions in MeOH with *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) and dimethylaminopyridine (DMAP) as catalyst to give **42** (Scheme 3.7). The protected thioglycoside **42** was isolated in 60% of yield after the two step sequence of oxidation and esterification. The remaining OH-4 was protected with levulinic acid under Steglich esterification conditions to generate the fully differentiated compound **43** in excellent 93% of yield. This thioglycoside could be either directly employed in glycosylation reactions or transformed in the corresponding trichloroacetimidate **44** after hydrolysis to the corresponding hemiacetal by treatment with *N*-iodosuccinimide and trifluoroacetic acid and activation with trichloroacetonitrile and DBU.



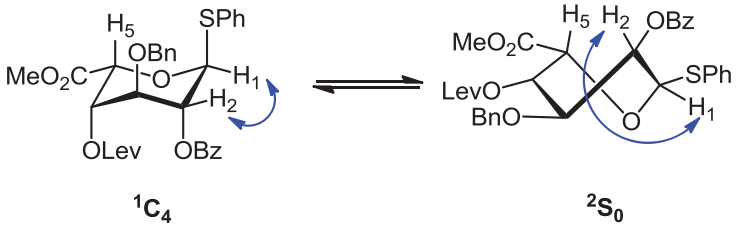
Scheme 3.7. Synthesis of iduronic acid building blocks. Reagents and conditions: a) TEMPO, BAIB, CH_2Cl_2/H_2O (3/1), 2h; b) MeOH, EDC·HCl, DMAP, CH_2Cl_2, 0 °C to rt, 60% in 2steps; c) LevOH, EDC·HCl, DMAP, CH_2Cl_2, 0 ºC to rt, 90%; d) NIS, TFA, CH_2Cl_2, 0 ºC, 15 min; e) Cl_3CCN, DBU, CH_2Cl_2, 0 °C to rt, 75% over two steps.

The pyranose ring of IdoA monosaccharides may generally adopt $^{1}C_4$ and $^{4}C_1$ chair conformations, or a $^{2}S_0$ skew-boat conformation (Scheme 3.8).[18,19,20] IdoA derivatives could adopt either a $^{1}C_4$ conformation or a skewed boat $^{2}S_0$ conformation depending upon the substituents on the pyranoid ring.[19,6] For IdoA units bearing a sulfate group at C2, the $^{1}C_4$ chair or $^{2}S_0$ skew-boat conformations are preferred. Unsubstituted IdA units, however, reside predominantly in the $^{1}C_4$ form. The flexibility of the IdoA residues can be an important factor for specific GAG-protein interactions.



Scheme 3.8. Conformational flexibility of IdoA rings.

The assignment of the conformation of the IdoA derivatives could be performed by analyzing the coupling constants between vicinal H-1 and H-2 protons in the ^{1}H NMR spectra since it is well known that vicinal proton–proton coupling constants (J_{H1-H2}) depend on the dihedral angle between them according to the Karplus equation.[21] The ^{1}H NMR spectra of the IdoA derivatives prepared in this thesis showed small coupling constants between these protons ($^{3}J_{H1-H2}$ ~0 Hz) and the signal of H-1was always observed as a broad signal[20], thus indicating the presence of a major $^{1}C_4$ conformation of the pyranoid ring for all of them.

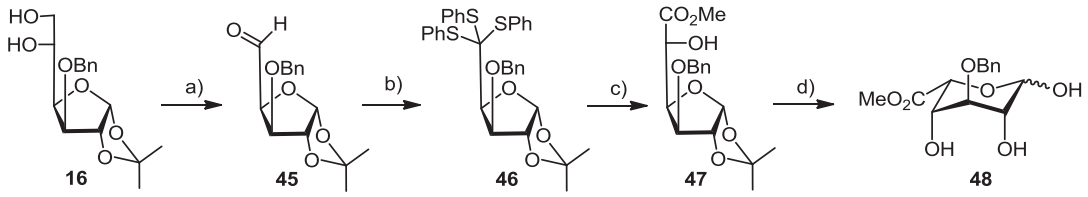


Scheme 3.9. $^{1}C_4$ -$^{2}S_0$ Conformational equilibrium of IdoA rings in compound **43**.

3.2.2.1 Synthesis of *n*- pentenyl orthoesters (NPOEs) as IdoA donors

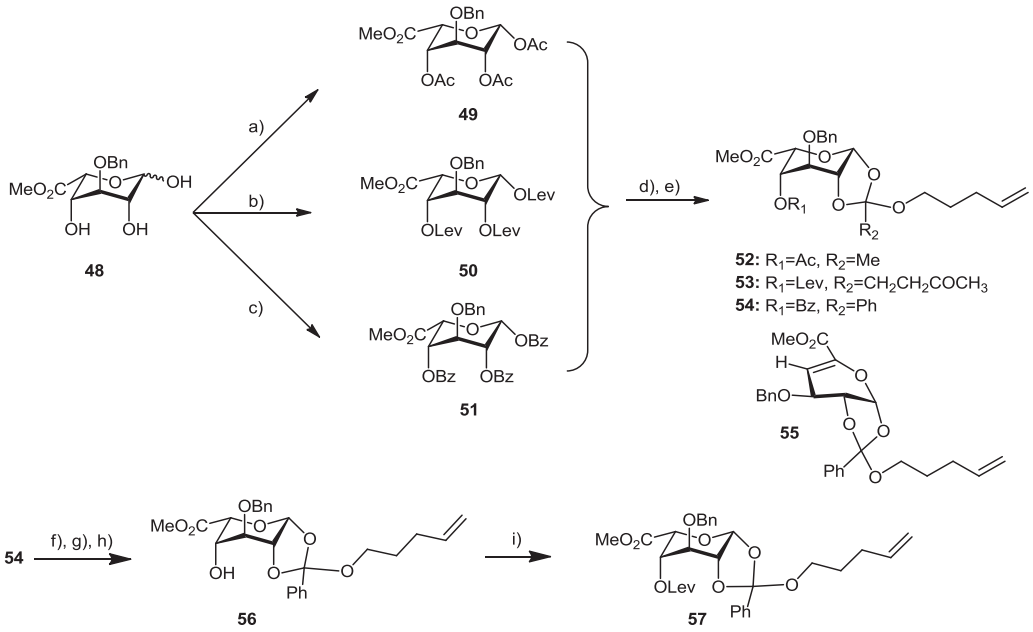
n-Pentenyl orthoesters (NPOEs) have been described by Fraser–Reid *et al.* as potent glycosyl donors of mannose, [22] glucose [22] and galactose.[23] Depending on the monosaccharide configuration, *n*-pentenyl glycosides (NPGs) and $NPOE_S$ can show very distinct reactivities and react through different charged intermediates. For example the mannose-NPOE, with a 1,2-cis diol configuration, found also in the stable L-idose-NPOE $^1C_4$ conformer, was found to be nearly three times more reactive than the corresponding 2-*O*-acyl NPG.[24] Activation of idose and iduronic acid as NPOEs simultaneously activates the anomeric position as a glycosyl donor and differentiates positions C-2 and C-4, a feature that would otherwise require multiple additional steps for other glycosyl donor types. A further hallmark of NPOEs is their facile preparation from per-acylated anomeric bromides by treatment with a soft base in the presence of n-pentenol.[22] Surprisingly, in spite of these potential advantages, NPOEs have not been evaluated up to date as potential glycosyl donors for the synthesis of HS oligosaccharides, while NPGs have been investigated previously.[25]

We synthesized orthoesters **52-57** via the corresponding bromosugars from triol **48**,[26] which was prepared in 6 steps with an overall yield of 50% by the method described by Bonaffé (Scheme 3.10). The key step of this route is the preparation of the L-ido diastereomer by addition of $(PhS)_3CLi$ to the aldehyde **45** at -78°C with complete stereoselectivity. Orthothioester **46** was then converted to the corresponding methyl ester **47** using a copper salt mixture $CuO/CuCl_2$ in 86% and subsequently rearranged to the pyranose **48** with position 3 protected as Bn ether by treatment with 90% of trifluoroacetic acid.



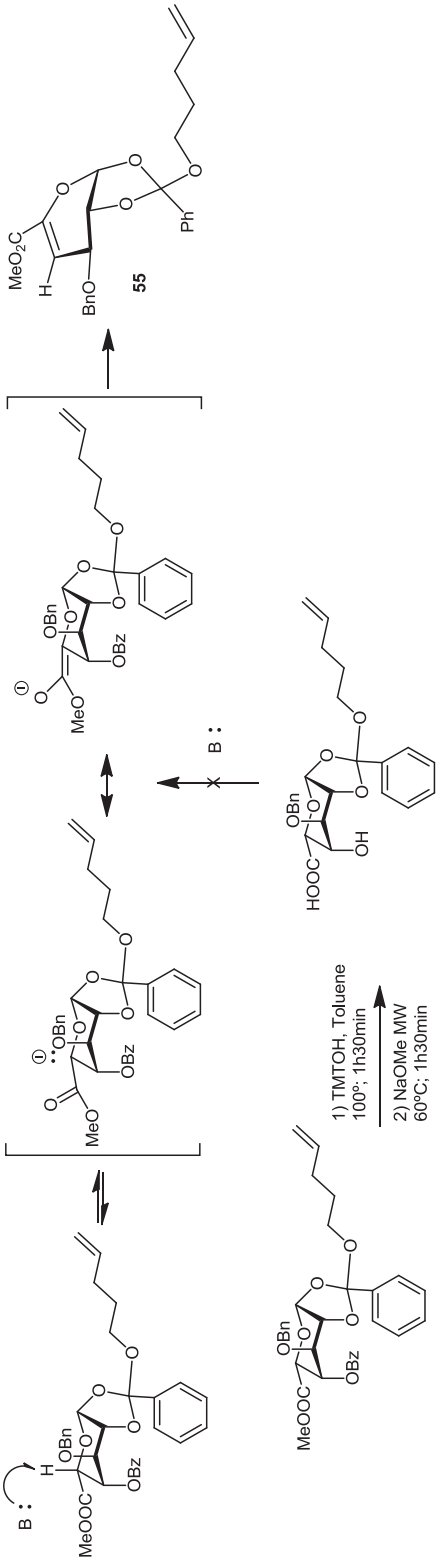
Scheme 3.10. Synthesis of triol **48**. Reagents and conditions: a) $NaIO_4$, silica gel, H_2O/CH_2Cl_2, quantitative; b) $(PhPS)_3CLi$, THF, 78% c) $CuO/CuCl_2$, $MeOH/CH_2Cl_2/H_2O$, 89%; d) 90% CF_3CO_2H, quantitative.

Triol **48** as central precursor was then acylated either with acetic anhydride, levulinic acid or benzoyl chloride to afford the corresponding triacyl compounds **49**,[27] **50** and **51** respectively. Tribenzoate **51** was most readily obtained and easily crystallized while acetate **49** and levulinate **50** required very careful adjustment of reaction conditions to suppress the competing furanoside formation. The corresponding 1-bromosugars from **49-51** were obtained in high yield by either treatment with HBr/AcOH or $TiBr_4$, and rapidly reacted with *n*-pentenol to give mixed orthoesters **52-54** (Scheme 3.11).[25]



Scheme 3.11. Synthesis of orthoesters with increasing steric bulk at the exocyclic carbon. Reagents and conditions: a) AcCl, pyridine, DMAP, CH_2Cl_2, -40 °C to rt ,78% ; b) LevOH, EDC·HCl, DMAP, -25 °C to rt, 80%; c) BzCl, pyridine, CH_2Cl_2, -40 °C to rt, 91%, d) HBr/AcOH, CH_2Cl_2 or $TiBr_4$ (1.3 eq.), CH_2Cl_2; e) 4-pentenol, 2,6-lutidine, CH_2Cl_2; f) Trimethyltin hydroxide, toluene, microwave 100°C; g) NaOMe/ MeOH, microwave 60°C; h) MeOH, EDC·HCl, DMAP, 60% over 3 steps; i) LevOH, EDC·HCl, DMAP, CH_2Cl_2, 90%.

In order to transform orthoester **54** into 4-*O*-levulinoyl ester protected derivative **57**, the benzoate group in **54** was removed under Zemplén deacylation conditions. Unfortunately, the hydrolysis of the benzoate group using sodium methoxide was accompanied by extensive elimination to form alkene **55**. This elimination reaction has been described as a usual transformation in heparin chains exposed at basic pH[28]. The elimination is proposed to take place via a E1cB mechanism, which is specially favoured when there are acidic protons with electron attracting groups in α position such as (COOMe), that could stabilize the carbanion formed (Scheme 3.12).



Scheme 3.12 Mechanism proposed for the elimination reaction

The use of lithium peroxide has been proposed in order to avoid this elimination reaction, but in our case, it was applied without any success. Nevertheless, mild ester hydrolysis of compound **54** using trimethyltinhydroxide, [29] allowed debenzoylation without any elimination. Re-esterification of the carboxylic acid intermediate under Steglich esterification conditions using MeOH, cleanly afforded **56** although care had to be taken in the workup to avoid the cleavage of the acid labile orthoester function. Final levulination of the OH-4 in **56** gave rise to the fully differentiated orthoester glycosyl donor **57**.

As indicated previously the conformation of the pyranoid ring in all IdoA glycosyl donors described so far, including NPOEs **52-57**, was unambigously established as $^{1}C_{4}$ by analysis of the coupling constant in the NMR spectra.

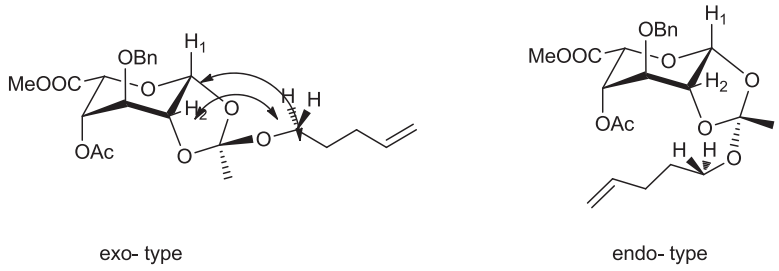
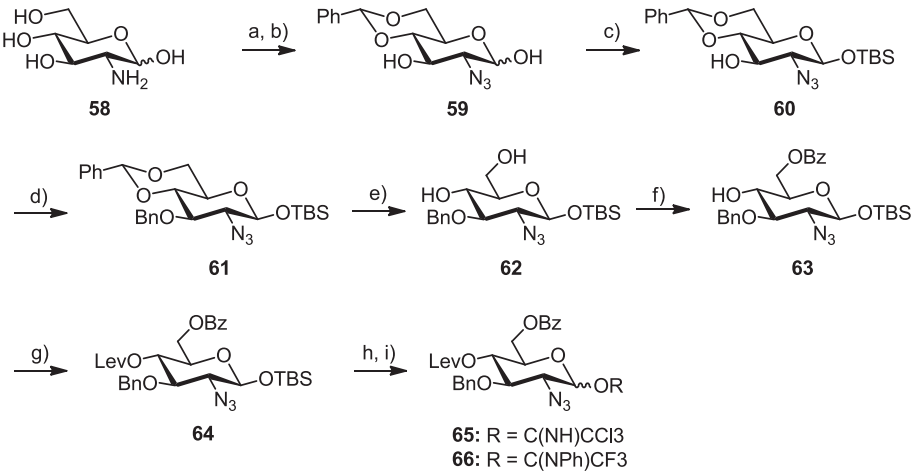


Figure 3.1. Possible configuration of the n-pentenyl orthoester.

The formation of 1,2-orthoesters involves the generation of a new chiral center and two isomers, *endo*-type and *exo*-type, could be formed (Figure 3.1). All synthesized NPOEs were *exo*-type isomers as determined by NOE measurements which showed the proximity of the $-OCH_{2pent}$ protons to protons H-1 and H-2 of the pyranoid ring as could be expected for *exo*-type structures.

3.2.3 Synthesis of 2-azido-D-glucopyranose (Glc) building blocks

The glucosamine building blocks **65** and **66** were obtained from commercially available D-glucosamine hydrochloride **58** in 9 steps as described in Scheme 3.13. First, the amino group was masked as an azide by an azido transfer reaction using trifluoromethanesulfonyl azide (TfN_3), which was prepared *in situ* from sodium azide and trifluoromethanesulfonyl anhydride.[30,31] In a one pot procedure, positions OH-4 and OH-6 were then protected as a benzylidene acetal with benzaldehyde dimethyl acetal in the presence of catalytic amounts of *p*-toluenesulfonic acid to afford compound **59**. Selective silylation of the anomeric position in **59** with *tert*-butyldimethylsilyl chloride (TBSCl) at low temperature (-10 °C), gave compound **60** in 65% yield. Benzylation of **60** with benzyl bromide and sodium hydride to give **61** followed by removal of the 4,6-*O*-benzylidene acetal by transacetalisation with of *p*-toluenesulfonic acid and ethanethiol yielded diol **62** with 82% yield over the two steps. Compound **62** was then regioselectively benzoylated at OH-6 with benzoyl cyanide in the presence of a catalytic amount of triethylamine at -40 °C to afford compound **63**. Finally the OH-4 in **63** was protected as a levulinic ester (**64**) allowing orthogonal deprotection prior to subsequent activation of the anomeric position.

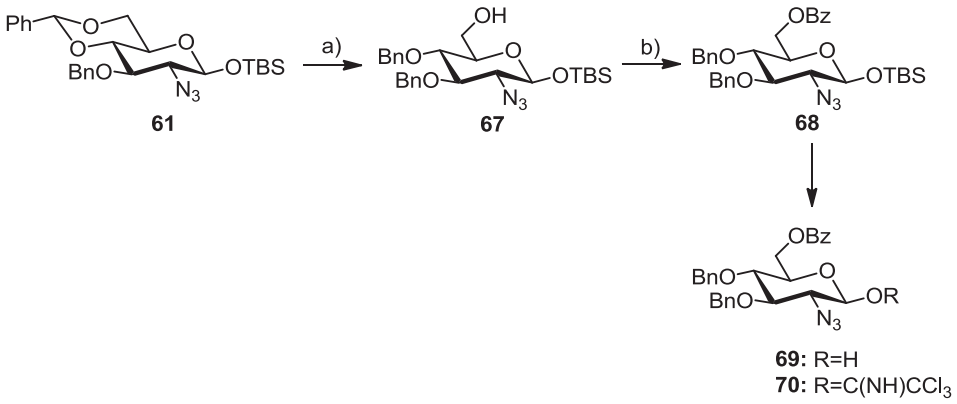


Scheme 3.13. Synthesis of acetimidates **65** and **66**. Reagents and conditions: a) MeONa, MeOH, TfN_3, DMAP; b) $PhCH(OMe)_2$, p-TsOH, DMF, 40 °C, 76% over 2 steps; b) TBSCl, imidazole, CH_2Cl_2, -20°C, 79%; c) BnBr, NaH, CH_2Cl_2, TBAI, 76%; e) EtSH, p-TsOH, CH_2Cl_2, 3h, rt, 89%; f) BzCN, cat. Et_3N, AcCN, 7h, - 40 °C, 90%; g) EDC·HCl, LevOH, cat. DMAP, CH_2Cl_2, rt, 91%; h) TBAF, AcOH, THF, 0 °C, 3h; i) for **65**: trichloroacetonitrile, DBU, CH_2Cl_2, 0 °C, 2h, 85% over two steps, for **66**: (*N*-Phenyl) trifluoroacetimidoyl chloride, K_2CO_3, acetone, rt, o/n, 91% over two steps.

Treatment of **64** with tetrabutylammonium fluoride solution (TBAF) in THF, buffered with acetic acid allowed the selective deprotection of the anomeric silyl group in the presence of levulinoyl ester group. The corresponding hemiacetal was activated either with trichloroacetonitrile in the presence of DBU as base to form trichloroacetimidate **65** or with (*N*-phenyl) trifluoroacetimidoyl chloride[32] using potassium carbonate as base to form (*N*-phenyl)trifluoro acetimidate **66** (Scheme 3.13).

3.2.3.1. Synthesis of the glucosamine non-reducing end building block

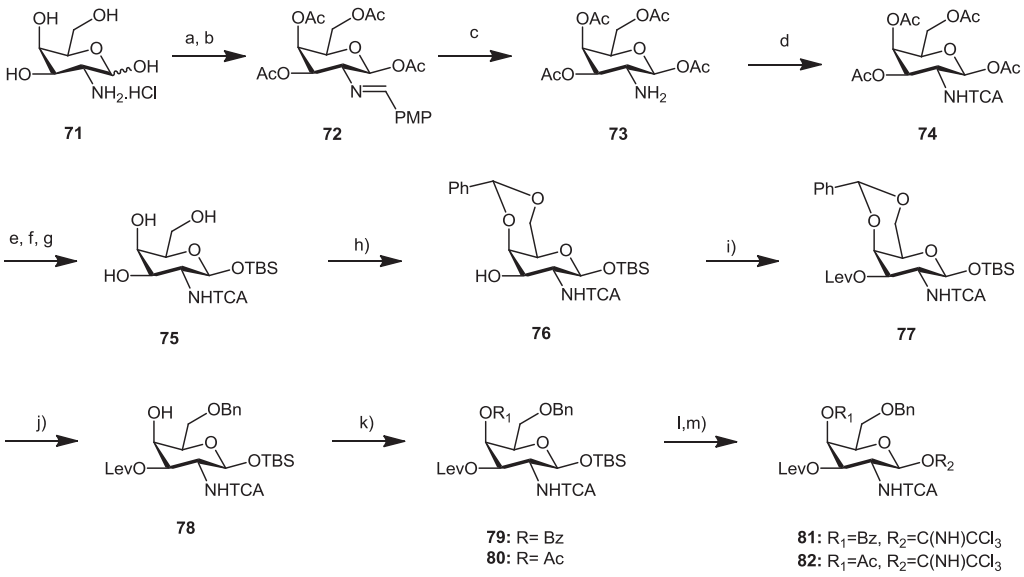
The synthesis of the capping building block **70** involved first the regioselective ring opening of benzylidene acetal intermediate **61** using 1M borane in THF solution and a catalytic amount of cupper triflate **to** afford intermediate alcohol **68**. Benzoylation of **68** employing benzoyl chloride in pyridine gave **69** and cleavage of the anomeric silyl protecting group in **69** with TBAF buffered with acetic acid in THF at 0 °C resulted in the formation of the corresponding hemiacetal, which was subsequently activated as trichloroacetimidate **70** using trichloroacetonitrile and DBU (Scheme 3.14).



Scheme 3.14. Synthesis of Non-reducing End Building Block of Glucosamine **70**. Reagents and conditions: a) BH_3·THF, $Cu(OTf)_2$, 85%; b) BzCl, pyridine, DMAP, CH_2Cl_2, 95%; c) TBAF, AcOH, THF, 0°C; d) CCl_3CN, DBU, 0°C to rt, CH_2Cl_2, 80% over two steps.

3.2.4 Synthesis of galactosamine building blocks

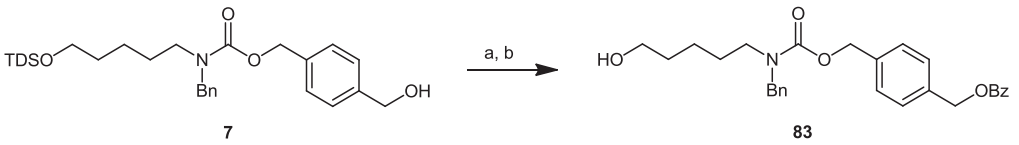
For the synthesis of the D-galactosamine protected building blocks, a reported procedure for D-glucosamine by Bergmann and Zervas was applied (Scheme 3.15) [33] The amino group of D-galactosamine hydrochloride (**71**) was protected as an imine by treatment with *p*-anisaldehyde in aqueous solution of NaOH (1M). The imine was peracetylated with acetic anhydride in pyridine to obtain **72** in 65% of yield. Hydrolysis of the imine by treatment of **72** with HCl 5N in acetone provided the free amine **73**, which was protected as trichloroacetamide **74** by treatment with trichloroacetyl chloride and triethylamine in good yield (91%). Selective removal of anomeric acetate of **74** using hydrazine acetate followed by treatment with *tert*-butyldimethylsilylchloride (TBDMSCl) and subsequent deproctecion of the acetyl groups under Zemplén deacetylation conditions using catalytic NaOMe in MeOH gave compound **75** in 86% yield over three steps. Treatment of **75** with an excess of benzaldehyde dimethylacetal (2.7 eq.), in the presence of camphorsulfonic acid (CSA) in acetonitrile afforded **76** in 93% yield. Compound **76** was levulinoylated by DMAP mediated esterification with levulinic acid to give **77** with a yield of 92%. The regioselective ring opening of benzylidene acetal **77** to form 6-*O*-benzyl derivative **78** needed to be optimized. Treatment of **78** with sodium cyanoborohydride and HCl (4M) in dioxane, resulted in the formation of byproducts in which the keto group in levulinate was reduced to an alcohol. The identity of this byproduct was confirmed by NMR which showed the disappearance of ketone ^{13}C NMR signal at δ 206 ppm thus indicating the simultaneous reduction of the ketone on to alcohol group. The use of borane tetrahydrofuran complex with TMSOTf[14] produced the same byproduct. Finally, the successful regioselective ring opening of the benzylidene acetal **77** was achieved using a mixture of triethylsilane and trifluoroacetic acid to give **78** in 69% yield.[12] The free hydroxyl group in **78** was protected both as benzoate (**79**) and as acetate (**80**) by treatment with acetic anhydride in pyridine. The hydrolysis of the anomeric TBS protecting group in **79** and **80** afforded the corresponding hemiacetals which were activated as trichloroacetimidates **81** and **82** (Scheme 3.15).



Scheme 3.15. Synthesis of galactosamine building blocks **81** and **82**. Reagents and conditions a) NaOH 1M, *p*-anisaldehyde, b) Ac_2O, pyridine, 65% in 2 steps; c) HCl 5N, acetone, 96%; d) trichloroacetyl chloride, Et_3N (cat.), CH_2Cl_2, 91%; e) $N_2H_4 \cdot AcOH$, DMF; f) TBDMSCl, imidazole, DMF; g) NaOMe, MeOH, 86% over three steps; h) $PhCH(OMe)_2$, CSA (cat.), acetonitrile, 93%; i) levulinic acid, EDC·HCl, DMAP, CH_2Cl_2, 92%; j) triethylsilane, trifluoroacetic acid, CH_2Cl_2, 69%; k) for **79**: benzoyl chloride, pyridine, CH_2Cl_2, 96%; for **80**: Ac_2O, pyridine, 82%; l) TBAF, AcOH, THF, 0 °C; m) CCl_3CN, DBU, 0 °C to rt, CH_2Cl_2,78% over two steps (**81**), 85% (**82**).

3.3. EVALUATION OF GLYCOSYL DONORS FOR THE SYNTHESIS OF HS OLIGOSACCHARIDES IN SOLUTION

With the different donors in hand, their performance in key glycosylation reactions involved in the assembly of HS oligosaccharides was first investigated in solution using a model carbamate spacer **83**. (Scheme 3.16). This linker was synthesized starting from **7** by treatment with benzoyl chloride in the presence of pyridine. TDS group was removed using HF pyridine complex to produce acceptor **83**. The benzoyl group in the linker would mimic the ester linkage towards the resin presented in the solid phase synthesis.



7 83

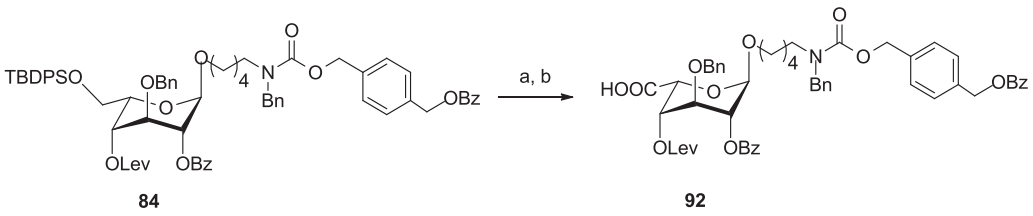
Scheme 3.16. Synthesis of a linker for solution synthesis evaluation. Reagents and conditions: a) BzCl, pyridine, 95%; b) HF-pyridine, 68%.

3.3.1 Glycosylation of the linker

First, the glycosylation of the carbamate spacer linker **83** with building blocks **25-27**, **35-38**, **43** and **57** was studied. The results are summarized in Table 3.1. Most of the donors provided the desired products in good yields (60-90%), with the only exception of 6-*O*-acetyl protected idose and iduronic acid (IdoA) derivatives .All reactions involving idose derivatives were carried out at –20 °C after temperature optimization in a series of test glycosylations employing the 6-*O*-*tert*-butyldiphenylsilyl (TBDPS)-derivative **25** (entries 1-3, (**84**)). Thioglycoside donors (**25-27, 35-38, 43** and **57**.) reacted to give the product in good to excellent yield independently of the size of the substituent at OH-6. Donors **37, 43,** and **57** with electron withdrawing substituents in position C6 performed less well under the same reaction conditions. Stronger activation conditions employing higher excess of NIS were required to achieve good yields (entry 11). In general, the glycosylation of the primary linker hydroxyl group proved to be straightforward for all glycosyl donors evaluated including orthoester derivative **57** (entry 12) performed equally well as the

corresponding phenyl thioglycoside **43** (entry 11). Analysis of the ^{1}H and ^{13}C NMR spectra of the isolated products **84-91** demonstrated an exclusive *trans* selectivity due to neighbouring group participation by the 2-*O*-benzoyl group.

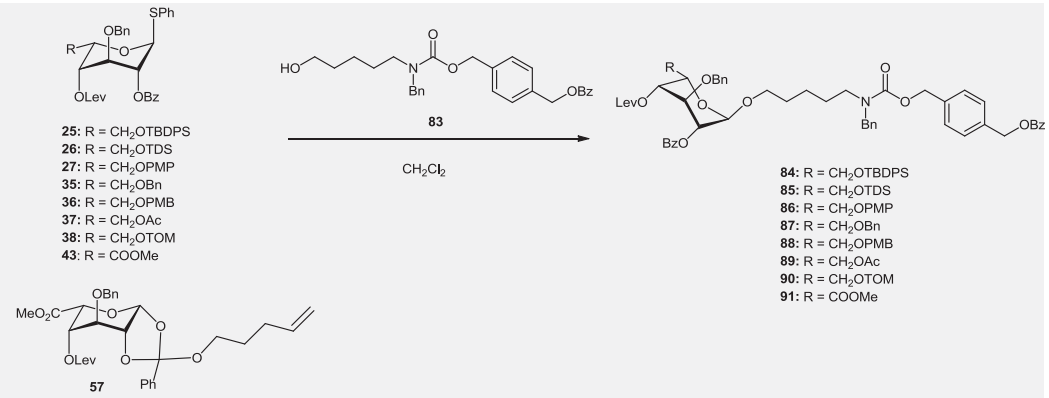
At this point, we wanted also to investigate a synthetic route that would allow the oxidation of L-idose residues to iduronic acids maintaining the protecting groups employed in the synthesis (Scheme 3.17). The oxidation of 6-OH position would be performed after cleavage of the assembled oligosaccharide from the resin.[34-37] This strategy was evaluated in solution on model compound **84**. The 6-*O*-TBDPS group in **84** was easily cleaved employing HF·pyridine complex and the resulting alcohol was oxidized using a catalytic amount of TEMPO and stoichiometric amounts of an organic oxidant [bis(acetoxy)-iodo]benzene (BAIB) in aqueous[16,38] and biphasic system.[34] The corresponding uronic acid **92** was isolated in good yield without affecting other protecting groups.



84 **92**

Scheme 3.17. Transformation of idose derivative **84** into corresponding iduronic acid derivative **92**. Reagents and conditions: a) HF·pyridine, THF, 0 ºC, 84% b). TEMPO/BAIB, CH_2Cl_2/H_2O (3:1) 80%.

Table 3.1. Test glycosylations of linker **83** in solution using glycosyl donors **25-27, 35-38, 43** and **57.**

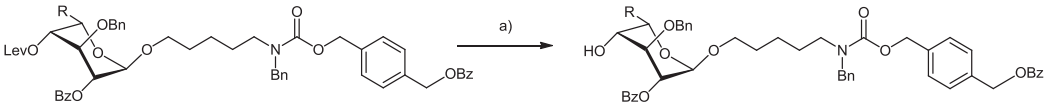


Entry	Donor	R	Temperature	Product	Yield (%)[a]
1	**25**	$CH_2OTBDPS$	-20°C to rt[b]	**84**	82%
2	**25**	$CH_2OTBDPS$	0°C to rt[b]	**84**	60%
3	**25**	$CH_2OTBDPS$	-45°C to rt[b]	**84**	63%
4	**26**	CH_2OTDS	-20°C to rt[b]	**85**	80%
5	**27**	CH_2OPMP	-20°C to rt[b]	**86**	79%
6	**35**	CH_2OBn	-20°C to rt[b]	**87**	82%
7	**36**	CH_2OPMB	-20°C to rt[b]	**88**	70%
8	**37**	CH_2OAc	-20°C to rt[c]	**43**	52%
9	**38**	CH_2OTOM	-20°C to rt[c]	**42**	90%
10	**43**	COOMe	0°C to rt[b]	**44**	55%
11	**43**	COOMe	rt[d]	**44**	66%
12	**57**	IdoANPOE	0°C to rt[d]	**44**	69%

[a] only α-anomer formed; [b] 1.5 eq. donor, 1.5 eq. NIS, 0.25 eq TMSOTf [c] 1.2 eq donor, 1.5 eq. NIS, 0.1 eq. TfOH; [d] 1.5 eq. donor, 3 eq. NIS, 0.25 eq TMSOTf.

3.3.2 Disaccharide synthesis

More pronounced differences in reactivity were expected in the following glycosylation of Ido and IdoA acceptors with glucosamine derived trichloroacetimidate **65** and trifluroroacetimidate **66**, due to the different protecting group regimes presented by the acceptors. The deprotection of 4-*O*-levulinic ester in glycosides **84-91** to liberate position OH-4 for subsequent glycosylation reaction was performed using hydrazine acetate in dichloromethane providing the required glycosyl acceptors **93- 100** in high yields ranging from 71 to 91% (Scheme 3.18).



84: R = $CH_2OTBDPS$
85: R = CH_2OTDS
86: R = CH_2OPMP
87: R = CH_2OBn
88: R = CH_2OPMB
89: R = CH_2OTOM
90: R = CH_2OAc
91: R = COOMe

93: R = $CH_2OTBDPS$
94: R = CH_2OTDS
95: R = CH_2OPMP
96: R = CH_2OBn
97: R = CH_2OPMB
98: R = CH_2OTOM
99: R = CH_2OAc
100: R = COOMe

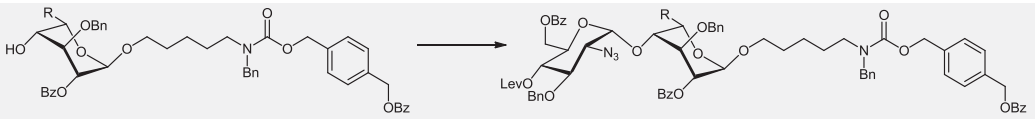
Scheme 3.18. Deprotection of 4-O-levulinic ester in glycosides **84-91**. Reagents and conditions: a) hydrazine acetate, CH_2Cl_2; 85% (**93**); 91% (**94**); 90% (**95**); 91% (**96**); 86% (**97**); 71% (**98**); 91% (**99**) and 85% (**100**).

The influence of the 6-*O*-protecting group on the reactivity of acceptors was studied in detail and the results are shown in Table 3.2. As a general trend, the glycosylation yields increased with the decreasing of steric hindrance. Substitution of OH-6 by bulky protecting groups such as TBDPS- (entries 1-2) or a tri-*iso*-propylsilyloxymethyl (TOM) group (entry 7), extensively employed in nucleic acid chemistry, hampered the accessibility of the acceptor site substantially and the disaccharides **101** and **106** were formed in yields below 50%. Changing the bulky TBDPS group for a smaller thexyldimethyl silyl (TDS) group (entry 3), led to a slight increase in the formation of the corresponding disaccharide (from 42% yield for **101** to 51% yield for **102**). An improvement was observed for 6-*O*-*p*-methoxyphenyl (-PMP), 6-*O*-benzyl, and 6-*O*-*p*-methoxybenzyl (-PMB) protected Ido acceptors (entries 4-6), which produced disaccharides **103-105** in yields around 60%. These protecting groups provide both good accessibility of axial acceptor and

additionally, the electro-donating character of these groups enhance the nucleophilicity of the acceptors.

This effect was reversed however, when smaller but electron-withdrawing groups were employed. Both, 6-*O*-acetate derivative **99** (entry 8) and IdoA derivative **100** (entry 9, 10) were poorer acceptors with yields for the corresponding disaccharides **107** and **108** below 50%, probably as result of decreased nucleophilicity of the axial OH-4 acceptor. Furthermore, in the case of the 6-*O*-acetate derivative **99**, an α/β mixture of anomers which could not be separated was obtained as a further consequence of its decreased reactivity. In a direct comparison of the imidate donors **65** and **66** (entries 1, 2 and 9, 10) the trichloroacetimidate **65** performed with both Ido and IdoA acceptors consistently better than the corresponding *N*-phenyl trifluorocetimidate **66**.

Table 3.2. Glycosylations of different Ido and IdoA acceptors with azido glucose donors **65** and **66** in solution.



93: R = $CH_2OTBDPS$
94: R = CH_2OTDS
95: R = CH_2OPMP
96: R = CH_2OBn
97: R = CH_2OPMB
98: R = CH_2OTOM
99: R = CH_2OAc
100: R = COOMe

101: R = $CH_2OTBDPS$
102: R = CH_2OTDS
103: R = CH_2OPMP
104: R = CH_2OBn
105: R = CH_2OPMB
106: R = CH_2OTOM
107: R = CH_2OAc
108: R = COOMe

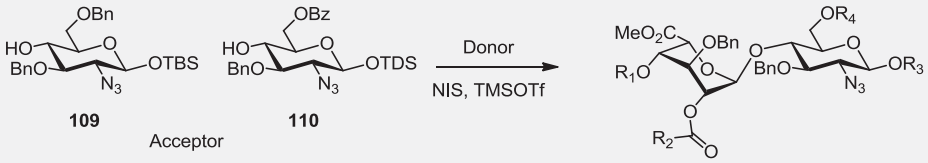
Entry	Donor	R	Temperature	TMSOTf (eq.)	Product	Yield (%)[a]
1	**65**	$CH_2OTBDPS$	-20 °C to rt	0.25	**101**	42%
2	**66**	$CH_2OTBDPS$	-20 °C to rt	0.25	**101**	32%[a]
3	**65**	CH_2OTDS	-20 °C to rt	0.25	**102**	51%
4	**65**	CH_2OPMP	-20 °C to rt	0.25	**103**	65%
5	**65**	CH_2OBn	-20 °C to rt	0.25	**104**	58%
6	**65**	CH_2OPMB	-20 °C to rt	0.25	**105**	61%
7	**65**	CH_2OTOM	-20 °C to rt	0.10	**106**	50%
8	**65**	CH_2OAc	-20 °C to rt	0.10	**107**	15%[b]
9	**66**	COOMe	0°C to rt	0.05	**108**	32%
10	**65**	COOMe	0°C to rt	0.05	**108**	48%

Reagents and conditions: 1.2-1.4 eq. donor. TMSOTf, CH_2Cl_2, [a] conversion determined by UPLC-MS analysis, [b] obtained as α/β mixture of anomers.

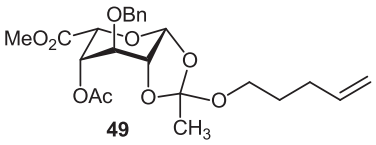
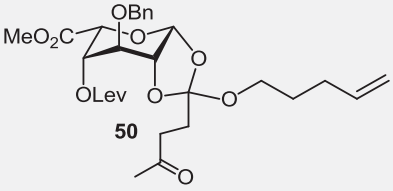
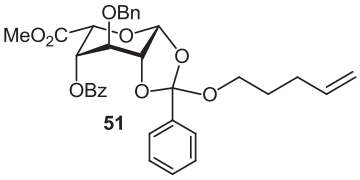
3.3.2.1 Evaluation of n-pentenyl orthoesters as of L-iduronic acid donors

A particular challenge in the chemical synthesis of HS oligosaccharides is the efficient glycosylation of OH-4 of glucosamine residues. While little difference between the reactivity of Ido and IdoA donors was found for the glycosylation of the primary hydroxyl-group in the aminopentyl linker **83** we were intrigued to investigate the performance of the IdoA orthoesters and thioglycosides in the notoriously more difficult glycosylation of the azido glucose acceptors **109**[39] and **110.** The *n*-pent-4-enyl orthoacetate **49**, ortholevulinoate **50** and orthobenzoate **51** were activated with NIS and a catalytic amount of TMSOTf and reacted with the azido glucose acceptors **109** and **110** (Table 3.3). Glycosylations involving the orthoacetate **49** and ortholevulinoate **50** produced the disaccharides **111** and **112** in 30% and 36% respectively. These compounds were accompanied by up to 30% of a disaccharide with undetermined stereochemistry lacking the OH-2 protecting group, which had presumably formed via an orthoester exchange mechanism after attack on the exocyclic carbon.[40,41]

Table 3.3. Comparison of *n*-pentenyl orthoesters **49-51** in the key glycosylation with different azido glucose acceptors **109-110**.[a]

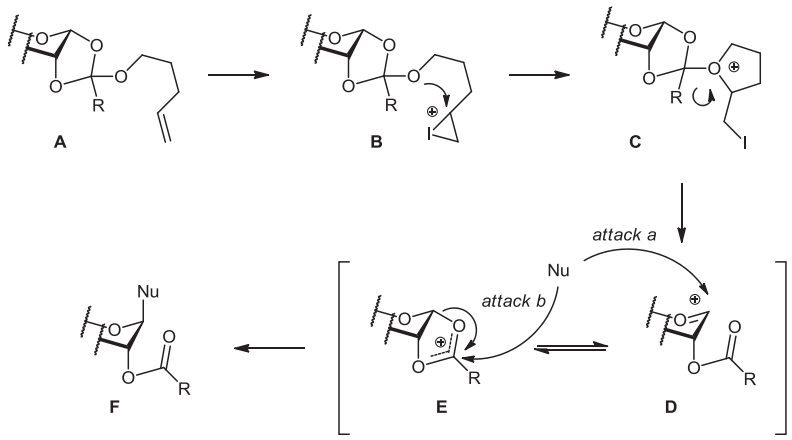


111: R_1=Ac, R_2=Me, R_3= TBS, R_4= Bn
112: R_1=Lev, R_2=$CH_2CH_2COCH_3$, R_3= TDS, R_4= Bz
113: R_1=Bz, R_2=Ph, R_3= TDS, R_4= Bz

Entry	Donor	Acceptor	Conditions	Product	Yield (%)
1	 **49**	**109**	a, 0°C to rt	**111**	30
2	 **50**	**110**	a, 0°C to rt	**112**	36
3	 **51**	**109**	b, 0°C to rt	**113**	85

[a] Reagents and conditions: a) 1.2 eq. donor, 1.5 eq. NIS, 0.2 eq. TMSOTf; b) 1.2 – 1.5 eq. donor, 3.0 eq. NIS, 0.2 eq. TMSOTf.

Scheme 3.19 shows the activation of an NPOE by the electrophilic addition of a halogenium ion, typically iodonium or bromonium, to the double bound (**B**), its subsequent cyclisation to the iodo or bromomethylfuran (**C**) and the formation of an oxocarbenium ion **D**, which can react with the sugar nucleophile (Nu, pathway a) (Scheme 3.19). Under Lewis acid catalysis the NPOE **A** can react also in different ways.[24] Depending on the size of the acyl group in O2 and the stability of the intermediate dioxolenium ion **E** the attack of the nucleophile can preferentially occur on the anomeric carbon (a) leading to irreversible and trans-selective glycoside formation **F** or attack on the exocyclic carbon (b) which can react in a multitude of modes including glycoside anomerisation, transacylation of the acceptor or hydrolysis.[40]

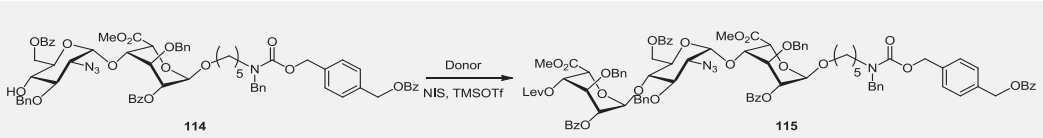


Scheme 3.19. Schematic representation of activation of *n*-pentenyl orthoesters by iodonium ions.

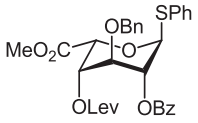
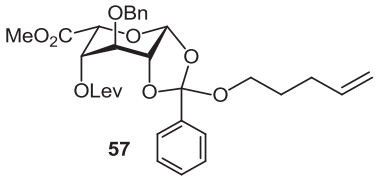
We reasoned that the increasing of the bulk of the orthoester alkyl substituent from methyl to phenyl (compounds **49** and **50**, Table 3.3) should favour the attack of the nucleophile at the anomeric (*attack a* of Scheme 3.19) rather than the exocyclic carbon (*attack b* of Scheme 3.19), leading to a stronger stereo-control in the glycosylation. Indeed, after activating the orthobenzoate **51** with NIS/TMSOTf the disaccharide **113** was produced in an excellent 85% yield as pure α-anomer.

After this promising result, we compared the performance of IdoA thioglycoside **43** and *n*-pent-4-enyl orthobenzoate donor **57** in the glycosylation of more complex disaccharide acceptor **114**. Both reactions involving thioglycoside **43** and orthoester **57** proceeded less well than expected from the previous results with less than 50% yield of trisaccharide **115,** obtained after chromatographic purification. In any case, the orthoester **57** performed slightly better (45% yield) than the thioglycoside **43** derivative (34% yield) in this trial. With all this information about reactivity in solution, we moved on to evaluate performance as glycosyl donors of the orthoesters and thioglycosidesin the solid-phase assembly of building blocks taking advantage of the possibility of increasing the reaction conversion by using excess of donor and various cycles of glycosylation.

Table 3.4. Glycosylation of disaccharide acceptor **114**.[a]



114 115

Entry	Donor	Acceptor	Conditions	Product	Yield (%)
1	 43	**114**	a, rt	**115**	34
2	 57	**114**	a, 0°C to rt	**115**	45

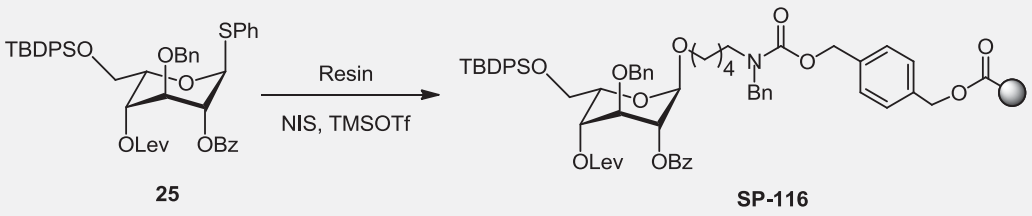
[a] Reagents and conditions: a) 1.2 eq. donor, 3.0 eq. NIS, 0.2 eq. TMSOTf

3.4 EVALUATION OF GLYCOSYL DONORS IN THE SOLID PHASE SYNTHESIS OF HS PRECURSORS.

3.4.1 Initial studies: trisaccharide solid phase synthesis using idose TBDPS

As a first attempt, L-idopyranosyl thioglycoside **25**[36,6] bearing 6-*O*-TBDPS protecting group was used in the glycosylation of different linker functionalized resins **SP-9 (SP-9$_{PS0.2}$, SP-9$_{PS0.4}$, SP-9$_{TENT0.2}$ and SP-9$_{TENT0.4}$,** see section 3.1.1) to afford α-neoglycoconjugate funtionalized material **SP-116 (SP-116$_{PS0.2}$, SP-116$_{PS0.4}$, SP-116$_{TENT0.2}$ and SP-116$_{TENT0.4}$)**. The glycosylation of different resins was performed employing 2 equivalents of donor **25** activated with NIS in the presence of catalytic amounts of TMSOTf acid. The glycosylation yield was determined by UPLC-MS after treatment of the glycosylated resin with NaOMe/MeOH. The results of these trials are summarized in Table 3.5. The use of Tentagel at two different loading capacities (0.2 and 0.4 mmol/g) afforded low conversion (30 %) at high loading capacity (entry 4) and no reaction when employing the lower loading resin (entry 2). On the other hand, the use of polystyrene (PS) resin resulted in very high conversion for both loading capacities (Table 3.5). This difference in resin reactivity could be related to the better swelling properties of polystyrene in dichloromethane compared with Tentagel.[42] Based on previous result, the resins **SP-116$_{PS0.2}$** and **SP-116$_{PS0.4}$** were selected as the best candidates for the solid phase synthesis of HS precursors.

Table 3.5. Evaluation of the linker functionalized solid support in the glycosylation of thioglycoside **25**. [a]



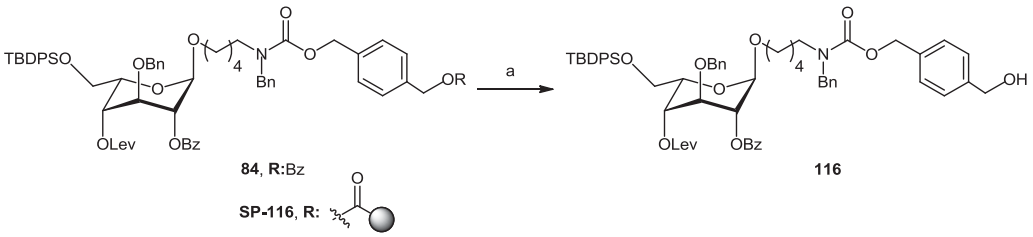
Entry	Resin	Loading	UPLC-MS conversion (%)
1	Polystyrene (**SP-9$_{PS0.2}$**)	0.2 mmol/g	86
2	Tentagel (**SP-9$_{TENT0.2}$**)		No reaction
3	Polystyrene (**SP-9$_{PS0.4}$**)	0.4 mmol/g	95
4	Tentagel (**SP-9$_{TENT0.4}$**)		30

[a] Reagents and conditions: a) **25**, NIS (2.5 eq.), TMSOTf, -20ºC to r.t CH_2Cl_2

After the glycosylation of the linker functionalized resin with **25**, it was necessary to cap the possible non reacted hydroxyl groups. This was achieved by acetylation with acetic anhydride in pyridine and a catalytic amount of DMAP. The OH-4 group of the resin bound L-idopyranosyl unit was then orthogonally deprotected using an excess of hydrazine acetate in CH_2Cl_2/MeOH (5/1) at room temperature. The efficiency of this delevulination step in **SP-116** was difficult to monitor as cleavage from the resin employing standard conditions (NaOMe catalytic in MeOH) resulted in the concomitant delevulination and an alternative method for cleavage need to be developed.

For this reason, we investigated the use of dibutyltin oxide (DBTO) mediated transesterification of carboxylic acid esters[43] on model compound **84.** As shown in Scheme 3.20, the treatment of **84** with dibutyltin oxide in dichloromethane/methanol at 120 ºC allowed the cleavage of the primary benzoate ester with high selectivity affording alcohol **116**, in which the 4-*O*-levulinic ester remained intact. Likewise, microwave assisted irradiation of idose bound resin **SP-116** with 5 eq of DBTO at

120 °C afforded the idose derivative **116** with all protecting groups in place after 10 min.



Scheme 3.20. Transesterification reaction for the cleavage of primary benzoate groups. Reagents and conditions: a) Bu_2SnO, CH_2Cl_2, MeOH, MW 120 °C.

Therefore, the hydrazine-acetate mediated hydrolysis of the 4-*O*-levulinic ester group on **SP-116** could be efficiently monitored after DBTO mediated cleavage of an analytical sample from the resin (**116**, see Scheme 3.21).

In Scheme 3.21 is shown the synthesis on solid phase of a trisaccharide HS precursor. The disaccharide synthesis was performed on resin **SP-118$_{PS0.2}$** using 5 equivalents of the 2-azido-D- glucopyranosyl donor **65** under TMSOTf catalysis affording resin **SP-119** with a 53% conversion after 2 cycles as determined by UPLC-MS analysis (**119**). This yield could be raised to 82% after three additional glycosylation cycles (see Figure 3.2). In comparison, the solution phase reaction (previously described) with equimolar amounts of reactants produced disaccharide in only 42% yield, evidencing one of the advantages of employing solid phase synthesis in the preparation of complex HS precursors.

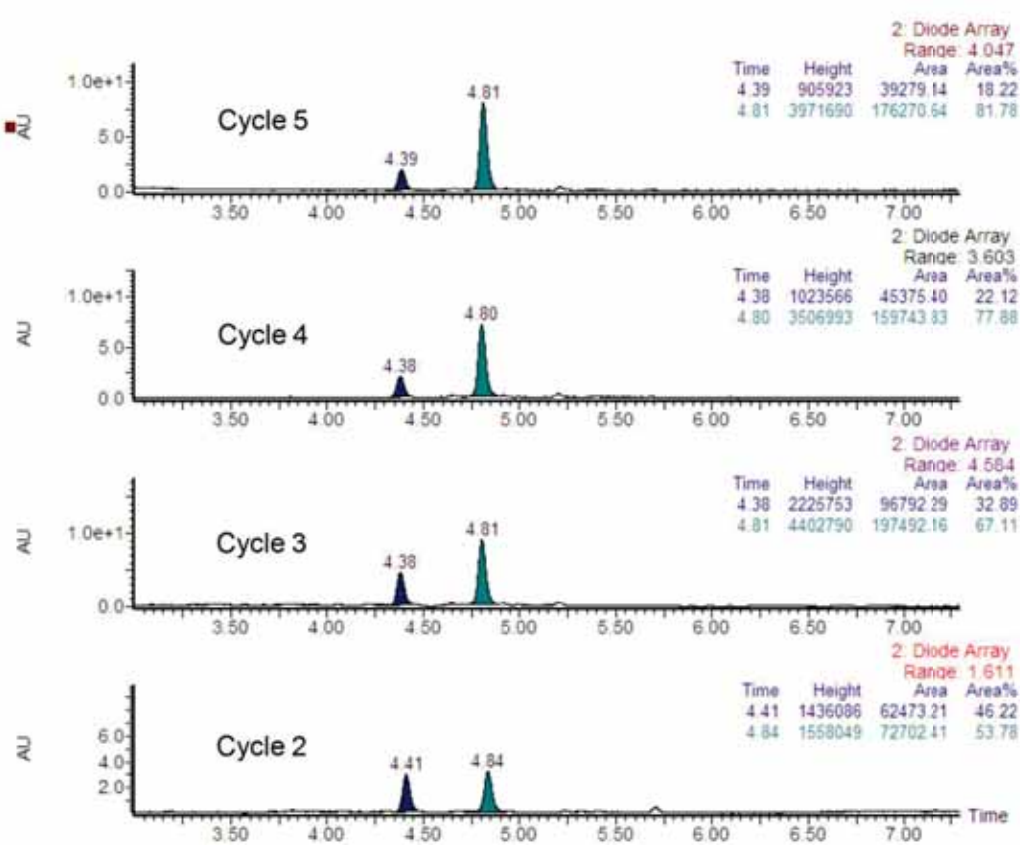
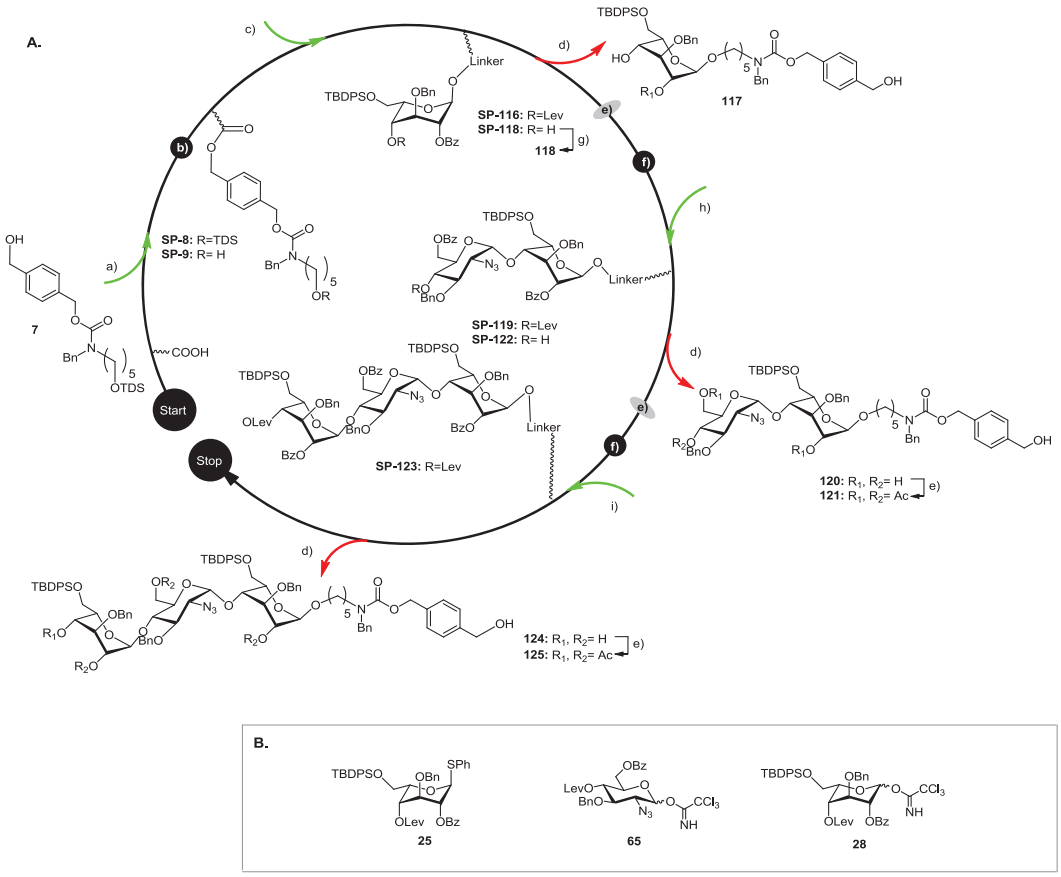


Figure 3.2. UPLC-MS data for conversion of monosaccharide **SP-116** (average retention time=4.39 min) to the disaccharide **SP-119** (average retention time=4.81 min) derivative

Treatment of **SP-119** with hydrazine acetate to remove levulinoyl group, afforded the free acceptor **SP-122**. (Scheme 3.21). The glycosylation of **SP-122** with **28** under NIS/TfOH activation produced trisaccharide **SP-123** in a moderate 50% yield.



Scheme 3.21. A) Synthesis of a trisaccharide HS precursor **125**. Reagents and conditions: a) carboxypolystyrene resin, DIC, DMAP, CH_2Cl_2, then Me_3SiCHN_2, THF, MeOH; b) HF·pyridine, THF; c) **25**, NIS, TMSOTf, CH_2Cl_2; d) NaOMe, MeOH; e) Ac_2O, pyridine, DMAP f) hydrazine acetate, CH_2Cl_2; g) Bu_2SnO, MeOH, CH_2Cl_2; h) **65**, TMSOTf, CH_2Cl_2, for conversion see Figure 3.2; h) **28**, NIS, TMSOTf, CH_2Cl_2; B) Monosaccharide protected idose donors **25**, **28** and glucosamine **65** building blocks used for HS trisaccharide synthesis.

Attempts to increase the conversion by using subsequent glycosylation cycles led to substantial breaking of both α- glycosidic linkages. In order to investigate in detail this secondary reaction, model glycoconjugate **84** was subjected to the same NIS/TfOH excess treatment in solution. The crude product was analyzed by UPLC-MS and both anomerisation and partial cleavage of glycosidic linkages accompanied by iodination were observed.

On the other hand, the use of dimethyl(methylthio)sulfonium (DMTST) as activator for L-idopyranosyl thioglycoside **25** on the solid support did not alter the glycosidic linkage, but was less efficient as only 40% of glycoconjugate **SP-116** resin was obtained after one reaction cycle. More reactive trichloroacetimidate donor **28** was employed for the glycosylation of **SP-122**. Trichloroacetimidate **28** was activated with a catalytic amount of TMSOTf and cleanly produced the trisaccharide **SP-123** with 53% conversion after 4 cycles of glycosylation reactions. Under these experimental conditions no competing cleavage of labile L-idopyranosyl glycosidic bonds was observed. Cleavage from the resin employing sodium methoxide in methanol produced trisaccharide **124**. In order to facilitate the purification, the crude product after resin cleavage was subjected to acetylation. This allowed the full characterisation of the protected trisaccharide **125**.

Based on this preliminary result, we can conclude that monosaccharide building blocks can be successfully assembled on a solid support to produce HS glycan precursors, thereby avoiding the use of far more advanced disaccharides. The novel ester type linker incorporating a carbamate protected C5-spacer for later ligand immobilisation proved to be stable throughout the synthesis and it was easily cleaved from the resin by a choice of two complementary methods (DBTO and NaOMe/MeOH). The lability of L-idopyranosyl residues under NIS/TfOH activation makes thioglycoside donors less attractive for the solid-phase synthesis of heparan sulfate oligosaccharide precursors. L-Idopyranosyl and 2-azido-D-glucopyranosyl trichloroacetimidates, however, were activated without any cleavage of glycosidic bonds.

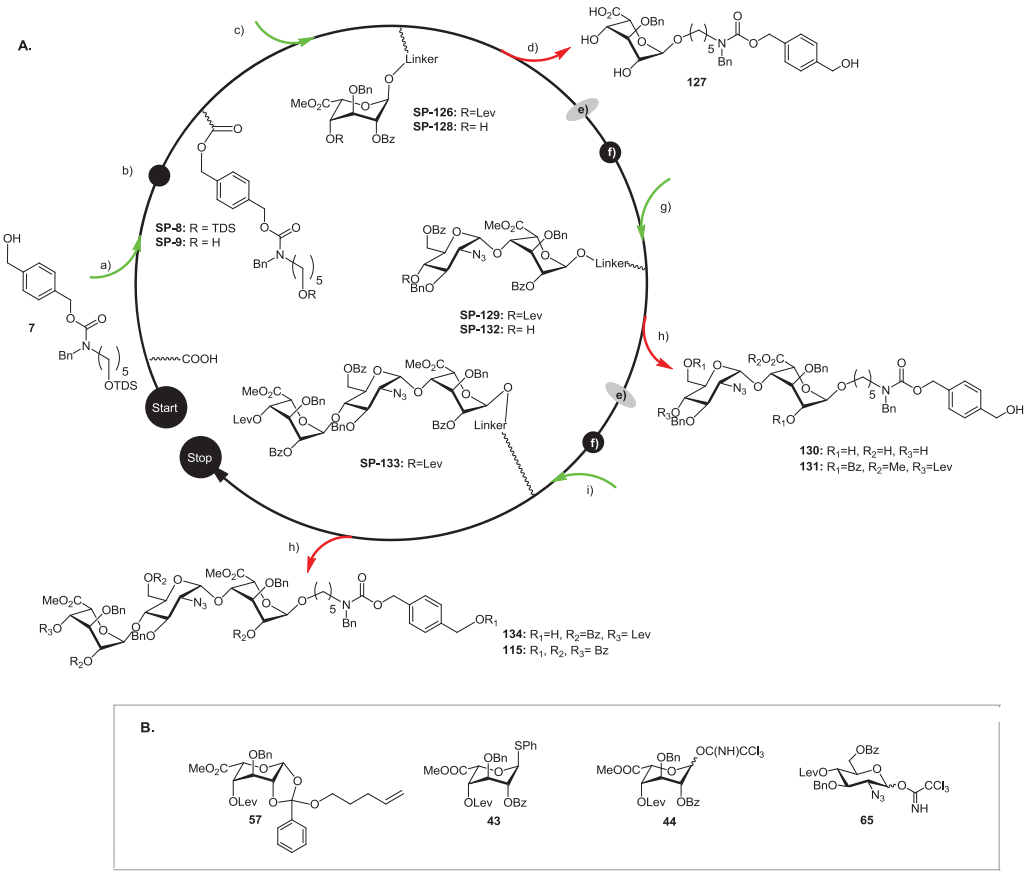
3.4.2 Solid phase synthesis of HS precursor trisaccharide using n-pentenyl orthoester of iduronic acid as glycosyl donors

Based on our previous results on disaccharide synthesis in solution with n-pentenyl orthoesters as L-IdoA donors, orthoester **57** was evaluated for solid phase synthesis of a HS trisaccharide precursor. The direct use of IdoA donors in the synthesis would avoid the need of deprotection and oxidation steps to produce the natural structure.

Glycosylation of the linker modified resin **SP-9** with 5 equivalents of the IdoA orthoester **57** under conventional NIS/TMSOTf activation provided the **SP-126** resin (Scheme 3.22). UPLC-MS analysis of a cleaved aliquot of the resin confirmed the formation of compound **127** with 85% yield after one glycosylation cycle. (See Table 3.6, entry 3). The use of trichloroacetimidate **44** under TMSOTf catalysis at -40 °C also afforded resin-bound monosaccharide **SP-126**, that after analytical cleavage also produced compound **127** in 84% yield as determined by UPLC-MS analysis. Similar yield was obtained with 5 eq. of thioglycoside **43** under conventional NIS/TMSOTf activation, thus reproducing the high yields of the solution-phase experiments for this reaction with the primary hydroxyl group of resin immobilized linker.

The following deprotection of the 4-*O*-levulinic ester of **SP-126** with 3 equivalents of hydrazine acetate to the resin-bound acceptor **SP-128** was monitored by dibutyltinoxide (DBTO) mediated[43] cleavage of an analytical sample of the resin. Subsequent glycosylation of **SP-128** with 3 equivalents of trichloroacetimidate **65** at -20 °C afforded resin **SP-129**. Cleavage of a resin aliquot with sodium methoxide showed the formation of disaccharide **130** with only 21% yield after one cycle. After a second cycle of glycosylation, the formation of elimination byproducts was detected by UPLC-MS analysis after cleavage from the resin. As previously reported for solution phase synthesis, the sodium methoxide cleavage of iduronic acids is accompanied by elimination products. The use of lithium peroxide described by

another authors[44] to avoid elimination side reactions was then used without success possibly due to the lack of proper resin swelling under the required aqueous reaction conditions. Also trimethyltinhydroxide (TMTOH) as in the case of NPOE donor was unsuccessfully applied. Finally, the use of dibutyltin oxide (DBTO) in CH_2Cl_2/MeOH (2/1) as solvent at 120 ºC under microwave irradiation for 10 minutes allowed the analytical cleavage without any elimination. Several heating cycles were applied until no further released of compound was detected by thin layer chromatography (TLC).



Scheme 3.22. A) Solid-phase synthesis of a trisaccharide employing IdoA donors. Reagents and conditions: a) carboxypolystyrene resin, DIC, DMAP, CH_2Cl_2, then Me_3SiCHN_2, THF, MeOH; b) HF·pyridine, THF; c) **57**, **43**, or **44**, NIS, TMSOTf, CH_2Cl_2; d) NaOMe, MeOH; e) Ac_2O, pyridine, DMAP; f) hydrazine acetate, CH_2Cl_2; g) **65**, TMSOTf, CH_2Cl_2; h) Bu_2SnO, MeOH, CH_2Cl_2; i) **57**, NIS, TMSOTf, CH_2Cl_2, for conversions see Table 4. B) Monosaccharide protected idose donors **57**, **43, 44** and glucosamine **65** building blocks used for HS trisaccharide synthesis.

Table 3.6. Evaluation of iduronic acid derivatives **57**, **43**, and **44** in the solid-phase synthesis of HS trisaccharide precursors.

Entry	Donor	Acceptor	Conditions	Product	Conversion[a]
1	**44**	**SP-9**	1 x 3 eq., -40 °C to rt, 10%TMSOTf	**127**	84%
2	**43**	**SP-9**	1x 5 eq., rt, NIS (3.5eq.), 15% TMSOTf	**127**	87%
3	**57**	**SP-9**	1 x 5 eq., 0 °C to rt, NIS (3.5eq.), 15% TMSOTf	**127**	85%
4	**65**	**SP-128**	1 x 3eq., -20 °C to rt , 15% TMSOTf	**130**	21%
5	**65**	**SP-128**	4 x 3eq., -20 °C to rt , 15% TMSOTf	**130**	84%
6	**57**	**SP-132**	3 x 3eq., 0 °C to rt, 15%TMSOTf	**134**	76%

[a] conversion was determined by UPLC-MS analysis after cleavage from the resin.

After 4 cycles the conversion of disaccharide **131** (cleaved with dibutyltin oxide (DBTO)) was increased to 86%. Again, 4-*O*-levulinic ester was deprotected by hydrazinolysis affording resin bound acceptor **SP-132** which was condensed with the *n*-pent-4-enyl orthoester donor **57** after activation with NIS and TMSOTf at 0 °C. The glycosylation was repeated twice affording resin **SP-133** and UPLC-MS analysis of a cleaved sample **134** showed a conversion of around 76% after three cycles of glycosylation. The preparative scale cleavage of the trisaccharide **134** from the resin with DBTO to avoid elimination was less effective than expected from the analytical trial and had to be repeated several times for complete recovery of the target trisaccharide. The use of lithium peroxide to hydrolyse the ester groups prior to the methoxide mediated cleavage from the resin was hampered by the low swelling of the hydrophobic resin in the aqueous solution. Also the use of trimethyltinhydroxide

as in the case of the NPOE in solution (previously described) was applied to compound **SP-133** without success. In the course of the reaction using DBTO, 2-*O*-benzoate groups were partially cleaved, but as they were not fully removed, we decided to treat the crude product with benzoyl chloride in pyridine in order to recover a more homogeneus product and to facilitate purification by preparative TLC. The protected trisaccharide **115** was isolated in 8% overall yield over 8 steps.

In conclusion, we evaluated the use of *n*-pentenyl orthoester donors for the solid phase synthesis of a trisaccharide heparin sulphate precursor. We have found that *n*-pentenyl orthoester based donors of IdoA could be employed successfully in glycosylation reactions to construct of HS protected oligosaccharides on the solid support. Nevertheless, the use of Ido A donors for the solid phase HS oligosaccharides synthesis was not compatible with the strong basic conditions required to cleave our ester linker and important elimination of IdoA ester residues to the corresponding alkene was observed. A change to more basic labile linkers, cleavable under milder conditions, or to orthogonal photolinkers as employed by Seeberger group[45] could permit the direct use of IdoA donors in the preparation of HS oligosaccharides.

3.4.3 Solid phase synthesis of HS trisaccharide precursor using L- idopyranosyl donors

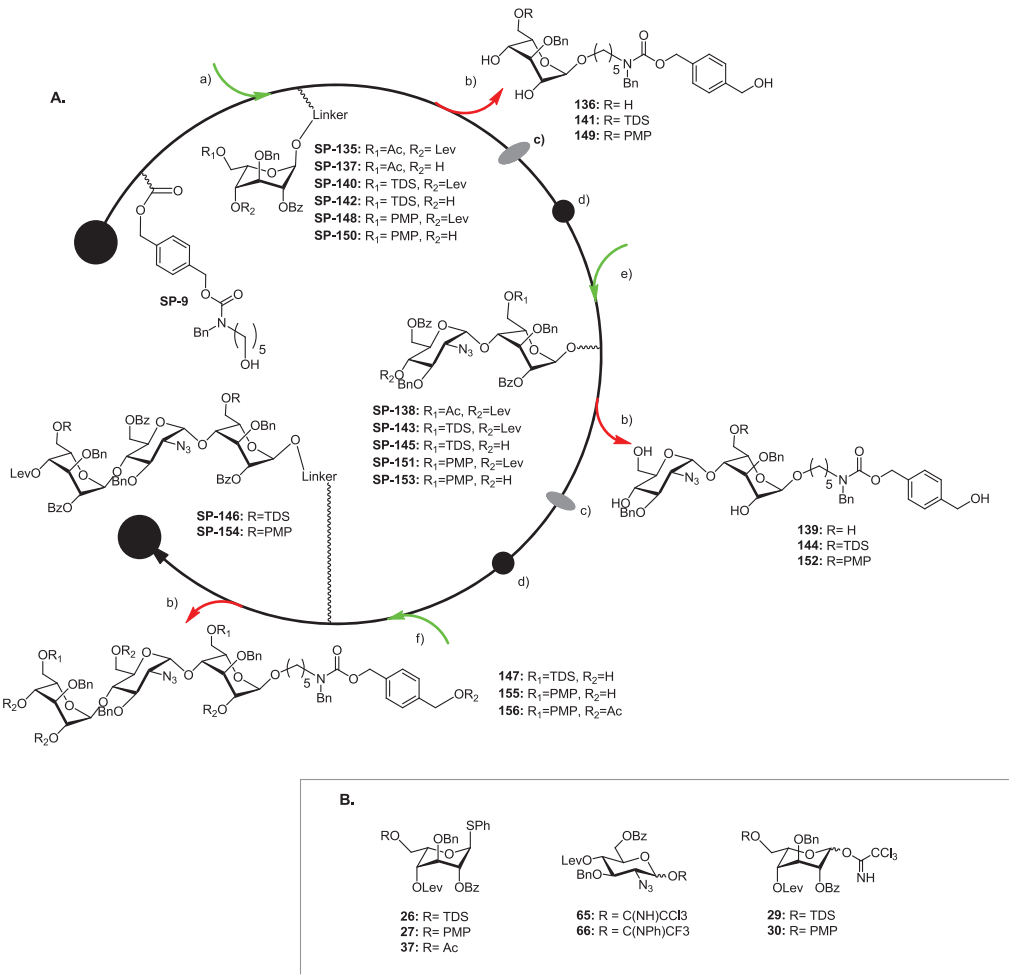
The incompatibility of the iduronic acid derivatives with the effective methoxide-promoted linker cleavage conditions led us to investigate L-idopyranosyl donors for the solid phase synthesis of a HS trisaccharide precursor.

First, resin bound glycoside **SP-135** was synthesised in near-quantitative yield, as the analysis of the methoxide cleavage product **136** suggested, using thioglycoside **37** as glycosylating agent (Scheme 3.25). Hydrazinolysis afforded the acceptor **SP-137** which was reacted with **65** to produce **SP-138**, albeit in only 22% as detected by UPLC-MS analysis of the cleaved compound **139**. Apparently, the electronic effect of the 6-*O*-acetate group on the nucleophilicity of the OH-4 acceptor compensated any favourable reduction of the steric bulk at C6 leading to an overall poor yield in the glycosylation and confirming the results of the solution-phase glycosylation (previously described), albeit with complete α-selectivity.

Next, employing the 6-*O*-thexyldimethylsilyl (TDS) protected thioglycoside **26** we prepared resin **SP-140** in near quantitative yield (**141**). Hydrazinolysis to **SP- 142** was followed by three cycles of glycosylation with three equivalents of the imidate **65** giving rise to the resin bound disaccharide **SP-143** with excellent conversion (77%, **144**). Removal of 4-*O*-levulinic ester provided acceptor **SP-145,** that was subjected to glycosylation reaction with trichoroacetimidate **29**. In this case, thioglycoside was not used in order to avoid the cleavage of the glycosidic bond observed previously. After 4 cycles of glycosylation with 3 equivalents of donor **29**, under TMSOTf activation, **SP-146** was formed in 70% yield (**147**). This result is comparable to the yield obtained with orthoester IdoA **57**.

Based on these promising results, we decided to scale up this reaction but unfortunately, during the scale up, we could observed partial loss of 6-*O*-TDS protecting group due to the high concentration of acid activator (TMSOTf) and subsequent overglycosylation in 6-OH was detected by UPLC-MS. So finally, we

focused our attention in 6-*O*-PMP protected L-idopyranosyl donors. Compound **27** would offer high stability under Lewis acid conditions required for the glycosylation reactions and also had demonstrated excellent acceptor properties in the solution based glycosylation trials (Table 3.7, entry 4).



Scheme 3.23. A) Solid-phase synthesis of trisaccharide precursors involving idose donors. Reagents and conditions: a) **26**, **27** or **37**, NIS, TMSOTf; b) NaOMe, MeOH; c) Ac_2O, pyridine, DMAP; d) hydrazine acetate, CH_2Cl_2; e) **65** or **66**, TMSOTf, CH_2Cl_2; f) **29** or **30,** NIS, TMSOTf, CH_2Cl_2; for conversions see Table 5. B) Monosaccharide protected idose donors (**26, 27, 29, 30** and **37**) and glucosamine **65** and **66** building blocks used for HS trisaccharide synthesis.

Preparation of resin bound glycoside **SP-148** proceed with excellent conversion (**152**). Deprotection of the 4-*O*-levulinic ester (**SP-150**) and glycosylation with trifluoroacetimidate **66** produced resin bound disaccharide **SP-151** in 71 % yield (**152**, entry 7 of Table 3.7)

Table 3.7. Evaluation of Idose derivatives **26**, **37**, **29**, **30** and **37** in the solid-phase synthesis of HS trisaccharide precursors

Entry	Donor	Acceptor	Conditions	Product	Conversion[a]
1	**37**	**SP-9**	1x 5eq., -20ºC to rt, NIS (6.5eq.), 10% TMSOTf	**136**	96%
2	**65**	**SP-137**	1x 3eq., -20 ºC to rt, 10% TMSOTf	**139**	22%
3	**26**	**SP-9**	1 x 5 eq., -20 °C to rt, NIS (6.5eq.), 10% TMSOTf	**141**	>95%
4	**65**	**SP-142**	3 x 3 eq. -20 °C to rt, 10% TMSOTf	**144**	77%
5	**29**	**SP-145**	4 x 3 eq. -20 °C to rt, 10%TMSOTf	**147**	70%
6	**27**	**SP-9**	1 x 5 eq., -20 °C to rt, NIS (6.5eq.), 10% TMSOTf	**149**	>95%
7	**66**	**SP-150**	2 x 6 eq. -20 °C to rt, 10% TMSOTf	**152**	71%
8	**65**	**SP-150**	4 x 3 eq. -20 °C to rt, 10% TMSOTf	**152**	80%
9	**65**	**SP-150**	2 x 6 eq. -20 °C to rt, 10%TMSOTf	**152**	85%
10	**65**	**SP-150**	1 x 12 eq. -20 °C to rt, 10% TMSOTf	**152**	68%
11	**65**	**SP-150**	1 x 12 eq. + 1 x 6 eq. -20 °C to rt, 10% TMSOTf	**152**	85%
12	**65**	**SP-150**	3 x 6 eq. -20 °C to rt, 10% TMSOTf	**152**	90%
13	**30**	**SP-153**	2 x 6 eq. -20 °C to rt, 10% TMSOTf	**155**	94%

[a] conversion was determined by UPLC-MS analysis after cleavage from the resin.

Coupling with imidate **65** in 4 cycles of 3 equivalents raised the yield of **SP-151** to 80% (**152,** entry 8). A larger donor excess of 6 equivalents improved the yield slightly after only 2 cycles to 85%, while a single cycle of 12 equivalents resulted in a slightly lower yield (Table 4, entries 9 and 10). On the other hand, 90% conversion was achieved after 3 cycles employing 6 equivalents of donor **65** (entry 12). Finally, after removal of the levulinic ester, acceptor **SP-153** was coupled in 2 cycles with imidate **65** to afford the resin bound trisaccharide **SP-154** with 94% conversion (**155**, entry 13).

Once optimized the conditions for glycosylation and deprotection reactions, the full solid phase glycosylation procedure towards HS precursor trisaccharide formation was carried out. Following this strategy we could obtain 39 mg of the crude HS precursor **156**. In order to facilitate the purification process by column chromatography, cleaved crude trisaccharide **155** was acetylated providing **156** in 72% overall yield over 8 steps with an average yield of 95% per step (Scheme 3.23).

In order to unequivocally assign the configuration of all glycosidic bonds, the geminal coupling constants between ^{13}C-^{1}H in the anomeric positions were determined in heteronuclear single quantum correlation (HSQC) nuclear magnetic resonance experiment**.** Measurement of the three ^{13}C-^{1}H heteronuclear anomeric coupling constants confirmed a 1,2-cis configuration for all glycosidic linkages (idose $J_{C\text{-}1,\ H\text{-}1}$ = 169 Hz, 170 Hz and azido glucose $J_{C\text{-}1,\ H\text{-}1}$ = 171 Hz, see Figure 3.4).[46,47]

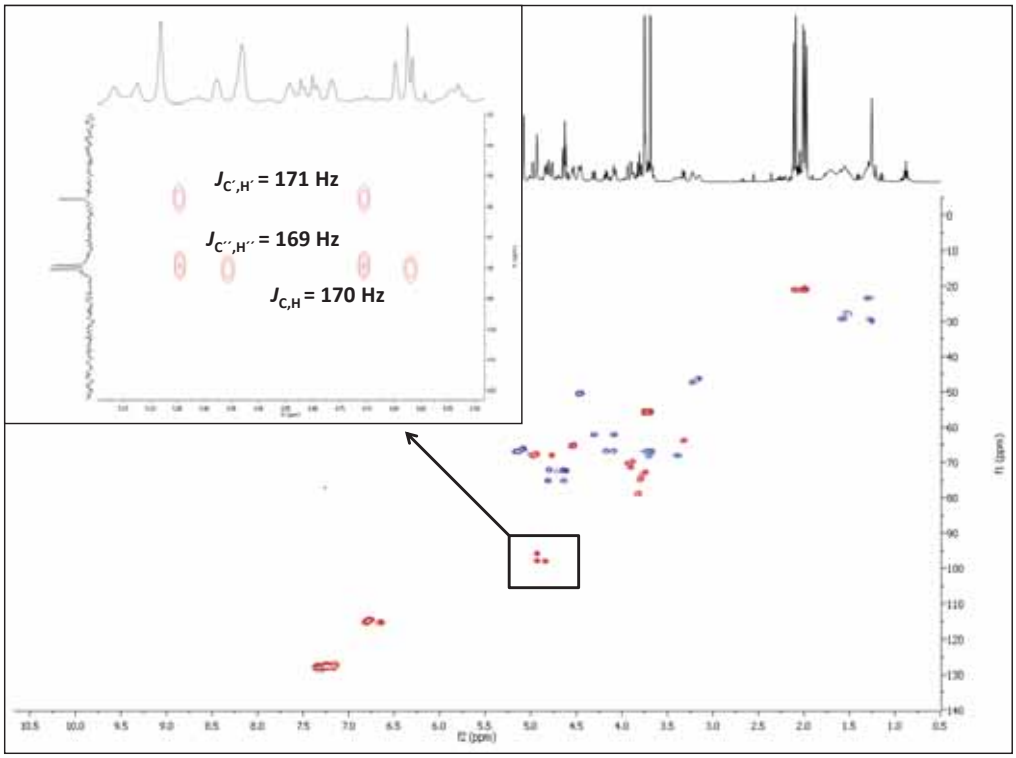
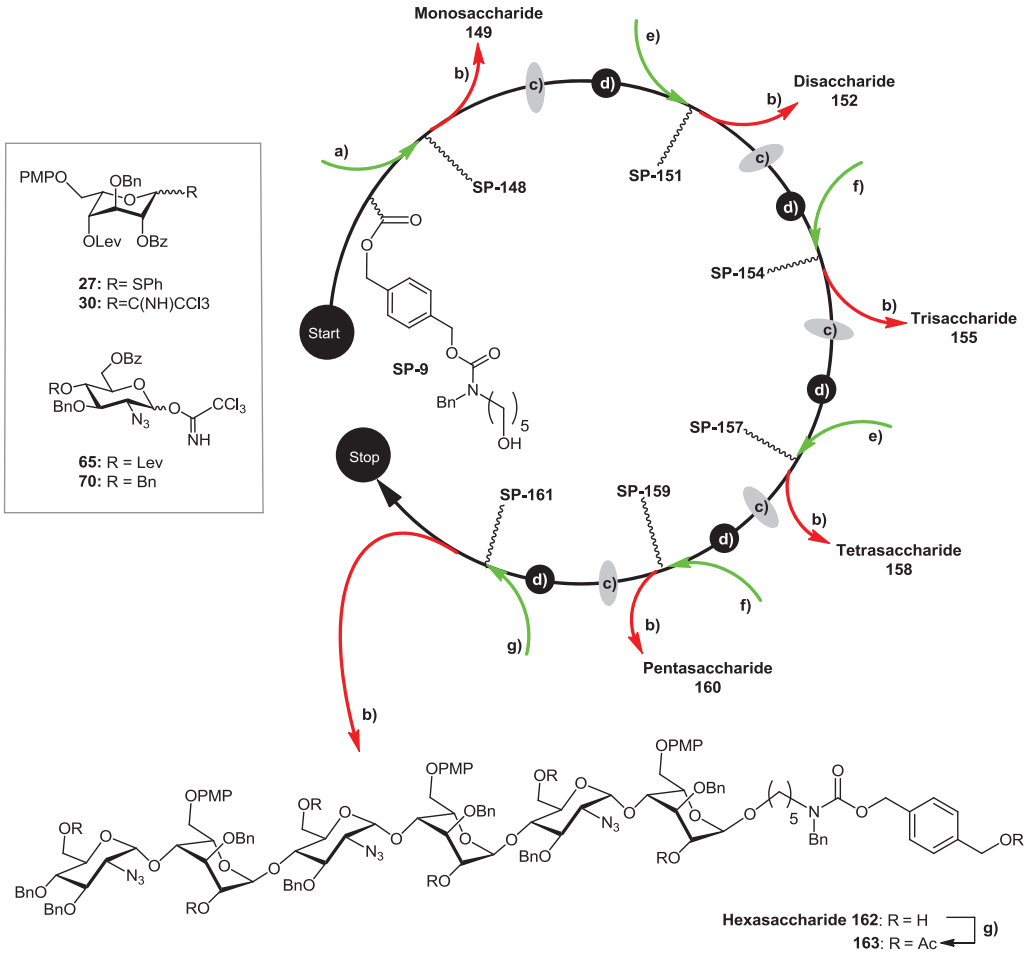


Figure 3.3. HSQC NMR spectra of heparin trisaccharide precursor **156**. $J_{C,H}$ coupling values (≈ 170 Hz) confirmed α-selectivity of the glycosylation reactions.

3.4.4 Solid phase synthesis of a HS hexasaccharide precursor.

Among the Ido and IdoA donors tested both in solution and solid-phase synthesis of a HS trisaccharide precursor (previously described), imidate **30** (Scheme 3.24) combined excellent stability and good reactivity and was selected for further studies targeting larger oligosaccharides.

Following up on these studies we now investigate the viability of a sequential assembly of monosaccharide building blocks to HS hexasaccharides on a solid support. For the synthesis of HS precursor **162**, Ido and GlcN donors **27, 30** and **65** were employed in an alternating fashion and donor **70** was used as a final capping block (Scheme 3.24).



Scheme 3.24 Solid-phase assembly of a heparan sulfate hexasaccharide precursor. a) **27**, N-iodosuccinimide, 20% TMSOTf, -20 °C to r.t; b) NaOMe (cat), MeOH, MW; c) Ac_2O, pyridine, DMAP; d) hydrazine acetate, CH_2Cl_2: MeOH (4:1); e) **65**, 20% TMSOTf, -20 °C to r.t; f) **30**, 20% TMSOTf, -20 °C to r.t; g) **70**, 20% TMSOTf, -20 °C to r.t; h) Ac_2O, pyridine, 0 °C to r.t.

Individual coupling efficiencies were estimated as previously by UPLC-MS after cleaving an analytical sample from the resin (see Table 3.8). Table 3.8 shows the apparent conversion for every coupling reaction and cycle. Reaction conditions were streamlined where possible to common procedures to evaluate the robustness of the chemistry for later translation to an automated synthesizer. The synthesis of the resin bound trisaccharide **SP-154** which had been described previously could be reproduced with similar conversion, namely with 92% and 94% respectively, for the di- and trisaccharide intermediates. Also in this case unreacted acceptor functions

were capped by acetylation to avoid the production of any deletion sequences. Successive capping, delevulination and alternate glycosylation cycles with **30** and **65** led to **SP-159** which was finally reacted with terminating capping donor **70**. High conversions were achieved after 3 cycles from trisaccharide **SP-154** to hexasaccharide **SP-161** UPLC-MS analysis of a cleaved analytical sample showed a conversion of 46% to the hexasaccharide **SP-161** (as **162**) in the first cycle which could be improved to an excellent 92% in a second cycle.

Table 3.8. UPLC-MS conversion for HS hexasaccharide.

Glycosylation	Donor	Product	UPLC-MS conversion (%)		
			Cycle 1	Cycle 2	Cycle 3
1	**27**	**149**	>99	-	-
2	**65**	**152**	n.a	n.a	92
3	**30**	**155**	n.a	94	-
4	**65**	**158**	28	62	78
5	**30**	**160**	62	72	76
6	**70**	**162**	46	92	92

Hexasaccharide **162** was cleaved off the resin by repeated treatment with sodium methoxide, acetylated with acetic anhydride and purified by preparative HPLC to afford the heparan sulfate hexasaccharide precursor **163** in 11% yield over the 14 steps (85% average yield for each step).

3.5 TOWARDS THE SOLID PHASE SYNTHESIS OF DERMATAN SULFATE

Dermatan Sulfate (DS) is a polyanionic linear polysaccharide composed of a repeating disaccharide unit of β-1,3 linked iduronic acid (IdoA) and N-acetyl galactosamine (GalNAc). DS is also commonly known as chondroitin sulfate B (CSB), because of the presence of GalNAc residues along its backbone. But the major difference to CS is the occurrence of iduronic acid (IdoA) moieties along its backbone highlighting structural similarities to heparin and HS, both of which too contain IdoA as a major building block.

DS polysaccharides are complex mixtures characterized by a broad range of chain lengths and heterogenous sulfation patterns along the backbone. The high presence of sulfate and carboxylate groups imparts DS with a high negative charge density facilitating its interaction with a wide array of different proteins. Sulfation in DS can occur at three major locations, the C2 position on IdoA (similar to HS or heparin) and the C4 and C6 of the galactosamine residues (similar to CSA and CSC). Most strategies for the solution-phase synthesis of GAGs have been based on the stereoselective assembly of conveniently protected disaccharide building blocks that allow the site-specific introduction of N- and O-sulfate groups.[48–54]

As mentioned in the Chapter 1, during the course of this work Seeberger reported the automated assembly of two CS hexasaccharide precursors employing monosaccharide building blocks in an overall yield of 8 and 13 %, respectively. This is a remarkable result, but a routine synthesis of GAGs on an automated synthesizer will require further improvement of coupling yields in order to outperform current solution-phase strategies.[55]

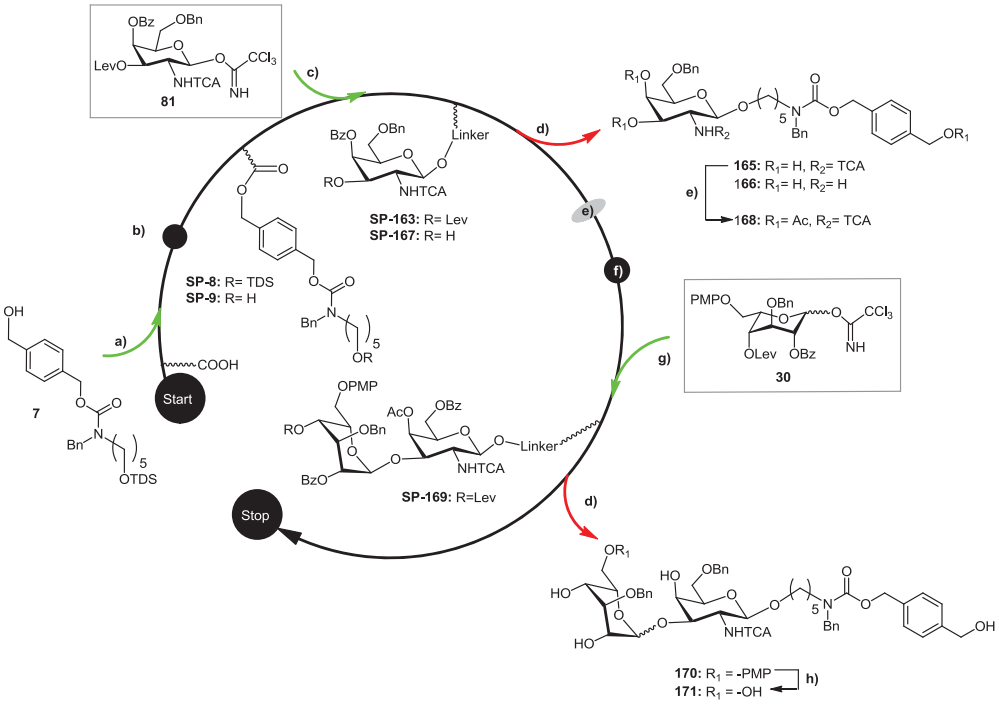
Based on excellent results obtained in the preparation of hexasaccharide precursor **163** of HS on the solid phase by the sequential assembly monosaccharide building blocks, we move towards the solid phase assembly of DS oligosaccharide precursors. The strategy would also be based in the sequential coupling of differentially protected monosaccharide building blocks on linker **SP-9** modified polystyrene resin.

3.5.1 Initial attemps on solid phase: disaccharide synthesis.

Solid-phase trials were performed on a polystyrene resin functionalized with the carbamate type linker **SP-9** at 0.2 mmol/g prepared as previously described. After methylation of the unreacted carboxylic acid functions using diazomethane, the TDS group of resin bound linker **SP-8** was deprotected with HF-pyridine to afford the acceptor **SP-9**. Glycosylation of primary hydroxyl group employing 5 equivalents of the N-trichloroacetimidate donor **81** under TMSOTf catalysis at -20 °C afforded resin bound monosaccharide **SP-163**. UPLC-MS analysis of an aliquot of the resin after cleavage with sodium methoxide at 55 ºC under microwave irradiation showed the formation of compound **165** with a conversion of 67% accompanied by a byproduct **166** in which the trichloroacetamide moiety was partially lost. The formation of this byproduct occurred during the harsh basic conditions applied for cleavage from the resin but could be reduced to less than 5% when the cleavage was carried out at 40°C for 5 min. The efficiency of these conditions proved to be efficient for the quantitative cleavage of the resin bound saccharides, we applied these conditions to monosaccharide **SP-163** which could be isolated in 89% yield after acetylation of crude product.

Compound **168** was confirmed to be a single isomer analysis as β-isomer. The ^{1}H NMR spectrum show signal of a doublet with a large coupling constant, ($J_{H1,2}$= 8.1 Hz) in agreement with a newly established 1, 2-trans glycosidic linkage. Selective deprotection of 3-*O*-levulinic ester (**SP-163**) by treatment with 5 equivalents of hydrazine acetate in one cycle afforded **SP-167** acceptor. Glycosylation of **SP-167** acceptor with 5 equivalents of trichloroacetimidate **30**

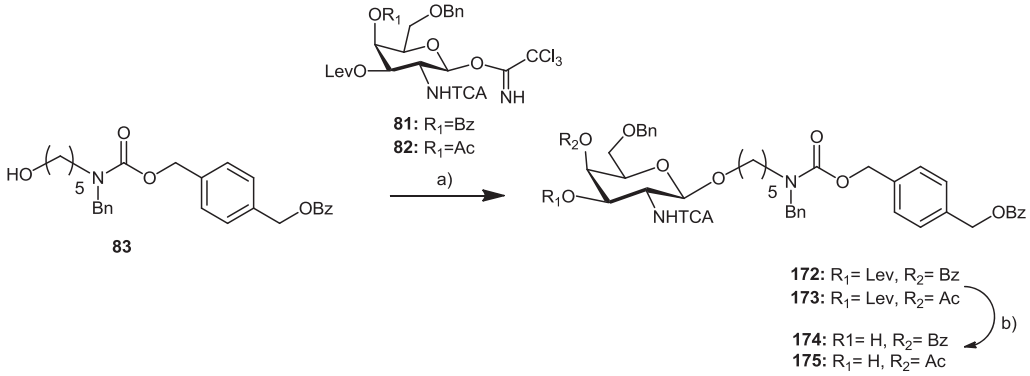
under TMSOTf catalysis (2 cycles), produced resin bound disaccharide **SP-169** in 97 % yield. Quantitative cleavage of the disaccharide **SP-169** from the resin gave **170** in 79 % yield. Unfortunately, ^{1}H and HSQC NMR analysis showed that an α/β mixture (9/1) in the newly formed idose anomeric position. Nevertheless, the mixture could be easily purified after deprotection of 6-*O*-PMP group using CAN in a mixture of acetonitrile/water (4/1).



Scheme 3.25. Disaccharide Dermatan Sulfate Solid-Phase Synthesis. Reagents and conditions: a) carboxypolystyrene resin, DIC, DMAP, CH_2Cl_2, then Me_3SiCHN_2, THF, MeOH; b) HF·pyridine, THF; c) **81**, 20% TMSOTf, -20 °C to rt; d) NaOMe (cat), MeOH, MW; e) Ac_2O, pyridine, DMAP, CH_2Cl_2; f) hydrazine acetate, CH_2Cl_2: MeOH (4:1); g) **30**, 20% TMSOTf, -20 °C to rt; h) cerium ammonium nitrate, acetonitrile/water (4/1), 0ºC to rt, 70% (as only α-anomer).

3.5.2 Solution synthesis of a dermatan sulphate disaccharide

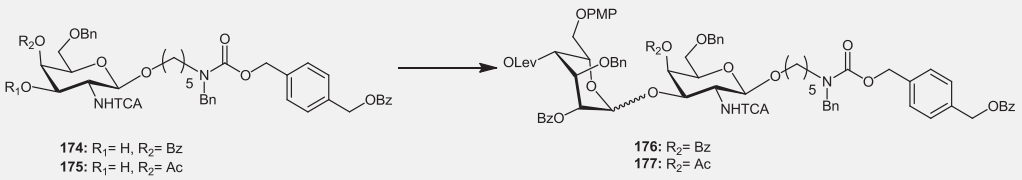
In order to improve the stereoselectivity of the reaction, the disaccharide formation was optimized in solution using different activation conditions and differently protected acceptors and donors (Table 3.9).



Scheme 3.26. Acceptor synthesis in solution. Reagents and conditions: a) donor **81** or **82**, TMSOTf, -20 ºC to rt; b) hydrazine acetate, CH_2Cl_2/MeOH (4/1).

Initially, galactosamine acceptors bearing different protecting groups in OH-4 were prepared. The model linker **84** was glycosylated with trichloroacetimidates **81**, **82** under TMSOTf catalysis to afford compounds **172**, **173** both in good yields 87% (Bz) and 85% (Ac), respectively (Scheme 3.26). After selective removal of 3-*O*-levulinic ester group with hydrazine acetate, acceptors **174** and **175** were ready for coupling trials with trichloroacetimidates **81** and **82**. Under the previously explored conditions on the solid phase disaccharide **176** was formed again as a mixture of anomers (α/β: 9/1, see Table 3.9). To avoid the formation of the β-isomer, different activator systems (entry 1, 2, 3) were explored and different donors (entry 4) were tried without success (Table 3.9). In all cases, the same ratio of disaccharide isomers was observed. Only when the galactosamine **177** with a less bulky 4-*O*-acetate group was employed as acceptor the compound **175** was obtained in 89% yield and with complete α-selectivity.

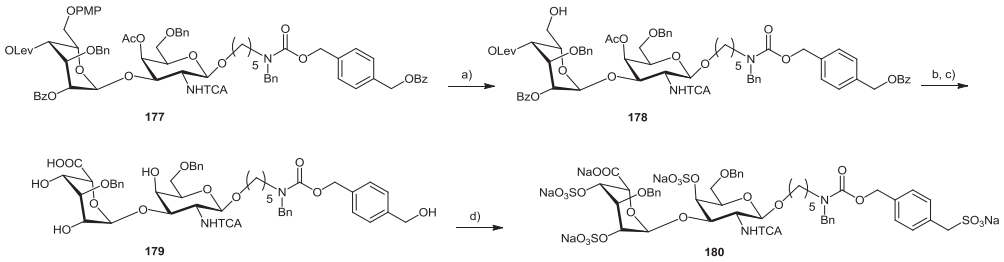
Table 3.9. Evaluation of different donors and activators in DS disaccharide synthesis.



Entry	Acceptor	Temperature	Activator	Yield (%)[a]	α/β ratio
1	**174**	-20 °C to r.t	TMSOTf (15%)	72	9/1
2	**174**	0 °C to r.t	$Cu(OTf)_2$	68	9/1
3	**174**	0 °C to r.t	$Yb(OTf)_3$	64	9/1
4	**174**	-20 °C to r.t	NIS, TMSOTf (10%)	70	9/1
5	**175**	-20 °C to r.t	TMSOTf (15%)	80	Only α

[a] conversion was determined by UPLC-MS analysis after cleavage from the resin.

After assembly of the disaccharide **177**, deprotection steps were tested early on to evaluate the overall strategy for the synthesis of sulfated oligosaccharides (Scheme 3.27). Therefore, **177** was treated with cerium ammonium nitrate (CAN) to selectively remove the *p*-methoxy phenyl group affording compound **178** in 82% of yield. Subsequently, the primary OH-6 hydroxyl group was oxidized to carboxylic acid with TEMPO/BAIB followed by the hydrolysis of all acyl groups with sodium methoxide obtaining disaccharide **179** in 70% over 2 steps. The pyridine sulfur trioxide complex in pyridine at 60 ºC in the microwave was used for the sulfation of free hydroxyl groups. The reaction crude was quenched by adding triethylamine and was purified by size exclusion chromatography. In order to form the sodium salt the crude was passed through an ion exchange resin. ^{1}H NMR analysis showed significant downfield shifts of H4 of the galactopyranosyl unit and H-4 and H-2 of idopyranosyl moiety: H-4 at 4.94 ppm (before at 4.04 ppm, 0.6 ppm at downfield than in the previous), H-4´ at 4.77 ppm (before at 4.14 ppm) and H-2´ at 4.44 ppm (before at 3.93 ppm).



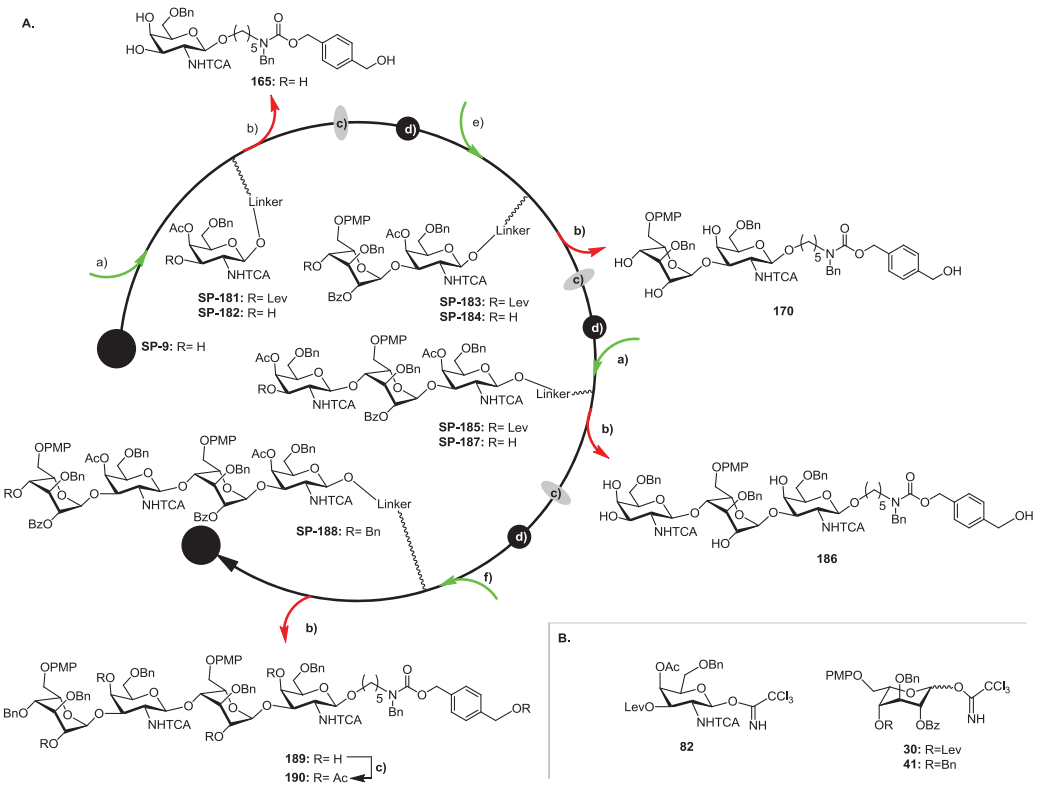
Scheme 3.27. Deprotection of disaccharide **177**. a) cerium ammonium nitrate, acetonitrile/water (4/1), 0 ºC, 82%; b) TEMPO/BAIB, acetonitrile/water (1/1), c) NaOMe/MeOH, 0 ºC to rt, 70%; d) Py·SO3, pyridine, 60 ºC, 73%.

Catalytic hydrogenation of **180** was anticipated to provide the natural product in good yields applying procedures previously reported for very similar intermediates.[56]

3.5.3 Solid phase synthesis of dermatan sulfate tetrasaccharide

Based on the previous result obtained for the synthesis of disaccharide **177** in solution trichloroacetimidate **82** was use in the solid phase synthesis of DS tetrasaccharide precursor. First, linker modified resin **SP-9** was glycosylated with imidate **82** to afford **SP-181** in near-quantitative yield as demonstrated by the analysis of the methoxide cleavage product **165** (Scheme 3.28). Hydrazinolysis afforded the acceptor **SP-182** which was reacted with trichloroacetimidare **30** to give **SP-183**, with excellent conversion (99%) after two cycles and complete α-selectivity. After capping reaction and delevulination, acceptor **SP-184** was glycosylated with trichloroacetimidate **22** affording **SP-185** in high yield (97%) after two glycosylation cycles. Subsequently, delevulination with hydrazine acetate was performed to afford resin **SP-187**. Glycosylation of **SP-187** was performed using the capping glycosyl donor **43** and **SP-188** was obtained in 90% of yield.

To facilitate the purification process crude tetrasaccharide **189** was acetylated with Ac_2O in pyridine after cleavage from the resin providing **190** in 72% overall yield over 8 steps with an average yield of 95% per step (Scheme 3.28). Measurement of the four C,H heteronuclear anomeric coupling constants confirmed a 1,2-cis configuration for idose glycosidic linkages (2xIdose $J_{C,H}$ = 170 Hz) and 1,2-trans configuration for galactosamine ($J_{C,H}$ = 163.0 Hz and 164.0 Hz, Figure 3.5).



Scheme 3.28. A) Synthesis of tetrasaccharide of DS on solid-phase. Reagents and conditions: a) **82**, 20% TMSOTf, -20 °C to r.t; b) NaOMe, MeOH; c) Ac_2O, pyridine, DMAP (cat.), CH_2Cl_2; d) hydrazine acetate, CH_2Cl_2/MeOH ;e) **30**, 20% TMSOTf, -20 °C to r.t; f); **41**, 20% TMSOTf, -20 °C to r.t; for conversions see Table 3.10. B) Monosaccharide building blocks of galactosamine (**82)** and of idose (**30** and **41**).

Table 3.10. UPLC-MS conversion for DS tetrasaccharide.

Glycosylation	Donor	Product	UPLC-MS conversion (%)	
			Cycle 1	Cycle 2
1	**82**	**165**	98	-
2	**30**	**170**	n.a	99
3	**82**	**186**	n.a	97
4	**41**	**189**	n.a	90

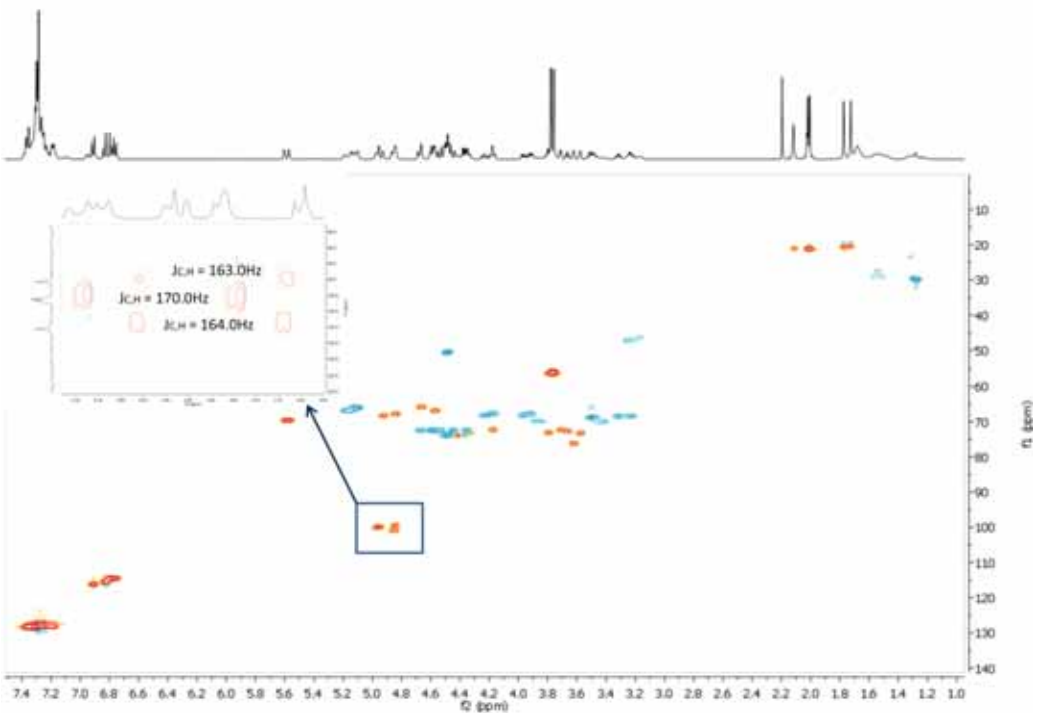
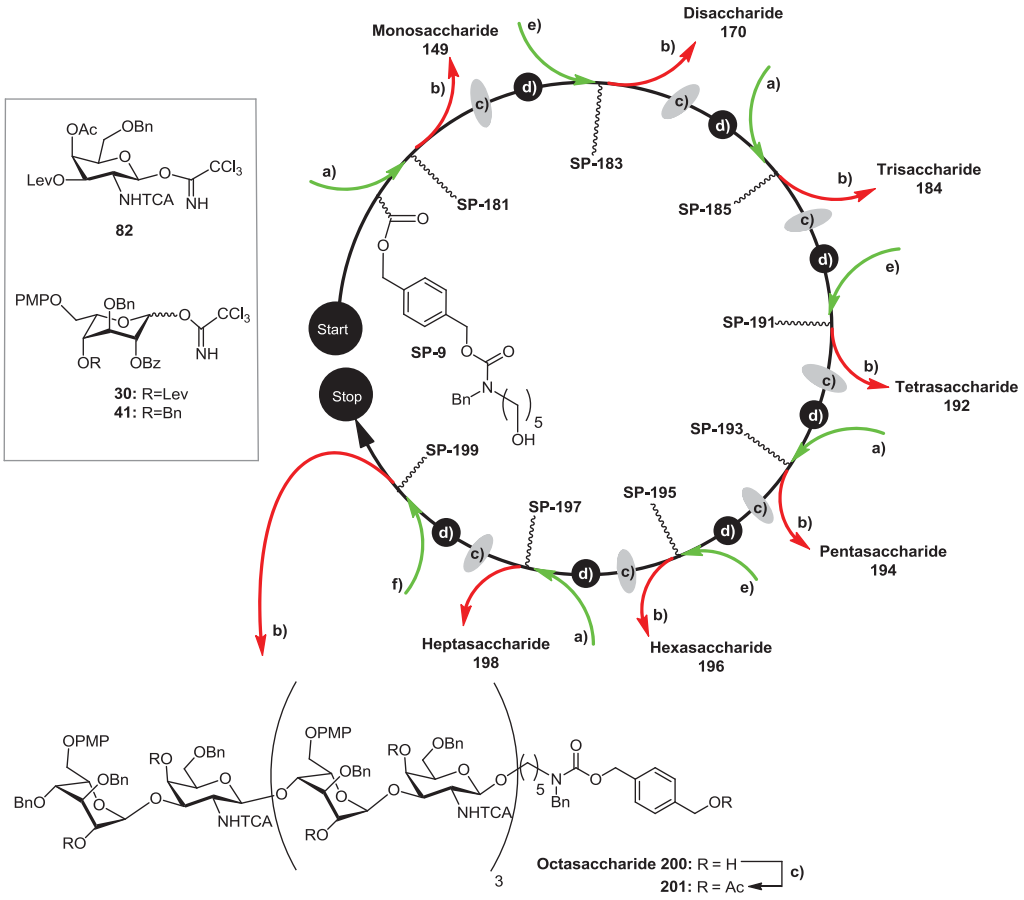


Figure 3.4. HSQC NMR spectra of heparin tetrasaccharide precursor **156**. $J_{C,H}$ coupling values confirmed the desired stereoselectivity.

3.5.4 Solid phase synthesis of a dermatan sulfate octasaccharide

Continuing with these studies we now show the viability of a sequential assembly of monosaccharide building blocks up to octasaccharide size on a solid support with good to excellent coupling yields. For the synthesis of DS precursor **200**, Ido and GalN donors **82** and **30** were employed in an alternating fashion and trichloroacetimidate **41** was used as a final capping block (Scheme 3.29).



Scheme 3.29. Solid-phase assembly of a dermatan sulfate octasaccharide precursor **201**. a) donor **82** (5eq.), TMSOTf (0.1eq.), -20 °C to r.t; b) NaOMe (cat.), MeOH, MW; c) Ac_2O, pyridine, DMAP; d) hydrazine acetate, CH_2Cl_2/MeOH (4/1); e) donor **30** (5eq.), TMSOTf (0.1eq.), -20 °C to r.t; f) capping donor **41** (5eq.), TMSOTf (0.1eq.), -20 °C.

Coupling yields were determined by UPLC-MS analysis of resin aliquots after individual coupling cycles (Table 3.11). Successive cycles of glycosylation, capping with acetic anhydride in pyridine, removal of the levulinic ester protection and alternated coupling with trichloroacetimidates **82** and **30** finally afforded resin bound heptasaccharide (**SP-197)**. After delevulination, the acceptor was coupled with the capping trichloroacetimidate **41** in a single cycle and 97% of conversion into octasaccharide (**SP-199)** as estimated by UPLC-MS.

Table 3.11. UPLC-MS conversion of DS octasaccharide.

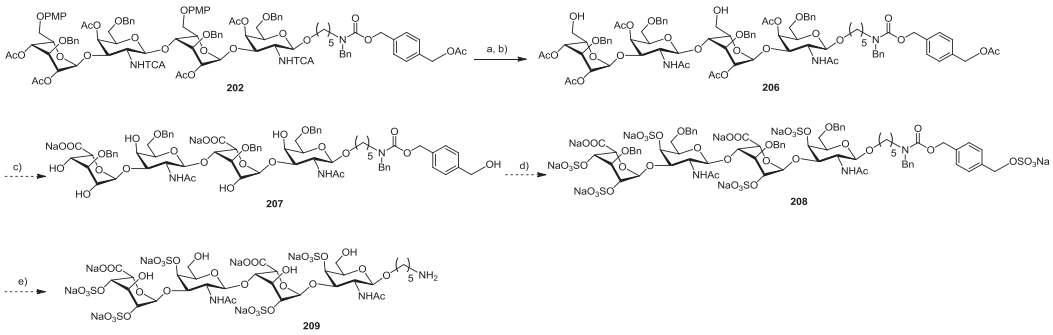
Glycosylation	Donor	Product	UPLC-MS conversion (%)	
			Cycle 1	Cycle 2
1	**82**	**149**	98	-
2	**30**	**170**	n.a	99
3	**82**	**184**	n.a	97
4	**30**	**192**	86	98
5	**82**	**194**	n.a	100
6	**30**	**196**	n.a	91
7	**82**	**198**	94	100
8	**41**	**200**	n.a	97

Preparative scale cleavage of resin with sodium methoxide followed by acetylation with acetic anhydride and purification by HPLC produced a mixture of the desired octasaccharide **201** and an octasaccharide presenting a mass increase of 90Da (**201+Bn**). Careful separation by HPLC afforded **201** in 9.4% overall yield and 5.6% of a presumably *N*-benzylated octasaccharide. *N*-benzylation of trichloroacetamides, which might be explained by a benzyl transfer under strong Lewis acid conditions, has been reported previously.[57] The additional benzyl group should be easily removed in the envisaged final removal of benzyl groups by

hydrogenation.[57] Compound **201** should be readily transformed to the final deprotected dermatan sulfate as had been shown for a similar derivative.[58, 10]

3.5.5 Deprotection schemes for DS tetra and octasaccharides.

The tetrasaccharide compound **202** will be subjected to a set of orthogonal deprotection conditions followed by sulfation at the designed positions (Scheme 3.30). Reduction of the trichloroacetamide groups to acetamide in compound **202** by treatment with a mixture of tributyltinhydride and the radical starter azobisisobutyronitrile (AIBN) and subsequent treatment of the with cerium ammonium nitrate in a mixture of acetonitrile/water (4/1) to remove the *p*-methoxyphenyl groups afforded **206**.

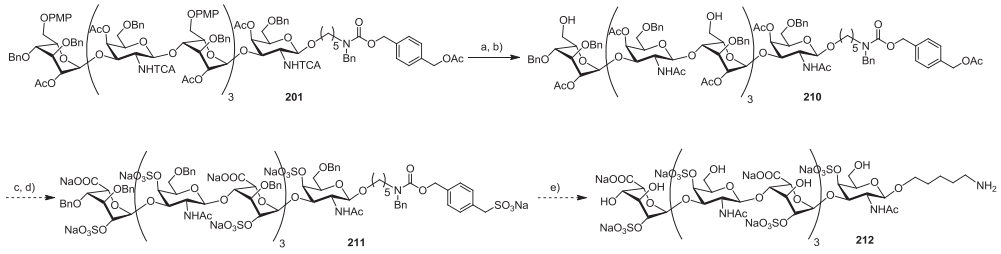


Scheme 3.30. Deprotection strategy for tetrasaccharide compound **202**. Reagent and conditions: a) tributyltinhydride, AIBN, toluene b) cerium ammonium nitrate, 54% over two steps; c) TEMPO, BAIB, acetonitrile/water (1/1), c) NaOMe/MeOH, 0 ºC to rt; d) Py·SO3, pyridine, DMF, 60 ºC; e) H_2, Pd-C.

This compound was then subjected to TEMPO oxidation in the presence of BAIB. UPLC-MS analysis of the reaction crude confirmed the presence of a major product with a +16 Da deviation over the expected mass for the target compound suggesting overoxidation

Next, treatment of the free hydroxyl groups with SO_3.Pyr complex in DMF would lead the sulphated intermediate. Finally, the benzyl and the benzyloxycarbonyl groups would be removed by catalytic hydrogenation.[58] Unfortunately, due to lack of sufficient material the final sequence to the natural products could not be completed.

In the same way, the octasaccharide **201** was treated with tributyltinhydride and azobisisobutyronitrile (AIBN) until no intermediates were detected by UPLC-MS. Next, this compound was treated with cerium ammonium nitrate in a mixture of acetonitrile/water (4/1) to remove the *p*-methoxyphenyl groups and purified by preparative HPLC to obtain 6 mg of compound **210**. The further oxidation and sulfation of the synthesized precursor compounds is anticipated to provide the natural products in good yields applying procedures reported for very similar intermediates.



Scheme 3.31. Deprotection strategy for octasaccharide compound **201**. Reagent and conditions: a) tributyltinhydride, AIBN, toluene b) cerium ammonium nitrate, acetonitrile/water (4/1); c) TEMPO, BAIB, acetonitrile/water (1/1), c) NaOMe/MeOH, 0 ºC to rt; d) Py·SO3, pyridine, DMF, 60 ºC; e) H_2, Pd-C.

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4. CONCLUSIONS

4. CONCLUSIONS

We have designed a novel ester type linker incorporating a carbamate protected C5-spacer for later ligand immobilization that proved stable through the synthesis and was easily cleaved from the resin by a choice of two complementary methods. We have found that the lability of the idose residue under NIS/TfOH activation makes thioglycoside donors less attractive for the solid phase synthesis of the heparin sulfate oligosaccharides precursors. On the other hand, idose and 2-azido-glucose trichloroacetimidates activated with lower amounts of promoter, did not lead to any cleavage of glycosidic bonds.

We have performed a systematic evaluation of IdoA and Ido glycosyl donors, with variation in the protecting group pattern and the leaving group, for the sequential solution- and solid-phase synthesis of glycosaminoglycans precursors. In this evaluation, we found that the sterically demanding TBDPS and other bulky substituents compromised yields in glycosylation when idose was used as acceptor.

For the first time, a series of idorunic acid *n*-pentenyl orthoester have been prepared and evaluated in the solution and solid-phase synthesis of trisaccharide heparin sulfate precursors including all major structural features of larger HS chains. We have found that the use of IdoA donors for the solid phase heparin sulfate synthesis was not compatible with the strong basic conditions required to cleave our ester linker, and considerable elimination of IdoA ester residues to the alkene was observed. A change to base-labile linkers cleaved under less harsh conditions or to orthogonal photolinkers in the future could permit the direct use of IdoA donors.

Our results also demonstrate that the idose donors with electron-donating groups and reduced steric demand at C-6 outperform iduronic acid as glycosyl donors and acceptors in the glycosylation with azidoglucose derivatives even though additional off-bead oxidation is required to arrive at the natural products. In

particular, the solution- and solid-phase involving the 6-O-PMP protected idose derivatives showed high yields for all conversions studied and therefore was selected for the synthesis of larger heparin sulfate and dermatan sulfate fragments.

An efficient strategy for the synthesis of dermatan sulfate disaccharide has been discussed above considering the results obtained in the initial attempts of DS on solid synthesis. A set of orthogonally protected idosyl donors and galactosamine acceptors were coupled to obtain the desired disaccharide in excellent yield and in a stereoselective manner. The substitution of the 4-*O*-benzoate ester group for a a less bulky 4-*O*-acetate ester of galactosamine effective and valuable to achieve full stereoselectivity in the glycosylation with idose donors both in solution and on the solid phase.

We have developed an effective strategy for the SP high yielding and high stereoselective assembly of simple monosaccharide building blocks into advanced oligosaccharide precursors up to the size of an octasaccharide for two important classes of GAGs. This is a significant progress in the ongoing development of a routine and automatable solid-phase synthesis of GAGs affording hexasaccharide and octasaccharide precursors in 2-3 weeks time, which in solution phase would have required several months. In addition, this strategy introduces a higher degree of flexibility for the generation of structurally diverse HS/ DS libraries and avoids the multi-step processing of elaborated disaccharide structures.

These results complement and considerably extend the last published automated syntheses of chondroitin sulfate (CS) hexasaccharide and DS tetrasaccharide precursors which utilize phosphate based glycosyl donors in the key glycosylation steps. The use of the alternative highly reactive trichloroacetimidates as glycosyl donors in the syntheses here reported has allowed the preparation of tetra- and octa-saccharides in the dermatan sulfate series in a highly efficient manner. We envisage that these trichloroacetimidate glycosyl donors can be easily introduced in an automated process for the assembly of GAG precursors.

The further deprotection and sulfation of the synthesized GAG precursors is anticipated to provide the natural products in good yields applying procedures previously reported for very similar intermediates. The deprotection of PMP followed by oxidation using TEMPO and bis(acetoxy)iodobenzene was successfully performed in a disaccharide model. The final deprotection of hexa and octasaccharide was initialized; however it could not be concluded due to constraints in time and amount of synthesized material.

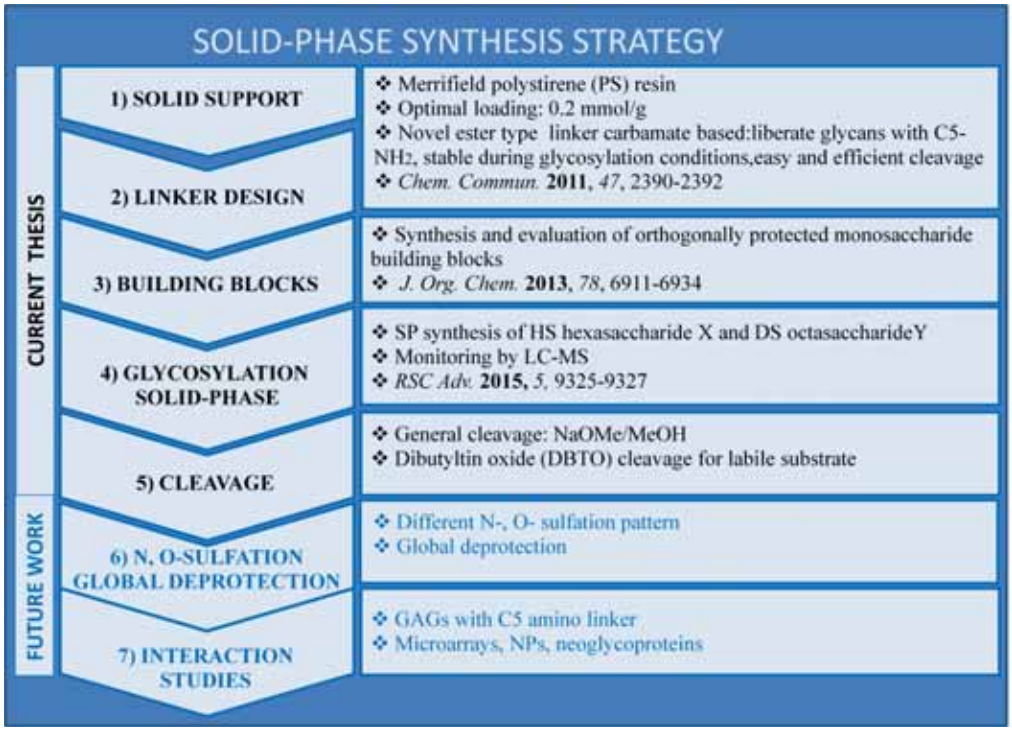


Figure 4.1. Thesis results and future work related to solid-phase synthesis strategy developed.

5. EXPERIMENTAL PART

5.1 GENERAL METHODS. All anhydrous reactions were performed in flame-dried or oven-dried glassware under a positive pressure of dry Argon. Air- or moisture-sensitive reagents and anhydrous solvents were transferred with dried syringes or cannulae. Purification of compounds was performed on a Biotage SP4 automated flash chromatography system, Biotage AB, Uppsala, Sweden or by conventional flash chromatography using Merck 62Å 230-400 mesh silica gel. Size exclusion chromatography was performed on Sephadex® LH-20. All solution-phase reactions were monitored using analytical thin-layer chromatography (TLC) with 0.2 mm pre-coated silica gel 60 F254 aluminium plates. Components were visualized by illumination with a short-wavelength (254 nm) ultraviolet light and/or by charring with vanillin, ceric ammonium molybdate, potassium permanganate, or phosphomolybdate staining solution. All solvents used for anhydrous reactions were distilled. Tetrahydrofuran (THF) was distilled from sodium/benzophenone under Argon. Dichloromethane and acetonitrile were distilled from calcium hydride. Methanol was distilled from calcium sulfate. *N*,*N*-dimethylformamide (DMF) was stored over activated 4 Å molecular sieves under Argon. Solid-phase reactions were performed in a normal Schlenck tube under an Argon atmosphere.

^{1}HNMR, DQF-COSY, HSQC and ^{13}C NMR spectra were recorded at ambient temperature on a Bruker 500 MHz spectrometer and chemical shifts (δ) are given in parts per million (ppm) relative to the residual signal of the solvent used. Deuterated chloroform ($CDCl_3$), methanol (CD_3OD), or water (D_2O) was used as the solvent for NMR experiments.Splitting patterns are designated as follows: s, singlet; ps, pseudo-singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet; b, broad. Low –resolution mass spectrometry (LRMS) was performed on a electrospray ionization time of flight mass spectrometer equipped with an electrospray source with a pump rate of 5μL/min using electrospray ionization (ESI) or a matrix-assisted desorption ionization time of flight (MALDI-TOF) mass spectrometer operated in the reflectron/positive ion mode with DHB in MeOH as MALDI matrix. High-resolution mass spectrometry (HRMS) data were acquired on a time of flight mass spectrometer. Samples in CH_2Cl_2/ MeOH were mixed with ES tuning mix for internal calibration and infused into the mass spectrometer at μL/min. Microwave irradiation was performed on Biotage Initiator monomode oven, Biotage AB, Uppsala, Sweden. UPLC-MS analysis were performed using Acquity UPLC coupled to a ESI-TOF LCT Premier XE (Waters, Milford, MA,

US), column Acquity BEH 100x2.1 mm 1.7 um particle size (Waters); flow rate 300 uL/min; PDA wavelength range: 195 - 500 nm; eluents (A) Ammonium formate 10mM / (B) MeOH. grad. B (10 min: Isocratic 99% A – 0.5 min. / 99-25% A – 2.5 min / 25-1% A – 3 min / isocratic 1% A – 4 min) or grad. D (20 min: Isocratic 99% A – 0.5 min. / 99-25% A – 5.5 min / 25-75% A – 10.5 min / isocratic 1% A – 4 min).

5.2 COMPOUND NUMBERING

Resin-bound compounds carry the prefix **SP-** (e.g. **SP-1**)

5.3 GENERAL PROCEDURES FOR SOLUTION SYNTHESIS

Procedure A (Levulination). To a solution of 4-hydroxy acceptor (1 eq.) in dry CH_2Cl_2 (~10 mL/mmol) a catalytic amount of DMAP was added at room temperature, followed by addition of levulinic acid (1.5-5 eq.) and EDC·HCl (1.5-5 eq.). After five hours, TLC control (hexane/ EtOAc 2/1 or 3/1) indicated full conversion of the starting material. The reaction mixture was diluted with CH_2Cl_2 (~ 150 mL) and subsequently washed with saturated $NaHCO_3$aq solution (2 x ~200 mL), 1 MHCl (~200 mL), water (~200 mL) and brine (~200 mL). After drying over anhydrous $MgSO_4$ and concentration the crude was purified by column chromatography on silica using a hexane/EtOAc gradient (1:0 to 1:1).

Procedure B (Glycosylation with thiophenyl idose derivatives and *n*-pentenyl orthoester derivatives). The linker acceptor and thiophenyl donor (1.2 – 1.5 eq.) were dissolved in dry CH_2Cl_2 (2 mL) under argon and then cooled to the desired temperature. *N*-iodosuccinimide and catalytic amount of trimethylsilyl triflate (TMSOTf) or triflic acid (TfOH) were added and the reaction mixture was allowed to warm to room temperature. After 1.5 – 3 h, the crude reaction mixture was quenched with saturated $NaHCO_3$ aq solution and solid $Na_2S_2O_3$. The mixture was filtered and washed with saturated $NaHCO_3$ aq solution, water and brine. The crude reaction mixture was purified by column chromatography.

Procedure C (Glycosylation with azido glucose trichloroacetimidate donor, disaccharide synthesis). The idose acceptor and azido glucose donor were dissolved in dry CH_2Cl_2 (15 mL/ mmol) under argon and then cooled to -20 °C (0 °C for iduronic acid). After addition of trimethylsilyl triflate (TMSOTf) the reaction mixture was

allowed to warm slowly to room temperature. After 2 h, the reaction mixture was quenched with triethylamine. The crude reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ aq solution, water and brine. The concentrated crude reaction was purified by column chromatography.

5.4 GENERAL PROCEDURES FOR SOLID PHASE SYNTHESIS

Procedure D (preparative cleavage from the resin). Preparative cleavage of the product from the resin was performed under alkaline conditions according toRoussel procedure. [1]

To a dry Schlenk flask under argon, dry resin loaded compound and NaOMe in MeOH (pH=8)/CH_2Cl_2 (1:4) solution were added. The resin was shaken at room temperature for 2 hours, filtered and the filtrate was neutralized with Amberlite® IR-120(H) ion exchange resin. The cleavage procedure was repeated 2 times and the filtrates were combined and concentrated.

Procedure E (analytical cleavage from the resin). To a 0.2-0.5 mL Biotage microwave reaction flask equipped with magnetic stir bar, After swelling the resin with 250 µL anhydrous CH_2Cl_2, 50 µL of a 0.2 M sodium methoxide solution was added. The mixture was irradiated in microwave oven for 5 min at 55 °C with pre-stirring of 30 sec. After cooling to room temperature the supernatant was transferred to an Eppendorf vial and the solution was concentrated to dryness by air stream. The residue was redissolved in 100 µL methanol (HPLC grade). A 1: 10 dilution of this solution was used for UPLC-MS analysis or MALDI-TOF MS.

Procedure F (Bu_2SnO mediated cleavage from the resin). To a 0.2-0.5 mL Biotage microwave reaction flask equipped with magnetic stir bar, 3-5mg of resin loaded compound was added. Bu_2SnO (1 eq) solution in MeOH was added and the sealed reaction vessel was heated in microwave at 120ºC for 10 minutes. After swelling of resin with 200 µL anhydrous dichloromethane 100 µL MeOH was added. The mixture was irradiated in microwave oven for 10 min at 120 °C with pre-stirring of 30 sec. After cooling to room temperature the supernatant was transferred to an Eppendorf vial and the solution was concentrated to dryness by air stream. The residue was resolved in 100 µL methanol (HPLC grade). A 1: 10 dilution of this solution was used for UPLC-MS analysis or MALDI-TOF MS.

Procedure G (Solid phase glycosylation). Solid phase glycosylations were performed in either a Schlenk tube fitted with a cooling jacket or a normal Schlenk tube under argon atmosphere. Unless otherwise noted, the resin was swollen with the glycosyl donor (thioglycoside or trichloroacetimidate) in dry CH_2Cl_2 (750 µL/ 100 mg resin). The reaction mixture was shaken for 10 minutes on a vortex or an orbital shaker. Then, the Schlenk tube with cooling jacket was connected to a cryostat and cooled to the specified temperature. The normal Schlenk tube was cooled by immersion in a dewar containing a cooling bath at the specified temperature. After additional shaking for 10 minutes the activator (NIS, TMSOTf/ TfOH for thioglycosides or TMSOTf for trichloroacetimidates) was added. The mixture was shaken for 10 minutes at the specified temperature. Then, the mixture was allowed to warm to room temperature and was shaking for 1 – 1.5 hours. The resin was washed with THF (5 x 3 mL/ 100 mg resin), CH_2Cl_2 (5 x 3 mL/ 100 mg resin) and dry diethyl ether (2 x 3 mL/ 100 mg resin) and dried in high vacuum. The THF washings were collected for possible recovery of the donor.

Procedure H (Solid phase capping and delevulination): Solid phase synthesis was performed either in a Schlenk tube fitted with a cooling jacket or a normal Schlenk tube under argon atmosphere. The resin was swollen in dry CH_2Cl_2 (1 mL / 100 mg resin) for 10 minutes, followed by addition of pyridine (300 µL / 100 mg resin), acetic anhydride (300 µL / 100 mg resin) and a catalytic amount of DMAP. After 5 h at room temperature resin was washed with CH_2Cl_2 (5 x 3 mL/ 100 mg resin), MeOH (5 x 3 mL/ 100 mg resin) and dry diethyl ether (2 x 3 mL/ 100 mg resin) and dried in high vacuum. The resin was used without further characterization for the delevulination: the resin was swollen in dry CH_2Cl_2 (1 mL / 100 mg resin) for 10 minutes, followed by addition hydrazine acetate (5 eq. in 200 µL methanol). After 5 h at room temperature the resin was washed with CH_2Cl_2 (5 x 3 mL/ 100 mg resin), methanol (5 x 3 mL/ 100 mg resin) and dry diethyl ether (2 x 3 mL/ 100 mg resin) and dried in high vacuum.

5.5 LINKER SYNTHESIS AND GLYCOSYLATION TRIALS ON SOLID SUPPORT

***N*-Benzyl-5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentan-1-amine (2):** To a solution of 5-(benzylamino)pentan-1-ol (**1**) (5.00 g, 25.9 mmol) in dry DMF (25 mL) at 0°C, imidazole (3.52 g, 51.7 mmol) and dimethylthexylsilyl chloride (6.09 mL, 31.0 mmol) were added respectively. The reaction mixture was stirred overnight, then diluted with ether and washed with a saturated solution of ammonium chloride and water. The organic layer was then dried over $MgSO_4$ and concentrated. Flash column chromatography (10% EtOAc/Toluene) afforded compound **2** as a clear oil (6.6 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30-7.15 (m, 5H), 3.73 (s, 2H), 3.52 (t, *J*=6.5 Hz, 2H), 2.58 (t, *J*=6.8 Hz, 2H), 1.62-1.51 (m, 1H), 1.51-1.41 (m, 4H), 1.36-1.24 (m, 2H), 0.83 (s, 3H), 0.82 (s, 3H), 0.78 (s, 6H), 0.02 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.58, 128.35, 128.08, 126.83, 62.79, 54.08, 49.46, 34.18, 32.69, 29.88, 25.12, 23.62, 20.36, 18.48, -3.39; HRMS (ESI): Calcd for $C_{20}H_{37}NOSi$ $[M+H]^+$ 336.2723, found 336.2708.

4-(Hydroxymethyl)benzyl 2,2,2-trichloroethyl carbonate (4): To 1,4-benzene-dimethanol (1.93 g, 14.0 mmol) in dry CH_3CN (50 mL) and pyridine (2.25 mL, 28.0 mmol), a solution of 2,2,2-trichloroethoxycarbonyl-chloride (1.88 mL, 14.0 mmol) in CH_3CN (125 mL) was added dropwise over a period of 3 hours. After 4 hours the reaction was diluted with EtOAc and washed with saturated solution of $CuSO_4$ and water. The organic layer was then dried over $MgSO_4$ and concentrated. Flash column chromatography (30% EtOAc/toluene) afforded **4** as a clear oil (2.23 g, 53%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46-7.33 (m, 4H), 5.24 (s, 2H), 4.77 (s, 2H), 4.71 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.96, 141.63, 133.88, 128.79, 127.18, 94.35, 76.88, 70.44, 64.91 ppm; HRMS (ESI): Calcd for $C_{11}H_{11}Cl_3O_4$ $[M+Na]^+$ 334.9621, found 334.9604.

4-(((2,2,2-Trichloroethoxy)carbonyloxy)methyl)benzyl *N*-benzyl *N*-(5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl) carbamate (6): To a solution of 4-(hydroxymethyl)benzyl 2,2,2-trichloroethyl carbonate (**4**) (4.57 g, 14.6 mmol) in CH_2Cl_2 (100 mL) at 0°C, pyridine (2.35 mL, 29.2 mmol) and 4-nitrophenyl-

chloroformate (3.52 g, 17.5 mmol) were added. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The solution was then concentrated and the resulting crystals were washed several times with hexanes to remove pyridine. Next, the dried solid was dissolved in DMF and *N*-benzyl-5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentan-1-amine (**2**) (5.87 g, 17.5 mmol) and DIPEA (3.31 mL, 19.0 mmol) were added at 0°C. The reaction mixture was stirred overnight, then diluted with diethyl ether and washed with saturated solution of NH_4Cl. The organic extract was dried over $MgSO_4$ and concentrated. Flash column chromatography (gradient of 10 to 50% EtOAc/hexanes) gave the title product in 79% yield as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50-7.10 (m, 9H), 5.31-5.12 (m, 4H), 4.79 (s, 2H), 4.55-4.47 (m, 2H), 3.61-3.50 (m, 2H), 3.34-3.16 (m, 2H), 1.68-1.41 (m, 5H), 1.38-1.22 (m, 2H), 0.89 (s, 3H), 0.88 (s, 3H), 0.85 (s, 6H), 0.08 (s, 6H,); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.59, 156.02, 153.90, 137.87, 137.59, 134.09, 128.59, 128.51, 127.99, 127.68, 127.24, 127.06, 94.32, 76.84, 70.33, 66.60, 62.63, 50.48, 50.19, 47.33, 46.25, 34.16, 32.42, 27.90, 27.49, 25.09, 23.13, 20.34, 18.48, -3.41; HRMS (ESI): Calcd for $C_{32}H_{46}Cl_3NO_6Si$ $[M+Na]^+$ 696,2057, found 696,1990.

4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl) carbamate (7): To a solution of 4-(((2,2,2trichloroethoxy)carbonyloxy)methyl)benzyl benzyl(5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl)carbamate (**6**) (6.16 g, 9.12 mmol) in 150 mL of AcOH/THF (1:10) at 0ºC was added freshly activated Zn dust (1.79 g, 27.4 mmol). The reaction mixture was stirred for 4 hours, diluted with EtOAc and the residual zinc was filtered off. The filtrate was concentrated and after flash column chromatography (hexane/EtOAc, 9/1 to 75/25), compound **6** was obtained as an oil (>95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45-7.10 (m, 9H), 5.24-5.07 (m, 2H), 4.75-4.63 (m, 2H), 4.56-4.41 (m, 2H), 3.61-3.44 (m, 2H), 3.33-3.10 (m, 2H), 1.71 (s, 1H), 1.66-1.38 (m, 5H), 1.36-1.15 (m, 2H,), 0.88 (s, 3H), 0.86 (s, 3H), 0.83 (s, 6H), 0.06 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.93, 128.50, 128.06, 127.79, 127.23, 127.05, 66.85, 65.06, 62.60, 50.18, 47.21, 46.24, 34.17, 32.43, 27.91, 27.52, 25.11, 23.12, 20.34, 18.48, -3.41; HRMS (ESI): Calcd for $C_{29}H_{45}NO_4Si$ $[M+Na]^+$ 522.3016, found 522.2964.

Resin bound 4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl) carbamate (SP-8): To a dry Schlenk flask 500mg of carboxypolystyrene resin (2.19 mmol/g capacity or 4.19 mmol/g) or Tentagel resin (2.19 mmol/g capacity or 4.19 mmol/g) was added under Argon. The resin was swollen with CH_2Cl_2 for 10 minutes then washed 3 times with CH_2Cl_2. To this resin in 23 mL of CH_2Cl_2, **7** (0.028 g, 0.110 mmol) dissolved in 2 mL of CH_2Cl_2, DIC (0.034 g, 0.548 mmol), and DMAP (0.002 g, 0.022 mmol) were added and the reaction mixture shaken overnight. After complete reaction of the linker with the solid support (determined by TLC), the reaction solution was filtered off and the resulting resin was washed with 3 cycles of CH_2Cl_2, MeOH and CH_2Cl_2. Finally, the resin was washed with 3 times THF and dried under vacuum. Capping of the unreacted carboxylate groups was performed according to Roussel and co-workers work.[1]

Resin bound 4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-hydroxypentyl) carbamate (SP-9): Dry resin **SP-8** (0.500 g) was transferred to a Teflon reaction vessel and swollen with THF (5 mL). Equal volume (5 mL) of HF pyridine (Note: this reagent is extremely toxic!) was added and the reaction mixture was shaken for 3 hours. The reaction mixture was filtered off and the resin was washed 3 times with THF, 3 cycles of MeOH then CH_2Cl_2, and 3 times CH_2Cl_2. The resin was then dried under vacuum.

4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-hydroxypentyl) carbamate (10): Loading of the linker on the solid phase support was determined by cleaving the linker from 0.100 g of the resin **SP-9** according to Procedure D. The loading of 0.2 mmol/g or 0.4 mmol/g was confirmed based on the obtained weight of **10**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44-7.08 (m, 9H), 5.22-5.07 (m, 2H), 4.67 (m, 2H), 4.53-4.43 (m, 2H), 3.64-3.38 (m, 2H,), 3.34-3.10 (m, 2H), 2.50-1.90 (m, 2H), 1.75-1.05 (m, 7H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.76, 156.34, 140.94, 140.76, 137.86, 136.15, 128.56, 128.42, 128.11, 127.83, 127.34, 127.26, 127.20, 127.08, 67.01, 64.96, 62.58, 50.52, 50.17, 46.94, 46.12, 32.21, 27.83, 27.38, 22.83; HRMS (ESI): Calcd for $C_{21}H_{27}NO_4Na$ $[M+Na]^+$ 380.1838, found 380.1814.

Resin bound 4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyloxy)pentyl) carbamate (SP-12): To a dry schlenk flask

under argon, resin loaded with the linker (0.10 g, 0.2 mmol/g), donor **11** (0.146 g, 0.22 mmol) and CH_2Cl_2 (1.5 mL) were added respectively. The resin was shaken for 15 minutes, and the reaction mixture was cooled to 0 ºC. TMSOTf (5 µL, 0.022 mmol) was added and the reaction mixture was allowed to warm to room temperature. After 1 hour, the reaction solution was filtered off, washed with 3 times with CH_2Cl_2 and the glycosylation cycle was repeated. The resin was then washed with 3 cycles of CH_2Cl_2 then MeOH, 3 times with CH_2Cl_2 and dried under vacuum.

4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyloxy)pentyl) carbamate (13): Compound **13** (10 mg, 79% yield) was obtained by preparative cleavage from 80 mg of resin **SP-12** according to Procedure D in 3 cycles of 2 hours. ^{1}H NMR (500 MHz, $CDCl_3$, 323 K) δ 7.38-7.00 (m, 24H), 5.14-5.02 (m, 2H), 4.80-4.65 (m, 2H), 4.64-4.50 (m, 5H), 4.49-4.34 (m, 4H), 3.95-3.84 (m, 1H), 3.82-3.70 (m, 2H), 3.70-3.57 (m, 3H), 3.57-3.42 (m, 1H), 3.35-3.02 (m, 3H), 2.75-1.65 (br s, 2H), 1.60-1.30 (m, 4H), 1.30-1.00 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.52, 141.00, 138.48, 138.41, 138.11, 137.98, 136.19, 128.51, 128.45, 128.28, 128.09, 127.90, 127.77, 127.58, 127.49, 127.28, 126.98, 99.27, 80.35, 75.06, 74.59, 73.50, 71.99, 71.30, 69.31, 68.54, 67.42, 66.96, 64.85, 50.54, 46.39, 28.99, 27.66, 23.32; HRMS (ESI): Calcd for $C_{48}H_{55}NO_9Na$ $[M+Na]^+$ 812.3774, found 812.3718.

5-Aminopentyl α-D-mannopyranoside (14): To a solution of **13** (40 mg, 0.05 mmol) in MeOH (2 mL) with 10% formic acid, palladium black (40 mg) was added and the reaction mixture was stirred overnight under H_2 atmosphere. The reaction mixture was then filtered over Celite, concentrated and the resulting residue was purified by Sephadex® LH-20 column chromatography to afford **14** in quantitative yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (br s, 1H), 4.74 (s, 1H), 3.82 (dd, *J*=4.5, 8.9 Hz, 1H), 3.80-3.63 (m, 4H), 3.60 (dd, *J*=9.5, 9.5 Hz, 1H), 3.55-3.42 (m, 2H), 2.99-2.88 (m, 2H), 1.76-1.58 (m, 4H,), 1.56-1.42 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.54, 101.71, 74.84, 72.79, 72.35, 72.31, 68.81, 68.20, 63.11, 40.81, 30.19, 28.50, 24.48; HRMS (ESI): Calcd for $C_{11}H_{23}NO_6$ $[M+Na]^+$ 288.1423, found 288.1424.

5.6 BUILDING BLOCK SYNTHESIS

5.6.1 Synthesis of Idose Building Block

3-*O*-Benzyl-1,2-di-*O*-isopropylidene-α-D-glucofuranose (16): To a cooled (0 ºC) solution of NaH (60% in mineral oil, 1.15 g, 28.8 mmol) in dry DMF (25 mL) was added a solution of compound **15** (5.00 g, 19.2 mmol) in dry DMF (8 mL), the resulting mixture was stirred at 0 ºC for 30 minutes before benzyl bromide (4.6 mL, 38.4 mmol) and TBAI (1.42 g, 3.84 mmol) were added. The reaction mixture was warmed to room temperature and stirred for 17 hours. The reaction was quenched by adding methanol, diluted with EtOAc (100 mL) and washed with water. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (hexane/EtOAc, 9/1 to 4/1) to give 3-*O*-Benzyl-1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose as white foam (6.3 g, 94%). A solution of this benzylated compound (6.3 g, 18 mmol) in AcOH (18 mL) and water (10 mL) was stirrred at 40 ºC overnight. The mixture was diluted with CH_2Cl_2 (200 mL) and neutralized with solid potassium carbonate. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases were washed with water and saturated NaCl solution. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The product was obtained as transparent oil and was used in the next step without further purification (5.5 g, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.32 (m, 5H, Ph), 5.95 (d, *J* = 3.7 Hz, 1H, H-1), 4.74 (d, *J* =11.8 Hz, 1H, C*H*$_a$H$_b$Ph), 4.64 (d, *J* =3.8 Hz, 1H, H-2), 4.54 (d, *J*=7.8 Hz, 1H, CH$_a$*H*$_b$Ph), 4.14 – 4.10 (m, 2H, H-3, H-4), 4.05 – 4.00 (m, 1H, H-5), 3.82 (dd, *J* = 3.5, 11.4Hz , 1H, H-6$_a$), 3.70 (dd, *J* = 5.6, 11.4 Hz, 1H, H-6$_b$), 1.49 (s, 3H, CH_3), 1.33 (s,3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.10(qC, Ar), 128.86, 128.35, 128.05(CH, Ar), 101.18 (CH, C-1), 82.03 (CH, C-2), 82.09 (CH, C-4), 78.0 (CH, C-3), 72.10 (*C*HPh), 69.39 (CH, C-5), 64.45 (CH, C-6), 26.77, 26.25 (CH_3, Ac) ppm.

6-*O*-Acetyl-3-*O*-Benzyl-1,2-*O*-isopropylidene-5-*O*-methanesulfonyl-α-D-glucofuranose (17): To a solution of **16** (5.30 g, 17.07 mmol) in pyridine (25.5 mL) was added methanesulfonyl chloride (3.1 mL) at 0 ºC. The resulting mixture was warmed to room temperature and was stirred overnight. The mixture was diluted with EtOAc (200 mL) and was washed with water (3x). The organic phase was dried over

anhydrous $MgSO_4$, filtered and concentrated. The crude product was triturated with hexane. The resulting product was filtered, dried under vacuum and was used in the next step without further purification (6.90 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.30 (m, 5H, Ph), 5.88 (d, *J* = 3.6Hz, 1H, H-1), 5.27 – 5.24 (m, 1H, H-5), 4.69 – 4.66 (m, 4H, H-2, H-6_a, CH_aH_bPh, CH_aH_bPh), 4.62 (dd, *J* = 5.7, 11.9Hz, 1H, H-6_b),4.60 (dd, *J* = 3.1, 7.4Hz , 1H, H-4), 4.13 (d, J = 3.11, 1H, H-3), 3.09 (s, 3H, CH_3), 3.01 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.32 (s, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.91(qC, Ar), 128.58, 128.23 (CH, Ar), 112.50 (qC, $C(CH_3)_2$), 105.31 (C-1), 81.52 (C-2), 81.04 (C-3), 78.08 (C-4), 74.35 (C-5), 72.49 (CH_2Ph), 68.96 (C-6), 39.107, 37.517 (CH_3, Ms), 26.86, 26.22 (CH_3, Ac). To a solution of the crude product (6.81 g, 14.60 mmol) in acetonitrile (135 mL) was added potassium acetate (13.51 g) and 18-crown-6 (0.39 g). The resulting mixture was stirred under reflux 43h. The mixture was diluted with dichlorometane (150 mL) and was washed with water (3x) and with NaCl sat. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. The crude product was crystallized from ethanol (100 mL) to give the title compound **17** as white solid (5.22 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.30 (m, 5H, Ph), 5.89 (d, *J* = 3.6Hz, 1H, H-1), 4.26 – 4.23 (m,1H, H-5), 4.71 – 4.66 (m, 2H, CH_aH_bPh, H-6_a), 4.61 – 4.59(m, 2H, CH_aH_bPh, H-6_b), 4.36(dd, *J*=7.8, 3.1Hz , 1H, H-4), 4.25 – 4.21 (m, 1H, H-6_b), 4.10(d, J = 3.1Hz, 1H, H-3), 3.02 (s, 3H, Ms), 2.09(s, 3H, Ac), 1.49 (s, 3H, CH_3), 1.31 (s,3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.33(qC, Ac), 137.11(qC, Ar), 128.51, 128.04 (CH, Ar), 112.30 (qC, $C(CH_3)_2$), 105.32 (C-1), 81.50 (C-2), 81.11 (C-3), 78.30 (C-4), 75.45 (C-5), 72.30 (CH_2Ph), 63.50 (C-6), 38.96 (CH_3, Ms), 26.87, 26.28 (CH_3), 20.79 (CH_3, Ac) ppm

3-*O*-Benzyl-1,2-epoxy-5,6-di-*O*-isopropylidene-α-D-glucofuranose (18): To a solution of compound **17** (0.696 g, 1.62 mmol) in CH_2Cl_2 (7 mL) was added a solution of ^{t}BuOK (364 mg, 3.24 mmol) in ^{t}BuOH (3.5 mL) at 0ºC. The resulting mixture was stirred at 0ºC for 75 minutes. The reaction was quenched by adding water, diluted with CH_2Cl_2 and was washed with water and NaCl saturated. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The crude product was used in the next step without further purification (452 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.30 (m, 5H, Ph), 6.0 (d, *J* = 3.8Hz, 1H, H-1), 4.75 (d, *J* =12.2Hz, 1H, C$H_a$$H_b$Ph), 4.64 (d, *J* = 3.8Hz, 1H, H-2), 4.52 (d, *J* =12.2Hz, 1H, C$H_a$$H_b$Ph), 3.96 (d, *J* = 3.5Hz, 1H, H-3), 3.81 (dd, J= 3.6, 6.1Hz, 1H, H-4), 3.29-3.26 (m, 1H, H-5), 2.77 (dd,

J = 4.4, 4.7Hz, 1H, H-6_b), 2.54 (dd, *J* = 2.7, 4.9Hz, 1H, H-6_a), 1.45 (s, 3H, CH_3), 1.32 (s,3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) 137.27 (qC, Ar), 128.55, 128.09, 127.71 (CH, Ar), 111.96 (qC, $C(CH_3)_2$), 105.47 (C-1), 82.71 (C-3), 82.43 (C-2), 82.07 (C-4), 71.94 (CH_2Ph), 50.20 (C-5), 43.19(C-6), 26.86, 26.34 (CH_3) ppm.

1,6-Anhydro-3-*O*-benzyl-β-L-idopyranose (19): A solution of **18** (2.30 g, 7.86 mmol) in 1M aq H_2SO_4 (2 mL) and dioxane (2 mL) was heated under MW for 40min. The mixture was neutralized with aq $Ba(OH)_2$, the solid was removed by filtration and was washed with EtOAc. The filtrate was extracted with EtOAc, the organic layers were combined and was washed with water (3x), dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by column Toluene/acetone (9/1 to 7/3) and a white solid was obtained (1.09 g; 55%).The spectroscopic data of the compound were in agreement with the data previously reported in literature. [2]

1,6-Anhydro-2-*O*-Benzoyl-3-*O*-benzyl-β-L-idopyranose (20): To a solution of **19** (0.417 g, 1.65 mmol) in CH_2Cl_2 (5 mL) was added pyridine (0.39 mL, 4.95 mmol) and benzoyl chloride (0.21 mL, 1.82 mmol) at 0ºC under argon. After being stirred overnight, the reaction was quenched with MeOH (0.3mL) and concentrated. The mixture was dissolved in EtOAc, and the solution was washed with aq 1M HCl, saturated aq $NaHCO_3$, water, and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by medium pressure column chromatography (7%- 60%; Hexane/EtOAc) to afford **20** as a white solid (517 mg, 88%). The spectroscopic data of the compound were in agreement with the data previously reported in literature. [3]

Phenyl 2-*O*-Benzoyl-3-*O*-Benzyl-1-thio-α-L-idopyranose (21): To a solution of 1,6-anhydro compound **20** (0.328 g, 0.920 mmol) in dry CH_2Cl_2 (3.5 mL), trimethyl(phenylthio)silane (0.57 mL, 3.0 mmol) and ZnI_2 (0.65 g, 1.8 mmol) were added. The mixture was stirred at room temperature overnight, filtered through a pad of celite. The filtrate was diluted with CH_2Cl_2, after which a solution of HCl in dioxane and water were added. The mixture was stirred at room temperature for 15 min, the organic layer was washed with aq 1 M HCl solution, saturated $NaHCO_3$ aq solution, and water, filtered and concentrated. The crude product was purified by flash

chromatography (hexane/EtOAc, 4/ 1 to 1/ 1) to obtain the compound **21** as a white solid (252 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.03 – 8.02 (m, 2H, aromatic); 7.58 – 7.24 (m, 15H, aromatic), 5.64 (s, 1H, H-1), 5.54 (s, 1H, H-2), 4.93 – 4.90 (d, *J* = 11.8Hz, 1H, CH_2Ph), 4.78 (m, 1H, H-5), 4.67 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 3.97 (dd, *J* = 11.8, 6.3 Hz, 1H, H-6a), 3.93 – 3.82 (m, 3H, H-6b, H-3, H-4), 3.10 – 2.51 (bs, 1H, OH), 2.04 – 1.65 (bs, 1H, OH) ppm; ^{13}C NMR (126 MHz, CDCl3) δ = 165.0, 137.2, 135.6, 133.7, 132.0, 129.7, 129.1, 128.7, 128.5, 128.0, 127.8, 127.7, 127.5, 86.7 (C-1), 74.0, 72.3 (CBn), 69.9 (C-2), 68.4, 68.2, 63.3 (C-6) ppm; HRMS (ESI) m/z calcd for $C_{26}H_{26}O_6S$ [M+Na]+ 489.1348, found 489.1320.

Phenyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-1-thio-α-L-idopyranoside (23): To a solution of thiophenyl glycoside **21** (1.02 g, 2.19 mmol) in dry pyridine (9 mL) a catalytic amount of DMAP was added, followed by addition of TDSCl (2.63 mmol). The reaction mixture was stirred overnight at room temperature. TLC control (hexane/EtOAc, 2/1) indicated the full conversion of the starting material. The mixture was diluted with CH_2Cl_2 (100 mL) and was washed with saturated $CuSO_4$ aq solution (3 x 100 mL), water (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude was purified by column chromatography on silica using a hexane/EtOAc (1/1 to 0/1). The title compound was obtained as a colorless oil (1.13 g, 85%). R_f = 0.56 (Hexane/EtOAc, 4/1); ^{1}H NMR (126 MHz, $CDCl_3$) δ = 8.04 – 7.98 (m, 2H, aromatic), 7.60 – 7.53 (m, 3H, aromatic), 7.46 – 7.22 (m, 10H, aromatic), 5.61 (bs, 1H, H-1), 5.50 – 5.48 (m, 1H, H-2), 4.91 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.83 (td, *J* = 5.3, 1.5 Hz, 1H, H-5), 4.78 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 3.94 – 3.85 (m, 4H, H-3, H-6ab, H-4), 3.01 (bs, OH), 1.68 – 1.60 (m, 1H, CH_{thexyl}), 0.89 (d, *J* = 6.9 Hz, 6H, $CH_{3thexyl}$), 0.87 (s, 6H, $CH_{3thexyl}$), 0.11 (s, 6H, $SiCH_3$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 167.1, 129.8, 129.7, 133.4 , 131.6, 128.8, 128.2, 127.5, 127.1, 85.9, 77.0, 72.7, 72.1, 69.8, 67.0, 67.2, 61.2, 37.7, 29.7, 27.8 ,27.7, 27.6, 20.1, 18.6, -3.4, -3.7 ppm; MALDI-TOF: m/z calc. $C_{34}H_{44}O_6SSi$: 631.25 $[M+Na]^+$, found 631.33 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{34}H_{44}O_6SSi$ $[M+Na]^+$ 631.2526, found 631.2534.

Phenyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-1-thio-α-L-idopyranoside (24): A solution of compound **21** (2.20 g, 1.85 mmol) and *p*-methoxyphenol (2.92 g, 23.5 mmol) in dry THF (6 mL) was added to a solution of DIAD (1.85 mL, 9.40 mmol), and triphenylphosphine (2.47 g, 9.40 mmol) in dry THF (5 mL). After stirring overnight

at room temperature the mixture was diluted with EtOAc and was washed with 1 M NaOH aq, water and brine. After drying over $MgSO_4$ and concentration under reduced pressure the crude material was purified by column chromatography on silica using hexane/ EtOAc (3/1 to 1/1). The title compound was obtained as a colorless syrup (1.75 g, 70%). R_f = 0.38 (hexane / EtOAc, 3/1); $[\alpha]_D^{20}$ = +8.8° (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.01 – 7.99 (m, 2H, aromatic), 7.66 – 7.65 (m, 2H, aromatic), 7.61 – 7.57 (m, 1H, aromatic), 7.49 – 7.29 (m, 10H, aromatic), 6.90 (d, *J* = 9.2 Hz, 2H, aromatic$_{PMP}$), 6.84 (d, *J* = 9.2 Hz, 2H, aromatic$_{PMP}$), 5.61 (s, 1H, H-1), 5.56 – 5.54 (m, 1H, H-2), 5.21 – 5.17 (m, 1H, H-5), 4.95 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.71 (d, *J* = 11.8 Hz, 2H, CH_2Ph), 4.25 (d, *J* = 5.9 Hz, 2H, H-6ab), 3.95 – 3.88 (m, 2H, H-3, H-4), 3.78 (s, 3H, CH_{3PMP}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 164.9, 154.1, 153.0, 137.3, 135.8, 133.7 - 127.8 (C_{arom}), 115.6 - 114.7 ($C_{aromPMP}$), 86.8 (C-1), 74.0 (C-3), 72.4 (C_{Bn}), 70.1 (C-2), 68.3 (C-6), 67.6 (C-4), 67.2 (C-5), 55.7 (CH_{3PMP}) ppm; MALDI-TOF *m/z* calcd for $C_{33}H_{32}O_7S$ 595.18 $[M+Na]^+$, found 595.44. HRMS (ESI) *m/z* calcd for $C_{33}H_{32}O_7S$ $[M+Na]^+$ 595.1766, found 595.1754.

Phenyl 2-*O*-Benzoyl-3-*O*-Benzyl-6-*O*-tert-butyldiphenylsilyl-4-*O*-levulinyl-1-thio-α-L-idopyranose (25): A solution of **21** (0.957 g, 2.05 mmol) in anhydrous pyridine (15 mL), TBDPSCl (0.8 mL, 3.08 mmol) was added. After stirring overnight at room temperature under nitrogen, the mixture was diluted in EtOAc, was washed with water, $CuSO_4$ sat. and brine. The resulting product **22** was filtered, dried under vacuum and was used in the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (m, 2H, aromatic), 7.71 (d, 4H, aromatic), 7.60 – 7.28 (m, 19H, aromatic), 7.25 – 7.18 (m, 3H, aromatic), 5.60 (s, 1H, H-1), 5.50 (s, 1H, H-2), 4.91 (d, *J* = 12.0Hz, 1H, CH_2Ph), 4.81 (t, *J* = 5.1Hz, 1H, H-5), 4.68 (d, *J* = 11.9Hz, 1H, CH_2Ph), 3.98 (d, *J* = 5.3, 2H, H-6), 3.88 (s, 2H, H-3, H-4), 3.01 (s, 1H, OH), 1.06 (s, 9H, $(CH_3)_3$C). Levulination was carried out according to general procedure A using the crude product, levulinic acid (0.84 mL, 8.2 mmol), EDC·HCl and a catalytic amount of DMAP in dry CH_2Cl_2 (3.5 mL). The mixture was stirred at room temperature overnight. The residue was purified by chromatography on silica gel (Hexane/EtOAc, 8/2) to afford the product **25** in 91% of yield (over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 – 8.08 (m, 2H, aromatic), 7.72-7.21 (m, 23H, aromatic), 5.66 (s, 1H, H-1), 5.49 (m, 1H, H-4), 5.18 (s, 1H, H-2), 5.01 – 4.93 (m, 2H, H-3, CH_2Ph), 4.84 (d, *J* = 11.8Hz, 1H, CH_2Ph), 4.03 (m, 1H, H-5), 3.89 (dd, *J* = 6.4Hz, 4.1Hz, 2H,H-6), 2.56 (m, 2H; CH_2(Lev)), 2.49 – 2.40 (m, 2H,

CH_2(Lev)), 2.06 (s, 3H, CH_3(Lev)), 1.09 (s, 9H, $(CH_3)_3C$). ^{13}C NMR (126 MHz, $CDCl_3$) δ: ^{13}C NMR (126 MHz, $CDCl_3$) δ: 205.53 (qC), 171.86 (qC), 165.11 (qC), 137.36, 135.88 (qC), 135.54, 133.37, 133.06, 133.01, 131.64, 129.74, 129.69, 129.47, 128.78, 128.34, 127.72, 127.66, 127.45, 127.24, 86.09 (C-1), 72.63, 72.23 (C-5), 69.72 (C-4), 67.14, 67.05 (C-2, C-3), 62.35 (C-6), 37.67 (CH_2Lev), 29.50 (CH_3(Lev)), 27.76 (CH_2Lev), 26.71 $(CH_3)_3C$), 19.07 (qC, TBDPS).

Phenyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-4-*O*-levulinoyl-1-thio-α-L-idopyranoside (26): The reaction was carried out according to general procedure A of section 4.3 using compound **23** (1.13 g, 1.86 mmol), levulinic acid (1.30 g, 11.2 mmol), EDC·HCl (2.15 g, 11.2 mmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (20 mL). The compound **20** was obtained as colorless syrup (860 mg, 65%). R_f = 0.33 (hexane / EtOAc, 4/1); $[\alpha]_D^{20}$ = -13.5° (c = 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ = 8.10 – 8.05 (m, 2H, aromatic), 7.59 – 7.55 (m, 3H, aromatic), 7.47 – 7.43 (m, 4H, aromatic), 7.39 – 7.36 (m, 2H, aromatic), 7.33 – 7.23 (m, 4H, aromatic), 5.64 (s, 1H, H-1), 5.46 – 5.44 (m, 1H, H-2), 5.10 (s, 1H, H-4), 4.89 (d, J = 11.9 Hz, 1H, CH_2Ph), 4.87 – 4.83 (td, J = 2.0, 6.5 Hz, 1H, H-5), 4.79 (d, J = 11.9 Hz, 1H, CH_2Ph), 3.99 – 3.95 (m, 1H, H-3), 3.82 – 3.77 (m, 2H, H-6ab), 2.67 – 2.52 (m, 3H, CH_{2Lev}), 2.47 – 2.38 (m, 1H, CH_{2Lev}), 2.07 (s, 3H, CH_{3Lev}), 1.66 – 1.58 (m, 1H, CH_{thexyl}), 0.90 (d, J = 6.9 Hz, 6H, $CH_{3thexyl}$), 0.85 (2s, 6H, $CH_{3thexyl}$), 0.13 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 205.9, 172.1, 165.2, 137.5, 136.4, 133.6 - 127.3 ($C_{aromatic}$), 86.1 (C-1), 72.8 (C_{Bn}), 72.3 (C-3), 70.0 (C-2), 67.4 (C-5), 67.2 (C-4), 61.4 (C-6), 37.8 (CH_{2Lev}), 34.2 (CH_{thexyl}), 29.7 (CH_{3Lev}), 28.0 (CH_{2Lev}), 25.2 (Cq_{thexyl}), 20.3, 20.2, 18.6 ($CH_{3thexyl}$), -3.5, -3.7 ($Si(CH_3)_2$) ppm; HRMS (ESI): *m/z* calcd. for $C_{39}H_{50}O_8SSi$ $[M+Na]^+$ 729.2893, found 729.2894.

Phenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-1-thio-α-L-idopyranoside (27): The reaction was carried out according to general procedure A of section 4.3 using compound **24** (246 mg, 420 µmol), levulinic acid (146 mg, 1.26 mmol), EDC·HCl (242 mg, 1.26 mmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (3 mL). The compound **11** was obtained as a colorless syrup (259 mg, 90%). R_f = 0.22 (hexane/ EtOAc, 3/1); $[\alpha]_D^{20}$ = - 15.1° (c = 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ = 7.89 – 7.80 (m, 2H, aromatic), 7.59 – 7.54 (m, 2H, aromatic), 7.52 – 7.48 (m, 1H, aromatic), 7.42 – 7.19 (m, 10H, aromatic), 6.88 (d, J = 9.2 Hz, 2H, $aromatic_{PMP}$), 6.83

(d, J = 9.2 Hz, 2H, aromatic$_{PMP}$), 5.59 (bs, 1H, H-1), 5.48 – 5.46 (m, 1H, H-2), 5.28 – 5.24 (m, 1H, H-5), 5.15 (bs, 1H, H-4), 4.93 (d, J = 11.8 Hz, 1H, CH_2-Ph), 4.79 (d, J = 11.7 Hz, 1H, CH_2-Ph), 4.18 (dd, J = 9.9, 7.2 Hz, 1H, H-6a), 4.10 (dd, J = 9.9, 5.2 Hz, 1H, H-6b), 3.96 – 3.94 (m, 1H, H-3), 3.77 (s, 3H, CH_{3PMP}), 2.64 – 2.48 (m, 3H, CH_{2Lev}), 2.40 – 2.34 (m, 1H, CH_{2Lev}), 2.06 (s, 3H, CH_{3Lev}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.1, 172.6, 165.3, 154.2, 152.9, 137.4, 135.9, 133.6 – 127.6 (C_{arom}), 115.8, 114.7 ($C_{arom\text{-}PMP}$), 86.3 (C-1), 72.8 (C_{Bn}), 72.2 (C-3), 69.3 (C-2), 67.7 (C-6), 67.5 (C-4), 65.5 (C-5), 55.8 (CH_{3PMP}), 37.8 (CH_{2Lev}), 29.7 (CH_{3Lev}), 28.0 (CH_{2Lev}) ppm; HRMS (ESI): *m/z* calcd for $C_{38}H_{38}O_9S$ $[M+Na]^+$ 693.2134 , found 693.2120.

2-*O*-Benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-4-*O*-levulinoyl-α/β-L-idopyranosyl trichloroacetimidate (29): The thioglycoside **26** (820 mg, 1.16 mmol) was hydrolyzed using NIS (662 mg, 2.94 mmol) and TfOH (11 µL, 0.12 mmol) in wet THF at room temperature for 6 h. The reaction mixture was quenched with saturated $NaHCO_3$aq solution and solid $Na_2S_2O_3$. The mixture was filtered and washed with saturated $NaHCO_3$ aq. solution, water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The reaction crude was purified by column chromatography on silica (hexane/ EtOAc, 4/1). The intermediate hemiacetal was obtained as a colorless syrup and used in the next step without further purification (534 mg, 75%). R_f = 0.23 (hexane/ EtOAc, 4/1); MALDI-TOF *m/z* calcd for $C_{33}H_{46}O_9Si$ $[M+Na]^+$ 637.28, found 637.57. 2-*O*-Benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-4-*O*-levulinoyl-α/β-L-idopyranose (753 mg, 1.25 mmol) and trichloroacetonitrile (2.51 mL, 25 mmol) were dissolved in anhydrous CH_2Cl_2 (10 mL) with activated 4 Å molecular sieves. After 30 minutes stirring the solution was cooled to 0 °C and DBU (55 µL, 0.38 mmol) was added. After 2 h, TLC (hexane/ EtOAc, 4/1) indicated complete conversion of the starting material. The mixture was concentrated under reduced pressure and purified by column chromatography on silica (hexane / EtOAc, 1/0 to 4/1 with 1% of triethylamine) to obtain the compound **29** as a colorless solid, α/β mixture (ratio 5.8/1) (683 mg, 72%). R_f = 0.23 (hexane/ EtOAc, 4/1); α-anomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.63 (s, 1H, $OCNHCCl_3$), 8.10 (d, J = 7.7 Hz, 2H, aromatic), 7.59 (t, J = 7.4 Hz, 1H, aromatic), 7.49 – 7.24 (m, 7H, aromatic), 6.41 (s, 1H, H-1), 5.38 (s, 1H, H-2), 5.12 (bs, 1H, H-4), 4.84 (d, J = 11.6Hz, 1H, CH_2Ph), 4.75 (d, J = 11.6 Hz, 1H, CH_2Ph), 4.55 (t, J = 6.5 Hz, 1H, H-5), 3.99 (s, 1H, H-3), 3.80 – 3.70 (m, 2H, H-6), 2.64 – 2.36

(m, 4H, $2CH_{2Lev}$), 2.07 (s, 3H, CH_{3Lev}), 1.28 – 1.24 (m, 1H, CH_{thexyl}), 0.85 (d, J = 6.7 Hz, 2 x 3H, $CH_{3thexyl}$), 0.81 (s, 6H, $CH_{3thexyl}$), 0.08 (s, 3H, $SiCH_3$), 0.07 (s, 3H, $SiCH_3$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 205.4, 171.9, 165.0, 129.7 – 127.4 (C_{arom}), 94.9 (C-1), 72.3 (C_{Bn}), 71.9 (C-3), 68.5 (C-5), 66.5 (C-2), 66.3 (C-4), 61.1 (C-6), 37.7 (CH_{2Lev}), 33.9 (CH_{thexyl}) 29.7 (CH_{3Lev}) 27.9 (CH_{2Lev}), 27.8 (CH_{2Lev}), 20.2 ($CH_{3thexyl}$), 18.5 ($CH_{3thexyl}$), -4.1 ($SiCH_3$) ppm; MALDI-TOF *m/z* calcd for $C_{35}H_{46}Cl_3NO_9Si$ $[M+Na]^+$ 780.19, found 780.10.

2-*O*-Benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α/β-L-idopyranosyl trichloroacetimidate (30): The thioglycoside **27** (2.10 g, 3.13 mmol) was hydrolyzed using NIS (1.76 g, 7.83 mmol) and TfOH (27 µL, 0.31 mmol) in wet THF at room temperature for 6 h. The reaction mixture was quenched with saturated $NaHCO_3$ aq solution and solid $Na_2S_2O_3$. The mixture was filtered and washed with saturated $NaHCO_3$ solution, water and brine. The reaction crude was purified by column chromatography on silica (hexane / EtOAc, 4/1). R_f = 0.21 (hexane/ EtOAc, 2/1); MALDI-TOF *m/z* calcd for $C_{33}H_{46}O_9Si$ $[M+Na]^+$ 637.28, found 637.57. 2-*O*-Benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α/β-L-idopyranose (3.13 mmol) and trichloroacetonitrile (3.14 mL, 31.3 mmol) were dissolved in anhydrous CH_2Cl_2 (16 mL) with activated 4 Å molecular sieves. After 30 minutes stirring the solution was cooled to 0 °C and DBU (417 µL, 0.32 mmol) was added. After 2 h, TLC (hexane/ EtOAc, 3/1) indicated complete conversion was the starting material. The mixture was concentrated under reduced pressure and was purified by column chromatography on silica (hexane 100% → hexane/ EtOAc, 2/1) to obtain the compound **30** as a colorless syrup, α/β mixture (ratio 2/1) (1.27 g, 56% over 2 steps). R_f = 0.40 (hexane/ EtOAc, 2/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.61 (s, 1H, $OCNHCCl_3$), 8.09 – 7.93 (m, 2H, aromatic), 7.56 – 7.45 (m, 1H, aromatic), 7.43 – 7.20 (m, 7H, aromatic), 6.83 – 6.65 (m, 4H, $aromatic_{PMP}$), 6.38 (s, 1H, H-1), 5.33 (s, 1H, H-2), 5.13 (s, 1H, H-4), 4.81 (d, J = 11.6 Hz, 1H, CH_2Ph), 4.74 (s, 1H, H-5), 4.69 (d, J = 11.6 Hz, 1H, CH_2Ph), 4.10 – 4.07 (m, 1H, H-6a), 4.01 – 3.95 (m, 1H, H-6b), 3.93 (s, 1H, H-3), 3.68 (s, 3H, CH_{3PMP}), 2.55 – 2.22 (m, 4H, CH_{2Lev}), 1.97 (s, 3H, CH_{3Lev}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 205.9, 172.0, 165.5, 165.1, 160.6, 160.3, 154.2, 152.4, 133.6 - 128.2 (C_{arom}), 115.9 – 114.6 ($C_{aromPMP}$), 94.8 (C-1), 72.5 (C_{Bn}), 71.9 (C-3), 67.5 (C-2), 67.1 (C-6), 66.5 (C-5), 66.0 (C-4), 55.7 (CH_{3PMP}), 37.7 (CH_{2Lev}), 29.7 (CH_{3Lev}), 27.8 (CH_{2Lev}) ppm; MALDI-

TOF *m/z* calcd for $C_{35}H_{46}Cl_3NO_9Si$ $[M+Na]^+$ 780.19, found 780.10; HRMS (ESI): *m/z* calcd for $C_{34}H_{34}Cl_3NO_{10}$ $[M+Na]^+$ 744.1146, found 744.1146.

Phenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzyliden-1-thio-α-L-idopyranoside (31): To a solution of diol **21** (500 mg, 1.07 mmol) and benzyladehyde dimethyl acetal (10.7 mmol, 1.1 mL) in DMF (5 mL) a catalytic amount of CSA was added and the mixture was heated to 60 °C under reduced pressure for 5 h. After cooling to room temperature the mixture was diluted with CH_2Cl_2 (100 mL) and the organic layer was washed saturated NH_4Cl aq solution (3 x 100 mL), water (100 mL) and brine (100 mL). After drying over $MgSO_4$ and concentration under reduced pressure the crude material was purified by column chromatography on silica using hexane/ EtOAc (4/1 to 2/1). The title compound was obtained as a colorless syrup (468 mg, 79%). R_f = 0.56 (hexane / EtOAc, 3/1); $[\alpha]_D^{20}$ = +42.5° (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.88 (d, *J* = 8.3, 2H, aromatic), 7.51 – 7.07 (m, 18H, aromatic), 5.75 (bs, 1H, H-1), 5.52 (s, 1H, H_{acetal}), 5.48 – 5.43 (bs, 1H, H-2), 4.91 (d, *J* = 11.8 Hz, 1H, CH_{2Ph}), 4.64 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.45 (bs, 1H, H-5), 4.32 (d, *J* = 12.6 Hz, 1H, H-6a), 4.13 (d, *J* = 12.6 Hz, 1H, H-6b), 4.05 (bs, 1H, H-4), 3.85 (bs, 1H, H-3); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 165.7 (Cq), 137.8 - 126.3 (C_{arom}), 101.0 (C_{acetal}), 85.9 (C-1), 73.2 (C-4), 73.1 (C-3), 72.4 (C_{Bn}), 69.9 (C-6), 67.8 (C-2), 60.6 (C-5) ppm; MALDI-TOF *m/z* calcd for $C_{33}H_{30}O_6S$ 577.17 $[M+Na]^+$, found 577.36; HRMS (ESI) *m/z* calcd for $C_{33}H_{30}O_6S$ $[M+Na]^+$ 577.1661, found 577.1683.

Phenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-*p*-methoxybenzyliden-1-thio-α-L-idopyranoside (32): To a solution of diol **21** (250 mg, 536 µmol) and *p*-anisaldehyde (650 µL, 5.36 mmol) in toluene (5 mL), a catalytic amount of CSA was added and the mixture was heated under reflux using Dean-Stark aparatus for 2 h. After cooling to room temperature, the mixture was neutralized with triethylamine and the crude was concentrated under reduced pressure. The crude material was purified by column chromatography on silica using hexane/ EtOAc (4/1 to 2/1). The title compound was obtained as a colorless syrup (263 mg, 84%). R_f = 0.65 (hexane / EtOAc, 3/1); $[\alpha]_D^{20}$ = -77.3 ° (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 – 7.97 (m, 2H, aromatic), 7.60 – 7.20 (m, 15H, aromatic), 6.79 (m, 2H, $aromatic_{PMP}$), 5.83 (bs, 1H, H-1), 5.57 – 5.51 (m, 2H, H-2, H_{acetal}), 4.99 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.72 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.52 (bs, 1H, H-5), 4.38 (dd, *J* = 12.7, 1.7 Hz, 1H, H-6a), 4.19 (dd, *J* = 12.8,

2.0 Hz, 1H, H-6b), 4.11 (bs, 1H, H-4), 3.92 (bs, 1H, H-3), 3.80 (s, 3H, CH_{3PMP}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 165.8, 160.3, 137.4, 136.7, 133.2, 130.6, 130.6, 130.2, 129.7, 129.1, 128.7, 128.3, 128.2, 128.0, 127.8, 127.1, 113.5, 101.2 (C_{acetal}), 86.1 (C-1), 73.3 (C-3), 73.3 (C-4), 72.6 (C_{Bn}), 70.1 (C-6), 68.1 (C-2), 60.7 (C-5), 55.4 (CH_{3PMP}) ppm. MALDI-TOF *m/z* calcd for $C_{34}H_{32}O_7S$ 607.18 $[M+Na]^+$, found 607.9; HRMS (ESI) *m/z* calcd for $C_{34}H_{32}O_7S$ $[M+Na]^+$ 607.1766, found 607.1776.

Phenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-α-L-idopyranoside (33): Under argon atmosphere trifluoroacetic acid (172 µL, 2.25 mmol) was added slowly to a solution of the acetal **22** (250 mg, 451 µmol) and triethylsilane (360 µL, 2.25 mmol) in dry THF (2 mL) at 0 °C and stirred for 2 h. The mixture was diluted with CH_2Cl_2 (25 mL) and washed with saturated $NaHCO_3$ aq solution. After drying over $MgSO_4$ and concentration under reduced pressure the crude material was purified by column chromatography on silica using hexane/ EtOAc (3/1 to 1/1). The title compound was obtained as a colorless syrup (175 mg, 70%). R_f = 0.40 (hexane / EtOAc, 3:1); $[\alpha]_D^{20}$ = +25.9° (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.03 – 7.99 (m, 2H, aromatic), 7.62 – 7.56 (m, 3H, aromatic), 7.48 – 7.42 (m, 4H, aromatic), 7.40 – 7.28 (m, 8H, aromatic), 7.26 – 7.20 (m, 3H, aromatic), 5.62 (s, 1H, H-1), 5.54 – 5.51 (m, 1H, H-2), 5.03 – 4.99 (m, 1H, H-5), 4.93 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.68 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.64 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.59 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 3.92 – 3.81 (m, 1H, H-3, H-4, H-6ab), 2.72 (bs, 1H, OH) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 165.2, 138.1, 137.5, 136.0, 133.7 – 127.7 ($C_{aromatic}$), 86.9 (C-1), 74.3 (C-3), 73.6 (C_{Bn}), 72.5 (C_{Bn}), 70.5 (C-6), 70.1 (C-2), 68.1 (C-4), 67.4 (C-5) ppm; MALDI-TOF *m/z* calc $C_{33}H_{32}O_6S$ $[M+Na]^+$ 579.18, found 579.25; HRMS (ESI) *m/z* calcd for $C_{33}H_{32}O_6S$ $[M+Na]^+$ 579.1817, found 579.1809.

Phenyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-1-thio-α-L-idopyranoside (34): Under argon atmosphere iodine (416 mg, 1.64 mmol) was added portionwise in 5 min to a solution of the acetal **32** (240 mg, 410 µmol) and sodium cyanoborohydride (258 mg, 4.10 mmol) in dry CH_2Cl_2 (2 mL) at -20 °C and stirred for 15 min. The mixture was diluted with CH_2Cl_2 (25 mL) and washed with saturated $NaHCO_3$ aq solution. After drying over $MgSO_4$ and concentration under reduced pressure the crude material was purified by column chromatography on silica using hexane/ EtOAc (3/1 to 1/1). The title compound was obtained as a colorless syrup (146 mg, 61%). R_f = 0.34

(hexane /EtOAc, 3:1); $[\alpha]_D^{20}$ = -97.7° (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 – 7.97 (m, 2H, aromatic), 7.61 – 7.55 (m, 3H, aromatic), 7.48 – 7.40 (m, 4H, aromatic), 7.40 – 7.35 (m, 2H, aromatic), 7.34 – 7.21 (m, 6H, aromatic), 6.90 – 6.85 (m, 2H, aromatic$_{PMB}$), 5.61 (bs, 1H, H-1), 5.52 – 5.49 (m, 1H, H-2), 4.99 – 4.95 (m, 1H, H-5), 4.91 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.67 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.57 (d, *J* = 11.5 Hz, 1H, CH_{2PMB}), 4.51 (d, *J* = 11.4 Hz, 1H, CH_{2PMB}), 3.88 (td, *J* = 2.9, 1.4 Hz, 1H, H-3), 3.86 – 3.76 (m, 6H, H-4, H-6, CH_{3PMB}), 2.87 (bs, 1H, OH) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): 165.2, 159.4, 137.5, 136.0, 133.7, 132.2, 130.2, 129.9, 129.5, 129.5, 129.4, 129.0, 128.8, 128.7, 128.6, 128.1, 128.0, 127.9, 127.7, 113.9, 86.9 (C-1), 74.3 (C-3), 73.3 (C_{Bn}), 72.5 (C_{Bn}), 70.2 (C-6), 70.0 (C-2), 68.2 (C-4), 67.4 (C-5), 55.4 (CH_{3PMB}) ppm; MALDI-TOF *m/z* calcd for $C_{34}H_{34}O_7S$ $[M+Na]^+$ 609.19, found 608.96; HRMS (ESI) *m/z* calcd for $C_{34}H_{34}O_7S$ $[2M+NH_4]^+$ 1190.4389, found 1190.4389.

Phenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-levulinoyl-1-thio-α-L-idopyranoside (35): The reaction was carried out according to general procedure A of section 4.3 using compound **33** (220 mg, 395 µmol), levulinic acid (140 mg, 1.18 mmol), EDC·HCl (225 mg, 1.18 mmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (3 mL). The compound **9** was obtained as colorless syrup (258 mg, 94%). R_f = 0.45 (hexane/ EtOAc, 3/1); $[\alpha]_D^{20}$ = -10.7° (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25) δ = 8.08 – 8.06 (m, 2H, aromatic), 7.59 – 7.54 (m, 3H, aromatic), 7.42 – 7.19 (m, 15H, aromatic), 5.62 (s, 1H, H-1), 5.45 – 5.42 (m, 1H, H-2), 5.13 – 5.09 (m, 1H, H-5), 5.08 – 5.06 (m, 1H, H-4), 4.90 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.77 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.58 (d, J = 11.4 Hz, 1H, CH_2Ph), 4.53 (d, J = 11.8 Hz, 1H, CH_2Ph), 3.92 – 3.90 (m, 1H, H-3), 3.76 – 3.71 (m, 1H, H-6a), 3.71 – 3.66 (m, 1H, H-6b), 2.65 – 2.43 (m, 3H, CH_{2Lev}), 2.40 – 2.31 (m, 1H, CH_{2Lev}), 2.06 (s, 3H, CH_{3Lev}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 205.8, 171.9, 165.2, 133.3 – 125.1 (C_{arom}), 86.1 (C-1), 72.7 (C_{Bn}), 71.9 (C_{Bn}), 71.5 (C-3), 70.7 (C-2), 68.5 (C-6), 67.2 (C-4), 66.4 (C-5), 37.7 (CH_{2Lev}), 28.5 (CH_{3Lev}), 29.2 – 21.0 (CH_{2Lev}) ppm; HRMS (ESI): *m/z* calcd. for $C_{38}H_{38}O_8S$ $[M+Na]^+$ 677.2180, found 677.2155.

Phenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxybenzyl-1-thio-α-L-idopyranoside (36): The reaction was carried out according to general procedure A of section 4.3 using compound **34** (127 mg, 216 µmol), levulinic acid (50mg, 433 µmol), EDC·HCl (83 mg, 0.433 µmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (2 mL).

The compound **36** was obtained as a colorless syrup (120 mg, 81%). R_f = 0.25 (hexane/EtOAc, 3/1); $[\alpha]_D^{20}$ = -49.6° (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 – 8.05 (m, 2H, aromatic), 7.61 – 7.54 (m, 3H, aromatic), 7.49 – 7.40 (m, 4H, aromatic), 7.40 – 7.36 (m, 2H, aromatic), 7.34 – 7.29 (m, 1H, aromatic), 7.28 – 7.21 (m, 5H, aromatic), 6.87 (d, *J* = 8.6 Hz, 1H, aromatic$_{PMB}$), 5.62 (bs, 1H, H-1), 5.44 (m, 1H, H-2), 5.11 – 5.03 (m, 2H, H-4, H-5), 4.90 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.77 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.52 (d, *J* = 11.4 Hz, 1H, CH_{2PMB}), 4.46 (d, *J* = 11.5 Hz, 1H, CH_{2PMB}), 3.93 – 3.90 (m, 1H, H-3), 3.80 (s, 3H, CH_{3PMB}), 3.72 (dd, *J* = 9.9, 6.8 Hz, 1H, H-6a), 3.66 (dd, *J* = 10.0, 5.4 Hz, 1H, H-6b), 2.66 – 2.57 (m, 1H, CH_{2Lev}), 2.57 – 2.44 (m, 2H, CH_{2Lev}), 2.39 – 2.31 (m, 1H, CH_{2Lev}), 2.07 (s, 3H, CH_{3Lev}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): 206.0, 172.1, 165.3, 159.3, 137.4, 136.0, 133.6, 132.1, 132.1, 130.3, 130.0, 130.0, 129.7, 129.6, 129.5, 129.1, 129.1, 129.0, 128.6, 128.5, 127.9, 127.7, 127.7, 127.6, 113.8 ($C_{aromaticPMB}$), 86.3 (C-1), 73.1 (CH_{2PMB}), 72.8 (C_{Bn}), 72.3 (C-3), 69.5 (C-2), 68.7 (C-6), 67.6 (C-4), 65.8 (C-5), 55.4 (CH_{3PMB}), 37.9 (CH_{2Lev}), 29.8 (CH_{3Lev}), 27.9 (CH_{2Lev}) ppm; HRMS (ESI): *m/z* calcd for $C_{39}H_{40}O_9S$ $[M+NH_4]^+$ 702.2731 found 702.2722.

Phenyl 6-*O*-acetyl-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-1-thio-α-L-idopyranoside (37): To a solution of **25** (400 mg, 0.498 mmol) in THF (2 mL) at 0 °C, HF-pyr (1 mL) was added. The reaction mixture was allowed to warm to room temperature and it was stirred overnight. Next, the reaction was quenched by addition of saturated $NaHCO_3$aq solution and solid $NaHCO_3$ and was washed with saturated $NaHCO_3$aq solution and water. The organic layer was dried over $MgSO_4$ and concentrated. Flash chromatography (hexane/EtOAc, 1/0 to 8/2) afforded phenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-1-thio-α-L-idopyranoside (141 mg, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.16 – 8.08 (m, 2H, aromatic), 7.64 – 7.56 (m , 3H, aromatic), 7.54 – 7.26 (m, 10H, aromatic), 5.64 (s, 1H, H-1), 5.52 – 5.48 (m, 1H, H-2), 5.14 – 5.10 (m, 1H, H-5), 4.99 – 4.91 (m, 1H, H-4), 4.94 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.80 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 3.96 – 3.91 (m, 1H, H-3), 3.85 (dd, *J* = 11.6, 6.9 Hz, 1H, H-6a), 3.76 (dd, *J* = 11.6, 6.3 Hz, 1H, H-6b), 2.79 – 2.69 (m, 1H, CH_{2Lev}), 2.69 – 2.56 (m, 2H, CH_{2Lev}), 2.44 – 2.34 (m, 1H, CH_{2Lev}), 2.12 (s, 3H, CH_{3Lev}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.4, 172.5, 165.1, 137.1, 135.6, 133.5, 131.8, 129.7, 129.4, 129.0, 128.4, 128.4, 127.8, 127.5, 86.1, 72.5, 72.1, 69.1, 67.2, 66.8, 61.1, 37.8, 29.5, 27.8 ppm; MALDI-TOF *m/z* calcd for $C_{31}H_{32}O_8S$ $[M+Na]^+$ 587.2, found 587.4. To a solution of

phenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-1-thio-α-L-idopyranoside (0.095 g, 0.168 mmol) in CH_2Cl_2 (1 mL) at 0 °C, pyridine (0.020 mL, 0.252 mmol) and acetic anhydride (0.021 g, 0.202 mmol) were added. The reaction was allowed to warm to room temperature and was stirred until completion. The mixture was diluted with CH_2Cl_2, washed with saturated $CuSO_4$ aq solution and water. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuum. Flash column chromatography (hexane/EtOAc, 1:0 to 7:3) afforded **37** as colorless syrup (102 mg, quantitative). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.15 – 8.08 (m, 2H, aromatic), 7.65 – 7.56 (m, 3H, aromatic), 7.54 – 7.25 (m, 10H, aromatic), 5.70 – 5.64 (m, 1H, H-1), 5.50 – 5.44 (m, 1H, H-2), 5.16 – 5.09 (m, 1H, H-5), 5.07 – 5.02 (m, 1H, H-4), 4.94 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.79 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.33 (dd, *J* = 7.9, 11.5 Hz, 1H, H-6a), 4.28 (dd, *J* = 5.0, 11.5 Hz, 1H, H-6b), 3.98 – 3.92 (m, 1H, H-3), 2.72 – 2.52 (m, 3H, CH_{2Lev}), 2.47 – 2.37 (m, 1H, CH_{2Lev}), 2.11 (s, 3H, CH_{3Lev}), 2.07 (s, 3H, CH_{3Ac}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.7, 171.9, 170.5, 165.0, 137.1, 135.7, 133.5, 131.7, 129.8, 129.4, 128.8, 128.4, 128.3, 127.9, 127.6, 127.5, 86.0 (C-1), 72.7 (C_{Bn}), 71.9 (C-3), 68.8 (C-2), 67.0 (C-4), 64.6 (C-5), 62.7 (C-6), 37.7 (CH_{2Lev}), 29.5 (CH_{3Lev}), 27.8 (CH_{2Lev}), 20.7 (CH_{3Ac}) ppm; LRMS (MALDI-TOF) *m/z* calcd for $C_{33}H_{34}O_9S$ $[M+Na]^+$ 629.2, found 629.7; HRMS (ESI): *m/z* calcd for $C_{33}H_{34}O_9S$ $[M+Na]^+$ 629.1821, found 629.1844.

Phenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-1-thio-6-*O*-tri-*iso*-propylsiloxymethyl-α-L-idopyranoside (38): To a solution of **25** (260 mg, 0.460 mmol) in CH_2Cl_2 (2 mL) at 0 °C, freshly distilled DIPEA (0.402 mL, 2.302 mmol) and (triisopropylsiloxy)methyl chloride (0.260 mL, 0.921 mmol) were added. The reaction was allowed to warm to room temperature and was stirred until completion. The mixture was diluted with CH_2Cl_2, washed with water, dried over $MgSO_4$ and concentrated in vacuum. Flash column chromatography (hexane/EtOAc, 1:0 to 8:2) afforded **38** as a colorless syrup (220 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 8.15 – 7.10 (m, 15H, aromatic), 5.63 (s, 1H, H-1), 5.50 – 5.44 (m, 1H, H-2), 5.11 – 5.05 (m, 2H, H-4, H-5), 4.99 – 4.93 (m, 2H, CH_{2TOM}), 4.92 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.81 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 3.98 – 3.93 (m, 1H, H-3), 3.88 – 3.78 (m, 2H, H-6ab), 2.71 – 2.52 (m, 3H, CH_{2Lev}), 2.49 – 2.39 (m, 1H, CH_{2Lev}), 2.10 (s, 3H, CH_{3Lev}), 1.11 – 1.07 (m, 21H, $6CH_{3TOM}$, $3CH_{TOM}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.7, 172.0, 165.2, 137.3,

135.9, 133.4, 131.94, 129.8, 129.5, 128.8, 128.4, 127.8, 127.5, 127.4, 90.1 (CH_{2TOM}), 86.2 (C-1), 72.6 (C_{Bn}), 72.0 (C-3), 69.4 (C-2), 67.4 (C-4), 66.3 (C-6), 65.6 (C-5), 37.8 (CH_{2Lev}), 29.6 (CH_{3Lev}), 27.8 (CH_{2Lev}), 17.8 (CH_{3TOM}), 12.0 (CH_{TOM}) ppm. LRMS (MALDI-TOF) *m/z* calcd for $C_{41}H_{54}O_9SSi$ $[M+Na]^+$ 774.00, found 773.95. HRMS (ESI): *m/z* calcd for $C_{41}H_{54}O_9SSi$ $[M+Na]^+$ 773.3156, found 773.3118.

5.6.1.1 Synthesis of the Ido non-reducing end building block

Phenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-1-thio-α-L-idopyranoside (39): 27 (0.637 g, 1.29 mmol) was dissolved in pyridine (2 mL) and TMSCl (0.4 mL, 7.8 mmol) was added at 0 °C. After stirring for 2h at room temperature, the reaction mixture was diluted with EtOAc (50 mL) and was washed with water, saturated $CuSO_4$ aq solution, water and brine (10 mL each). The crude was concentrated, dried under vacuum and used in the next reaction without further purification. To a solution of this intermediate in dry CH_2Cl_2 (13 mL), 4 Å molecular sieves (0.400 g) and benzaldehyde (0.16 mL, 1.55 mmol) were added and after stirring the suspension for 1h at room temperature. The reaction mixture was cooled at -78 °C, triethylsilane (0.25 mL, 1.55 mmol) and TMSOTf (23 µL, 0.13 mmol) were added via microsyringe. After stirred for 3h, TLC analysis showed the presence of some starting material. Additional volume of TMSOTf (0.5 eq) was added and the reaction mixture was gradually warmed up. After stirred overnight, the reaction was diluted with CH_2Cl_2 (50 mL) and was washed with saturated $NaHCO_3$ aq solution, water and brine. The crude product was purified by column chromatography (hexane/EtOAc, 95:5 to 80:20) to obtain compound **39** (600 mg, 70%). $[\alpha]_D^{20}$ - 43.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 – 8.01 (m, 2H, aromatic), 7.66 – 7.60 (m, 2H, aromatic), 7.56 – 7.46 (m, 3H, aromatic), 7.45 – 7.39 (m, 2H, aromatic), 7.38 – 7.18 (m, 9H, aromatic), 7.17 – 7.11 (m, 2H, aromatic), 6.91 – 6.83 (m, 4H, $aromatic_{PMP}$), 5.66 (s, 1H, H-1), 5.55 – 5.53 (m, 1H, H-2), 5.11 (td, *J* = 6.3, 2.0 Hz, 1H, H-5), 4.97 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.70 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.54 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.40 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.25 (d, *J* = 6.3 Hz 2H, H-6), 4.07 – 4.02 (m, 1H, H-3), 3.83 – 3.77 (s, 3H, CH_{3PMP}), 3.75 – 3.70 (m, 1H, H-4) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8, 154.1, 153.0, 137.6, 137.5, 136.2, 133.3, 131.7, 130.1, 129.7, 129.0, 128.6, 128.4, 128.4, 128.1, 128.0, 127.9, 127.4, 115.6, 114.7, 86.1, 73.4, 72.6, 72.5, 71.1, 69.5,

67.4, 67.0, 55.8 ppm; HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{40}H_{38}O_7SNa$ 685.2230; Found 685.2217.

2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α/β-L-idopyranosyl trichloroacetimidate (41): Compound **39** (527 mg, 0.79 mmol) was dissolved in acetone/ water (9/1; 11 mL) and freshly recristallyzed NBS (155 mg, 0.87 mmol) was added in portions by spatula over 10 min at room temperature with vigorous stirring. After 45 minutes, TLC analysis showed presence of some starting material and additional NBS (84 mg, 0.47 mmol) was added. After 30 min, the reaction was diluted with EtOAc and was washed with 1M $Na_2S_2O_3$ aq solution, water and brine. The reaction crude was dried over anhydrous $MgSO_4$, concentrated and purified by column chromatography (hexane/EtOAc, 1:0 to 8:2) to obtain an α/β (1/1) mixture of the hemiacetal **40** (324 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 – 7.94 (m, 2H, aromatic), 7.57 – 7.50 (m, 1H, aromatic), 7.42 – 7.29 (m, 7H, aromatic), 7.23 – 7.07 (m, 3H, aromatic), 7.06 – 7.03 (m, 1H, aromatic), 7.02 – 6.99 (m, 1H, aromatic), 6.89 – 6.81 (m, 4H, aromatic$_{PMP}$), 5.32 – 5.26 (m, 1H, H-1α, H-1β), 5.21 – 5.18 (m, 0.5H, H-2), 5.16 – 5.13 (m, 0.5H, H-2), 4.81 – 4.75 (m, 1H, CH_2Ph), 4.71 – 4.64 (m, 1H, CH_2Ph), 4.64 – 4.61 (m, 0.5H, H-5), 4.45 – 4.36 (m, 1.5H, H-5, CH_2Ph), 4.33 (d, *J* = 11.2 Hz, 0.5H, CH_2Ph), 4.28 (d, *J* = 11.2 Hz, 0.5H, CH_2Ph), 4.25 – 4.15 (m, 2H, H-6), 4.14 – 4.09 (m, 1H, H-3), 3.78 (2s, 3H, CH_{3PMP}), 3.68 (t, *J* = 2.7 Hz, 0.5H, H-4), 3.58 (t, *J* = 2.4 Hz, 0.5H, H-4). HRMS (ESI) *m/z:* $[M+Na]^+$ Calcd for $C_{34}H_{34}O_8$ 593.2151, Found 593.2185. The hemiacetal **40** (360 mg, 0.63 mmol) was dissolved in dry CH_2Cl_2 (6.3 mL), trichloroacetonitrile (0.95 mL, 9.46 mmol) and catalytic amount of DBU (19 µL, 0.126 mmol) were added at 0 °C. The reaction mixture was allowed to warm up to room temperature and was stirred for 2h until TLC analysis showed disappearance of starting material. The reaction crude was concentrated and purified by column chromatography (hexane/EtOAc, 9/1 to 7/3 containing 5% of triethylamine) to obtain compound **41** as α/β (4/6) mixture in 76% of yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 0.4H, $OCNHCl_3$), 8.52 (s, 0.6H, $OCNHCl_3$), 8.05 – 8.00 (m, 2H, aromatic), 7.57 – 7.53 (m, 1H, aromatic), 7.39 – 7.09 (m, 12H, aromatic), 6.87 – 6.80 (m, 4H, aromatic$_{PMP}$), 6.51 (d, J = 2.7 Hz, 1H, H-1β), 6.43 (s, 1H, H-1α), 5.50 – 5.46 (m, 1H, H-2α, H-2β), 4.90 – 4.89 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.83 – 4.81 (d, J = 11.5 Hz, 1H, CH_2Ph), 4.78 – 4.76 (m, 2H, H-5α, CH_2Ph), 4.66 – 4.64 (d, J = 11.8Hz, 1H, CH_2Phα), 4.63 – 4.52 (m, 5H,

CH_2Ph, H-5β, CH_2Ph), 4.43 – 4.41 (m, 1H, CH_2Ph), 4.39 – 4.31 (m, 2H, H-6β), 4.27 – 4.17 (m, 3H, H-6α, H-3β), 4.04 - 4.03 (m, 1H, H-3α), 3.90 – 3.85 (m, 1H, H-4β), 3.80 – 3.75 (m, 4H, CH_{3PMP}, H-4α); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.9, 165.7, 160.8, 160.7, 154.2, 154.1, 152.8, 152.7, 137.7, 137.6, 137.5, 133.5, 133.3, 130.2, 130.1, 129.5, 129.4, 128.6, 128.5, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.9, 115.8, 115.6, 114.7, 97.8, 95.6, 95.3, 75.4, 74.5, 74.1, 73.7, 73.4, 72.7, 72.4, 72.1, 71.0, 69.3, 68.0, 67.3, 66.8, 66.3, 55.9 ppm.

5.6.2 Synthesis of L-iduronic acid (IdoA) building blocks

Methyl [Phenyl 2-*O*-benzoyl-3-*O*-benzyl-1-thio-α-L-idopyranoside]uronate (42): To a solution of the compound **21** (106 mg, 0.23 mmol) in acetonitrile/water (1/1 v/v, 1 mL), TEMPO (7 mg, 0.045 mmol) and BAIB (183 mg, 0.57 mmol) were added. The mixture was stirred for 4h. The reaction mixture was quenched by the addition of 1M aqNa_2SO_3 (0.7 mL). The layers were separated and the aqueous layer was acidified with 1M aq HCl, and extracted with CH_2Cl_2 (3X). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. The residue was dissolved in dry MeOH, EDC·HCl, DMAP were added at 0 ºC and was stirred overnight. The mixture was quenched with CH_2Cl_2 and was washed with water, HCl 1M, $NaHCO_3$ sat. and brine. $MgSO_4$ anhydrous was added, filtered and concentrated. The crude was purified by flash column chromatography Hexane/EtOAc (8/2) to obtain compound **38** as a colorless syrup (67 mg, 60% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 - 7.26 (m, 15H, aromatic), 5.76 (s, 1H, H-1), 5.53 (m, J = 2.0 Hz, 1H, H-2), 5.44 (m, 1H, H-5), 4.93 (d, 1H, *J* = 11.8Hz, 1H, CH_2Ph), 4.73 (d, *J* = 11.8Hz, 1H, CH_2Ph), 4.19 (m,1H, H-4), 3.97 (m, 1H, H-3), 3.85 (s, 3H, CH_3ester), 2.77 (bs, 1H, OH).

Methyl [Phenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinyl-1-thio-α-L-idopyranoside]uronate (43): The reaction was carried out according to general procedure A using compound **42** (166 mg, 0.21mmol), levulinic acid (77 mg, mmol), EDC·HCl (127 mg, 0.66 mmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (2.5 mL). The crude was purified by flash column chromatography Hexane/EtOAc (7/3) to obtain compound **43** as a colorless syrup (177 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 - 7.26 (m, 15H, aromatic), 5.81 (s, 1H, H-1), 5.50 (d, J = 2.0 Hz, 1H, H-5), 5.45 (m, 1H, H-2), 5.34 (m, 1H, H-4), 4.93 (d, *J* = 11.8Hz, CH_2Ph), 4.82 (d, *J* = 11.8Hz,

CH_2Ph), 4.01 (1H,bs, H-3), 3.84 (3H, CH_3ester), 2.65 - 2.61 (2H, m, Lev), 2.45 - 2.35 (2H, m, Lev), 2.10 (3H, s, Lev). ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.68 (qC), 171.44 (qC), 168.53, 165.04, 136.89, 135.40, 133.51, 131.22, 129.79, 129.21 (qC), 128.98, 128.43, 128.32, 127.93 (qC), 127.56, 127.48, 86.30 (C-1), 72.73 (CH_2Ph), 71.59 (C-3), 68.72 (C-2), 67.82 (C-5/4), 66.82 (C-5/4), 52.47 (CH_3ester), 37.62, 29.48, 27.80 ppm.

Methyl (2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α/β-L-idopyranosyluronate) trichloroacetimidate (44): To a solution of thioglycoside **43** (110 mg, 0.18 mmol) in CH_2Cl_2 (1.86 mL), NIS (84 mg, 0.37 mmol) and trifluoroacetic acid (28 µL, 0.37 mmol) were added at 0 ºC. After 15 min TLC analysis showed complete consumption of the starting material, the reaction was quenched with saturated $Na_2S_2O_3$aq solution and was washed with saturated $NaHCO_3$ aq solution. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The crude was dissolved in dry CH_2Cl_2 (2.4 mL), trichloroacetonitrile (0.36 mL, 3.6 mmol) and catalytic amount of DBU (3.6 µL, 0.024 mmol) were added at 0 °C. After stirring for 1h at room temperature, the reaction mixture was concentrated. The residue was purified by column chromatography (hexane/EtOAc, 7/3 containing 1% of Et_3N) to yield **44** as a viscous oil (90 mg, 75%). ^1H NMR (500 MHz, $CDCl_3$) *δ* 8.74 (s, 0.8 H, N*H*CCCl$_3$), 8.69 (s, 0.2H, N*H*CCCl$_3$), 8.11 – 8.08 (m, 2H, aromatic), 7.59 – 7.53 (m, 1H, aromatic), 7.45 – 7.26 (m, 7H, aromatic), 6.56 (s, 0.8H, H-1α), 6.34 (s, *J* = 1.8Hz, H-1β), 5.51 (m, 0.2H, H-2β), 5.37 (m, 0.8H, H-2α), 5.34 (m, 0.8H, H-4α), 5.28 (m, 0.2H, H-4β), 5.12 (d, *J* = 1.9Hz, 0.8H, H-5α), 4.86 – 4.75 (m, 2.2H, CH_2Ph, H-5β), 4.14 (t, 0.2H, H-3β), 4.01 (m, 0.8H, H-3α), 3.79 (s, 3H, CH_{3COOMe}), 2.60 – 2.56 (m, 2H, CH_{2Lev}), 2.45 – 2.40 (m, 2H, CH_{2Lev}), 2.36 – 2.31 (m, 2H, CH_{2Lev}), 2.06 and 2.04 (2s, 3H, CH_{3Lev}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 205.7, 171.5, 168.0, 167.1, 165.4, 164.9, 160.2, 160.0, 137.1, 136.8, 133.8, 133.5, 129.9, 129.0, 128.6, 128.5, 128.3, 127.9, 127.6, 95.0 (C-1α), 94.5 (C-1β), 73.3 (C-5β), 73.1 (C-3β), 72.6, 71.5 (C-3α), 67.7 (C-5α), 67.4 (C-4α), 67.3 (C-4β), 66.0 (C-2β), 65.2 (C-2α), 52.7 (CH_{3COOMe}), 37.7, 29.5, 27.8 ppm.

5.6.2.1 Synthesis of *n*- pentenyl orthoesters (NPOEs) as IdoA donors

3-*O*-Benzyl-1,2-O-isopropiliden-α-D-xilo-dialdose (45): At first, silica gel (106 g) was suspended in CH_2Cl_2 (8.4 mL/g silica) and was stirred vigorously for 5min. After, a solution of $NaIO_4$ (14.4g) in water (101mL) was added. A solution of the diol **16** was

added slowly to the mixture before prepared and the reaction was stirred for 30min. When the starting material disappeared, the mixture was filtered, concentrated and was used in the next step without purification. The spectroscopic data of the compound were in agreement with the data previously reported in literature.[4]

Tris(thiophenyl) 3-*O*-Benzyl-1,2-*O*-isopropylidene-β-L-orthoidofuranuronate (46): *n*BuLi (195 mL of a 1.6M solution. in hexane, 6.51 mmol, 1.1 equiv.) was added to a cooled (-78 °C), stirred solution of tris(thiophenyl)methane (2.42 g, 7.11 mmol, 1.2 equiv.) in THF (9 mL). Upon addition of *n*BuLi the colour changed to bright yellow and a yellow solid precipitated. After 1.5 h of stirring at this temperature, a solution of **45** (1.65 g, 5.92 mmol) in THF (9 mL) was added dropwise. The mixture was stirred for 1 h at the same temperature and was then allowed to come to room temperature overnight. The reaction mixture was quenched by adding saturated aq NH_4Cl (500 mL), and the aqueous phase was extracted with Et_2O (3X). The combined organic layers were washed with water (2X), dried over anhydrous MgSO4, filtered, and concentrated. Flash chromatography of the residue (Hexane/ EtOAc, 9/1 to 8/2) gave **46** (2.72 g, 78%). The spectroscopic data of the compound were in agreement with the data previously reported in literature.[4]

Methyl 3-*O*-Benzyl-1,2-*O*-isopropylidene-β-L-idofuranuronate (47): Methanol (960mL), CuO (5.94 g, 74 mmol, 1.7 equiv.), $CuCl_2$ (22.4 g, 167.0 mmol, 3.8 equiv.), and water (80 mL) were successively added to a solution of compound **46** (27.2 g, 43.0 mmol) in CH_2Cl_2 (80 mL). The reaction mixture was vigorously shaken for 2 h, filtered through a Celite 545 pad and concentrated without warming above 30 °C. The residue was dissolved in CH_2Cl_2 (1 L), and water (500 mL) was added, giving a Cu salt precipitate that was eliminated by filtration through a pad of celite. The aqueous layer was extracted with CH_2Cl_2 (2 X 250 mL). The combined organic layers were washed with a saturated $NaHCO_3$ aq solution (200 mL) and water (200 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. Flash chromatography of the residue (Hexane/EtOAc, 7/3 to 1/1) gave **47** (13.2 g, 89%). The spectroscopic data of the compound were in agreement with the data previously reported in literature.[5]

Methyl 3-*O*-Benzyl-β-L-idofuranuronate (48): A solution of **47** (8.95 g, 26.45 mmol) in aqueous 90% trifluoroacetic acid (50 mL) was kept at room temperature for 15 min,

concentrated to dryness, and concentrated twice with water (2 x 20 mL) to give a white solid residue **48** (7.5 g, 95%), which was crystallized from EtOAc. The spectroscopic data of the compound were in agreement with the data previously reported in literature.[5]

Methyl 1,2,4-*tri*-*O*-acetyl-3-*O*-benzyl-β-L-idopyranurate (49): A solution of **48** (1g, 3.35 mmol) in dry CH_2Cl_2 at -40ºC, DMAP (0.1eq.), pyridine and acetyl chloride were added and the reaction was stirred overnight. The mixture was diluted with CH_2Cl_2 and the organic phase was washed with aqueous saturated $NaHCO_3$ solution (3X), water (2X), 1M H_2SO_4 solution (3X), water (2X), dried ($MgSO_4$) and concentrated. The residue was purified by columm of silica gel hexane/EtOAc to yield **49** (1.1 g, 78%). The spectroscopic data of the compound were in agreement with the data previously reported in literature.[5]

Methyl 1,2,4-*tri*-*O*-levulinoyl-3-*O*-benzyl-β-L-idopyranurate (50): The compound **48** (1.0 g, 3.35 mmol) was dissolved in dry CH_2Cl_2 and cooled to -25 °C.EDC·HCl (3.2 g, 16.75 mmol), DMAP (1.64 g, 13.4 mmol) and levulinic acid (1.7 mL, 16.75 mmol) were added. The mixture was stirred overnight at -25 °C and warmed to room temperature and stirred for additional 3h. The reaction was diluted with CH_2Cl_2 and washed with with HCl 1M, water, saturated $NaHCO_3$ aq solution and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The crude was purified by column chromatography (hexane/EtOAc, 7/3) and compound **50** was obtained (1.58 g, 80%). $[\alpha]_D^{20}$ = +2.2 ° (*c* = 1, $CHCl_3$) , ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.38 – 7.29 (m, 5H, aromatic), 6.05 (d, *J* = 1.7 Hz, H-1), 5.19 – 5.17 (m, 1H, H-4), 5.06 – 5.04 (m, 1H, H-2), 4.76 (d, *J* = 2.2 Hz, 1H, H-5), 4.72 (m, 2H, CH_2Ph), 3.94 (t, *J* = 3.0 Hz, 1H, H-3), 3.78 (s, 3H, CH_3), 2.86 – 2.49 (m, 12H, CH_{2Lev}), 2.17 (s, 6H, CH_{3Lev}), 2.16 (s, 3H, CH_{3Lev}); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.38 (C=O, Lev), 206.28 (2xC=O, Lev), 172.13, 171.91, 170.70, 167.34, 136.86, 128.65, 128.29, 127.90, 90.36 (C-1), 73.44 (C-3), 73.38 (C-5), 73.23 (C_{Bn}), 67.18 (C-4), 66.27 (C-2), 52.71 (CH_{3COOMe}), 37.83 (CH_{2Lev}), 37.77 (CH_{2Lev}), 37.69 (CH_{2Lev}), 29.93 (CH_{3Lev}), 29.88 (CH_{3Lev}), 29.84 (CH_{3Lev}), 27.97 (CH_{2Lev}), 27.93 (CH_{2Lev}), 27.89 (CH_{2Lev}) ppm; HRMS (ESI) *m/z* calcd for $C_{29}H_{36}O_{13}$ $[M+Na]^+$ 615.2054, found 615.2031.

Methyl 1,2,4-*tri*-*O*-benzoyl-3-*O*-benzyl-β-L-idopyranurate (51): The triol **48** (2.08g, 6.97 mmol) was dissolved in dry CH_2Cl_2 and cooled to -40 °C, benzoyl chloride (6.1 mL, 53 mmol), pyridine (5.6 mL, 69.7 mmol) and DMAP (0.1eq.) were added and the reaction was stirred overnight. The residue was dissolved in CH_2Cl_2, and water was carefully added with cooling and vigorous stirring to decompose the excess of benzoyl chloride. The product was extracted with CH_2Cl_2, washed with HCl 1M, saturated $NaHCO_3$ aq solution, water and brine, dried and concentrated. The crude was purified by column chromatography (hexane/ EtOAc, 8/2) to obtain the perbenzoylated compound **51** (3.86 g, 91%). $[\alpha]_D^{20}$ = -18.3 ° (*c* = 1, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.05 – 7.13 (m, 20H, aromatic), 6.49 (d, *J* = 1.3 Hz, H-1), 5.50 – 5.49 (m, 2H, H-2, H-4), 5.08 (d, $J_{4,5}$ = 1.9 Hz, 1H, H-5), 4.93 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.90 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.37 (t, *J* = 3.0 Hz, 1H, H-3), 3.73 (s, 3H, CH_3) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.70, 165.74, 165.66, 164.45, 136.89, 133.73, 133.63, 133.38, 130.34, 130.12, 129.22, 128.99, 128.82, 128.58, 128.48, 128.29, 128.10, 91.01 (C-1), 73.83, 73.79 (C-5, C-3), 73.50 (C_{Bn}), 68.09, 66.97 (C-2, C-4), 52.79 (CH_{3COOMe}) ppm; HRMS (ESI) *m/z* calcd for $C_{35}H_{30}O_{10}$ $[M+Na]^+$ 633.1736, found 633.1757.

Methyl (4-*O*-acetyl-3-*O*-benzyl-[1,2-*O*-(1-pent-4-enyloxyethylidene)]-β-L-idopyranuronate (52): The peracetylated compound **49** (109 mg, 0.257 mmol) was dissolved in dry CH_2Cl_2 (2.6 mL) and 30% HBr in AcOH (0.26 mL) was added at 0 °C and was stirred for 3h at this temperature. The reaction mixture was diluted with cold CH_2Cl_2 and washed with ice-cold water and cold saturated $NaHCO_3$ aq solution. The organic layer was dried over anhydrous $MgSO_4$, filtered, concentrated. The crude bromide productwas used in the next reaction without further purification. A solution of bromide in dry CH_2Cl_2 (0.5 mL) containing 2,6-lutidine (0.59 mL, 5.1 mmol) and *n*-pentenol (0.26 mL, 2.6 mmol) was stirred for 20 h at room temperature under argon. The mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ aq solution and water. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/ EtOAc, 6/4 containing 1% of triethylamine) to obtain the orthoacetate **52** as a viscous oil (71 mg, 0.15 mmol, 60% in 2 steps). $[\alpha]_D^{20}$ = -19.2 ° (*c* = 1, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 – 7.32 (m, 5H, aromatic), 5.83 – 5.75 (m, 1H, CH_{pent}), 5.53 (d, *J* = 2.7 Hz, H-1), 5.22 – 5.19 (m, 1H, H-4), 5.03 – 4.94 (m, 2H, CH_{2pent}), 4.81 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.68 (d, *J* = 11.7Hz, 1H, CH_2Ph), 4.54 (d, 1H, *J* = 1.3 Hz, H-5), 4.12 (t, *J* = 2.3 Hz, 1H, H-3),

4.05 – 4.04 (m, 1H, H-2), 3.78 (s, 3H, CH_{3COOMe}), 3.53 – 3.41 (m, 2H, CH_{2pent}), 2.12 – 2.07 (m, 2H, CH_{2pent}), 2.03 (s, 3H, CH_{3Ac}), 1.73 (s, 3H, CH_3), 1.66 – 1.62 (m, 2H, CH_{2pent}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.3, 168.3, 138.2 (CH_{pent}), 137.0, 128.7 – 128.1 (Caromatic), 124.1 (Cq, orthoester), 115.0 (CH_{2pent}), 96.7 (C-1), 76.0 (C-2), 73.04 (C_{Bn}), 71.5 (C-3), 69.8 (C-5), 67.0 (C-4), 61.3 (CH_{2pent}), 52.7 (CH_{3COOMe}), 30.3 (CH_{2pent}), 28.7 (CH_{2pent}), 25.3 (CH_3), 20.9 (CH_{3Ac}) ppm; HRMS (ESI) *m/z* calcd for $C_{23}H_{30}O_9$ $[M+Na]^+$ 473.1782, found 473.1763.

Methyl (3-*O*-benzyl-4-*O*-levulinoyl-[1,2-*O*-(1-pent-4-enyloxylevulinylidene)]-β-L-idopyranuronate (53): The perlevulinated compound **50** (133 mg, 0.22 mmol) was dissolved in dry CH_2Cl_2 and 30% HBr in AcOH (0.76 mL) was added at 0 °C and stirred for 3h. The mixture was diluted with cold CH_2Cl_2 and sequentially washed with ice-cold water and cold saturated $NaHCO_3$ aq solution. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The crude product was used in the next reaction without further purification. A solution of the bromide in dry CH_2Cl_2 (0.5 mL) containing 2,6-lutidine (0.52 mL, 4.47 mmol), *n*-pentenol (0.23 mL, 2.26 mmol) was stirred for 20 h at room temperature under argon. The mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ aq solution and water. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/EtOAc, 6/4 containing 1% of triethylamine) to obtain **53** as a viscous oil. (91 mg, 73%). $[\alpha]_D^{20}$ = -7.4 ° (*c* = 1, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 - 7.31 (m, 5H, aromatic), 5.82 – 5.74 (m, 1H, CH_{pent}), 5.49 (d, *J* = 2.7 Hz, 1H, H-1), 5.20 – 5.19 (m, 1H, H-4), 5.03 – 4.95 (m, 2H, CH_{2pent}), 4.81 (d, *J* = 11.7 Hz , 1H, CH_2Ph), 4.66 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.51 (d, 1H, *J* = 1.3 Hz, H-5), 4.08 - 4.05 (m, 2H, H-3, H-2), 3.79 (s, 3H, CH_{3COOMe}), 3.51 – 3.41 (m, 2H, CH_{2pent}), 2.81 – 2.69 (m, 4H, CH_{2Lev}), 2.54 – 2.51 (m, 2H, CH_{2Lev}), 2.29 – 2.25 (m, 2H, CH_{2Lev}), 2.18 (s, 3H, CH_{3Lev}), 2.16 (s, 3H, CH_{3Lev}), 2.10 – 2.06 (m, 2H, CH_{2pent}), 1.65 – 1.60 (m, 2H, $CH_{2,pent}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 208.4, 206.3, 172.0, 167.9, 138.1 (CH_{pent}), 137.0, 128.8, 128.4, 128.1, 124.4 (Cq, orthoester), 115.1 (CH_{2pent}), 96.5 (C-1), 75.6 (C-2), 73.1 (C_{Bn}), 71.8 (C-3), 69.7 (C-5), 67.0 (C-4), 61.5 (CH_{2pent}), 52.7 (CH_{3COOMe}), 38.7, 37.9 (CH_{2Lev}), 32.0 (CH_{2Lev}), 30.3 (CH_{2pent}), 30.0, 29.9 (CH_{3Lev}), 28.7 (CH_{2pent}), 28.1 (CH_{2Lev}) ppm; HRMS (ESI) *m/z* calcd for $C_{29}H_{38}O_{11}$ $[M+Na]^+$ 585.2312, found 585.2309.

Methyl (4-*O*-benzoyl-3-*O*-benzyl-[1,2-*O*-(1-pent-4-enyloxybenzylidene)]-β-L-idopyranuronate (54): The perbenzoylated compound **51** (310 mg, 0.508 mmol) was dissolved in dry CH_2Cl_2 (9.3 mL), 30% HBr in AcOH (1.72 mL) was added at 0 °C and the solution stirred for 3h. The mixture was diluted with cold CH_2Cl_2 and was washed with ice cold water and cold saturated $NaHCO_3$ aq solution. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The crude bromide was used in the next reaction without further purification. A solution of bromide in dry CH_2Cl_2 (1mL) containing 2,6-lutidine (1.18 mL, 10.2 mmol), *n*-pentenol (0.52 mL, 5.1 mmol) was stirred for 20 h at room temperature under argon. The mixture was diluted with CH_2Cl_2 (200 mL), washed with saturated $NaHCO_3$ aq solution and water. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/EtOAc, 7/3 containing 1% of triethylamine) to obtain compound **54** (214 mg, 73%). $[\alpha]_D^{20}$ = -1.7 ° (*c* = 1, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 – 7.21 (m, 16H, aromatic), 5.80 – 5.73 (m, 1H, CH_{pent}), 5.71 (d, *J* = 2.7 Hz, 1H, H-1), 5.47 – 5.46 (m, 1H, H-4), 5.01 – 4.97 (m, 3H, CH_{2pent}, CH_2Ph), 4.78 (d, *J* = 11.7 Hz, CH_2Ph), 4.68 (d, *J* = 1.3 Hz, 1H, H-5), 4.38 – 4.37 (m, 1H, H-2), 4.28 (t, *J* = 2.17 Hz, 1H, H-3), 3.69 (s, 3H, CH_{3COOMe}), 3.44 – 3.34 (m, 2H, CH_{2pent}), 2.11 – 2.05 (m, 2H, CH_{2pent}), 1.68 – 1.62 (m, 2H, CH_{2pent}); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.1, 165.9, 138.1 (CH_{pent}), 137.0, 136.9, 133.2, 130.0, 129.2, 128.8, 128.4, 128.4, 128.1, 127.1, 122.4 (Cq, orthoester), 115.0 (CH_{pent}), 96.9 (C-1), 75.4 (C-2), 73.2 (C_{Bn}), 72.1 (C-3), 70.2 (C-5), 67.1 (C-4), 63.5 (CH_{2pent}), 52.7 (CH_{3COOMe}), 30.3 (CH_{2pent}), 28.9 (CH_{2pent}); HRMS (ESI) *m/z* calcd for $C_{33}H_{34}O_9$ $[M+Na]^+$ 597.2101, found 597.2082.

Methyl (3-*O*-benzyl-[1,2-*O*-(1-pent-4-enyloxybenzylidene)]-β-L-*threo*-hex-4-enopyranuronate (55): The compound **54** (20 mg, 0.035 mmol) was dissolved in MeOH (0.5 mL) and was treated with catalytic amount of sodium methoxide (0.5 eq.) overnight. The crude was quenched with Amberlite® IR-120(H), filtered and concentrated to obtained compound **51** (14 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 – 7.31 (m, 10H, aromatic), 6.22 (dd, *J* = 1.1 Hz, 5.2 Hz, H-4), 5.96 (d, *J* = 4.0 Hz, H-1), 5.83 – 5.74 (m, 1H, CH_{pent}), 5.03 – 4.95 (m, 2H, CH_{2pent}), 4.80 – 4.76 (m, 1H, H-2), 4.67 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.60 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.27 – 4.25 (dd, *J* = 2.1, 5.2 Hz, 1H, H-3), 3.73 (s, 3H, CH_3), 3.52 – 3.43 (m, 2H, CH_{2pent}), 2.14 – 2.10 (m, 2H, CH_{2pent}), 1.71 – 1.66 (m, 2H, CH_{2pent}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.1, 143.6, 138.1 (CH_{pent}), 137.4, 137.1, 129.3, 128.7, 128.5, 128.3, 128.2, 128.1,

126.4, 122.3, 115.1 (CH_{2pent}), 108.1 (C-4), 96.8 (C-1), 71.2 (C_{Bn}), 67.1 (C-3), 63.2 (CH_{2pent}), 52.6 (CH_{3COOMe}), 30.3 (CH_{2pent}), 28.8 (CH_{2pent}) ppm.

Methyl 3-*O*-benzyl-[1,2-*O*-(1-pent-4-enyloxybenzylidene)]-β-L-idopyranuronate (56): To a solution of compound **54** (105 mg, 0.182 mmol) in dry toluene (0.4 mL), trimethyltinhydroxide (18 mg, 0.201 mmol) was added, the reaction mixture was stirred in the microwave at 100 °C until TLC indicated the disappearance of the starting material (1h). The mixture was concentrated and dried under high vacuum. The residue was dissolved in dry MeOH and a solution of 1M NaOMe (0.27 mL) was added. The mixture was placed in the microwave at 60 °C, and after 1h 30min. was quenched with Amberlite® IR-120(H) until pH=7. The reaction crude was dissolved in dry CH_2Cl_2 (0.4 mL), MeOH (1.1 mL), EDC·HCl (286 mg, 1.5 mmol) and DMAP (86 mg, 0.71 mmol) were added. After stirring overnight, the reaction mixture was concentrated and purified by flash chromatography (hexane/EtOAc , 6/4containing 1% of triethylamine) to obtain the compound **56** (52 mg, 60%). ^{1}H NMR (500 MHz, CD_3OD) δ = 7.72 – 7.32 (m, 10H, aromatic), 5.80 (m, 1H, CH_{pent}), 5.63 (d, J = 2.8 Hz, 1H, H-1), 5.05 – 4.92 (m, 2H, CH_{2pent}), 4.74 (m, 2H, CH_2Ph), 4.50 (d, J = 1.7 Hz, 1H, H-5), 4.36 – 4.32 (m, 1H, H-2), 4.07 – 4.02 (m, 2H, H-3, H-4), 3.73 (s, 3H, CH_3), 3.44 (m, 2H, CH_{2pent}), 2.11 (m, 2H, CH_{2pent}), 1.70 – 1.59 (m, 2H, CH_{2pent}) ppm; ^{13}C NMR (126 MHz, CD_3OD) δ = 170.8, 139.2, 139.0, 138.8, 130.2, 129.6, 129.2, 129.1, 129.0, 127.8, 123.5, 115.4, 98.6 (C-1), 77.4 (C-2), 76.0 (C-3), 73.8 (C_{Bn}), 73.0 (C-5), 68.0 (C-4), 64.1 (CH_{2pent}), 52.6 (CH_{3COOMe}), 31.3 (CH_{2pent}), 29.9 (CH_{2pent}) ppm; HRMS (ESI) *m/z* calcd for $C_{26}H_{30}O_8$ $[M+Na]^+$ 493.1838, found 493.1832.

Methyl (3-*O*-benzyl-4-*O*-levulinoyl-[1,2-*O*-(1-pent-4-enyloxybenzylidene)]-β-L-idopyranuronate (57): The reaction was carried out according to general procedure A using compound **56** (205 mg, 0.44 mmol), levulinic acid (152 mg, 1.31 mmol), EDC·HCl (250 mg, 1.31 mmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (4 mL). The crude was purified by flash chromatography (hexane/EtOAc, 8/2 containing 1% of triethylamine) to obtain the compound **57** as a viscous oil (225 mg, 90%). $[\alpha]_D^{20}$ = +19.7 ° (c = 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 – 7.73 (m, 2H, aromatic), 7.38 – 7.33 (m, 5H, aromatic), 5.80 – 5.74 (m, 1H, CH_{pent}), 5.66 (d, J = 2.8Hz, H-1), 5.17 (m, 1H, H-4), 5.02 – 4.94 (m, 2H, CH_{2pent}), 4.82 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.70 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.55 (d, J = 1.0 Hz, 1H, H-5), 4.31 – 4.30 (m, 1H, H-2),

4.14 – 4.13 (m, 1H, H-3), 3.75 (s, 3H, CH_{3COOMe}), 3.48 – 3.41 (m, 2H, CH_{2pent}), 2.34 – 2.18 (m, 2H, CH_{2Lev}), 2.13 – 2.08 (m, 2H, CH_{2Lev}), 2.00 (s, 3H, CH_{3Lev}), 1.70 – 1.64. (m, 2H, CH_{2pent}); ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.4, 171.7, 167.9, 138.1 (CH_{pent}), 137.4, 137.0, 129.2, 128.8, 128.4, 128.1, 128.0, 127.0, 122.4 (Cq, orthoester), 115.0 (CH_{2pent}), 96.8 (C-1), 75.4 (C-2), 73.1 (C_{Bn}), 71.6 (C-3), 69.7 (C-5), 66.8 (C-4), 63.3 (CH_{2pent}), 52.6 (CH_{3COOMe}), 37.6 (CH_{2Lev}), 30.3 (CH_{2Lev}), 29.7 (CH_{3Lev}), 28.9 (CH_{2Lev}), 28.1 (CH_{2Lev}) ppm; HRMS (ESI) *m/z* calcd for $C_{31}H_{36}O_{10}$ $[M+Na]^+$ 591.2206, found 591.2197.

5.6.3 Synthesis of 2-azido-D-glucopyranose (Glc) building blocks

2-Azido-4,6-*O*-benzylidene-2-*O*-deoxy-β-D-glucopyranoside (59): In a 1L three-neck round-botton flask, equipped with a dropping funnel, an internal thermometer and an argon inlet, NaN_3 (62.22g) was dissolved at room temperature in water. CH_2Cl_2 was added, the mixture was cooled to 0ºC and to the vigorously stirred solution Tf_2O (32mL) was added within 1h30min, while keeping the temperature unchanged. After completation of the addition, the mixture was stirred for 2h at 0 ºC, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2x). The combined organic layers were washed with saturated $NaHCO_3$ aq solution, dried over anhydrous $MgSO_4$ and filtered to obtain a 0.4 M TfN_3 solution.

In a 2L three-neck round-botton flask, equipped with a dropping funnel, a septum and a argon inlet, a suspension of D-glucosamine hydrochloride (10 g) in dry MeOH (200mL) was treated with a 0.5M solution of NaOMe in MeOH (110 mL).

After stirring for 30min. at room temperature, the mixture was diluted with MeOH and treated with 4-(dimethylamino)pyridine (6 g) to afford a clear and colourless solution, to wich 0.4M TfN_3 solution (350mL) was added dropwise at room temperature within 2h.(WARNING: TfN_3 has been reported to be explosive when not in solvent and should always be used as a solution). After stirring 48h at room temperature under argon, solvent was concentrated at 30ºC to furnish an oily residue wich was dissolved in MeOH, treated with NH_4Cl until pH 7, concentrated and finally filtered through a short silica column (4:1, CH_2Cl_2:CH_3OH). After concentration, a mixture of the residue, benzaldehyde dimethyl acetal (10.4mL) and a catalytic amount of 10-camphor-sulfonic acid in 60mL of dry DMF was stirred for 5h under reduced pressure. The mixture was

neutralized with solid $NaHCO_3$ and concentrated. The residue was purified by column chromatography (toluene/EtOAc, 8/2) to yield the product (10.3 g, 76%). The spectroscopic data of the compound were in agreement with the data previously reported in literature.[6]

Tert-butyldimethysilyl-2-Azido-4,6-*O*-benzylidene-2-*O*-deoxy-β-D-glucopyranoside (60): To a solution of **59 (**7.74 g, 26.39 mmol) in dry CH_2Cl_2 (10 mL) was added imidazole (better in solution), and at -20ºC TBDMSCl (4.37 g, 29.03 mmol) was added. The mixture was stirred for 6h and the reaction was partitioned betweenwater and CH_2Cl_2. The organic layer was washed with water and extracted with CH_2Cl_2 (2x25mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by column chromatography (Hexane/EtOAc, 7/1) to afford the product **60** (8.51 g, 20.88 mmol) in 79% of yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.50 – 7.45 (m, 2H, aromatic), 7.40 – 7.33 (m, 3H, aromatic), 5.54 (s, 1H, PhCHO), 4.66 (d, *J* = 7.6Hz, 1H, H-1), 4.30 (dd, *J* = 10.5Hz, 5.0Hz, 1H, H-6a), 3.79 (m, 1H, H-6b), 3.64 (m, 1H, H-3), 3.58 (m, 1H, H-4), 3.42 (m, 1H, H-5), 3.35 (m, 1H, H-2), 2.61 (s, 1H, OH), 0.94 (s, 9H, $(CH_3)_3C$), 0.17 (s, 6H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 136.85 (qC, TBS), 129.39, 128.39, 126.28 (Caromatic), 102.04 (PhCHO), 97.58 (C-1), 80.74 (C-4), 71.83 (C-3), 69.00 (C-2), 68.57 (C-6), 66.33 (C-5), 25.56 (CH_3, TBS), -4.35, -5.17 (CH_3, TBS) ppm.

Tert-butyldimethysilyl -2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-deoxy-β-D-glucopyranoside (61): NaH (60% dispersion in mineral oil) was added to a cooled (0 ºC) solution of **60** (5.85 g, 14.35 mmol) in dry CH_2Cl_2 (60 mL). After the mixture was stirred for 3h, benzyl bromide and catalytic TBAI were added. The reaction was quenched with MeOH, was diluted with CH_2Cl_2 and was washed with NH_4Cl solution and water. The aqueous layers were extracted with CH_2Cl_2, and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. The crude product was crystallized from ethanol to give the title compound **61** as white solid (5.46 g, 10.97 mmol, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (m, 2H, aromatic), 7.41 – 7.27 (m, 8H, aromatic), 4.90 (d, *J* = 11.4Hz, 1H, CH_2Ph), 4.79 (d, *J* = 11.4Hz, 1H, CH_2Ph), 4.59 (d, *J* = 7.7Hz, 1H, H-1), 4.29 (dd, *J* = 10.5Hz, 5.0Hz, 1H, H-6), 3.79 (m, 1H, H-6), 3.71 (m, 1H, H-3), 3.52 (m, 1H, H-4), 3.38 (m, 2H, H-5, H-2), 0.94 (s, 9H, $(CH_3)_3C$), 0.16 (s, 6H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 137.98 (qC), 137.20 (qC), 129.05, 128.36,

128.28, 128.12, 127.82, 126.03 (Caromatic), 101.34 (PhCHO), 97.51 (C-1), 81.65 (C-3), 78.84 (C-4), 74.84 (CH_2Ph), 68.76 (C-2), 68.63 (C-6), 66.34 (C-5), 25.57 (CH_3, TBS), -4.34, -5.17 (CH_3, TBS) ppm.

2-Azido-3-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy-β-D-glucopyranose (62): EtSH (8 mL, 103.52 mmol) and catalytic *p*TsOH were added to a solution of 2-azido-3-*O*-benzyl-4,6-*O*-benzyliden-1-*O*-tert-butyldimethylsilyl-2-deoxy-β-D-glucopyranose (**26**) (10.3 g, 20.70 mmol) in dry CH_2Cl_2. After stirring for 3h under argon, the mixture was neutralized with solid $NaHCO_3$, diluted with CH_2Cl_2 and washed with water. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to dryness. The residue was purified by column chromatography (hexane/EtOAc, 7/3) to yield **27** (7.25 g, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.38 – 7.29 (m, 5H, aromatic), 4.95 (d, J = 11.5 Hz, 1H, CH_2Ph), 4.71 (d, J = 11.4 Hz, 1H, CH_2Ph), 4.57 (d, J = 7.6 Hz, 1H, H-1), 3.83 (dd, J = 11.8, 3.7 Hz, 1H, H-6a), 3.74 (dd, J = 11.8, 5.0 Hz, 1H, H-6b), 3.58 (dd, J = 9.7, 8.7 Hz, 1H, H-4), 3.32 – 3.27 (m, 2H, H-2, H-5), 3.21 (dd, J = 9.9, 8.7 Hz, 1H, H-3), 0.95 (s, 9H, TBS), 0.17 and 0.16 (2s, 6H, TBS); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 138.2, 128.8, 128.3, 128.2, 97.4 (C-1), 82.5 (C-3), 75.3 (C-5), 75.1 (C_{Bn}), 70.6 (C-4), 68.4 (C-2), 62.7 (C-6), 25.7 (CH_{3TBS}), 18.1 (Cq_{TBS}), -4.2 (CH_{3TBS}), -5.0 ppm (CH_{3TBS}); HRMS (ESI) *m/z* calcd for $C_{19}H_{31}N_3O_5Si$ $[M+Na]^+$ 432.1931, found 432.1913.

2-Azido-6-*O*-benzoyl-3-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy-β-D-glucopyranose (63): BzCN (2.1 mL of 0.9 M solution in dry CH_3CN) and catalytic Et_3N (3.5 mL) were added to a cooled (-40 °C) solution of **62** (7.25 g, 17.70 mmol) in dry CH_3CN (35 mL). After 4h, additional BzCN was added (0.5 mL) until starting material had disappeared. After 7h, MeOH was added and the mixture was allowed to reach room temperature. The solvent was concentrated, and the residue was dissolved in MeOH and concentrated to dryness. The purification was carried out by column chromatography (hexane/ EtOAc, 4/1) to afford the product **63** (8.18 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.06 – 8.04 (m, 2H, aromatic), 7.59 – 7.56 (m, 1H, aromatic), 7.45 – 7.32 (m, 7H, aromatic), 5.96 (d, J = 11.4 Hz, 1H, CH_2Ph), 4.76 (d, J = 11.4 Hz, 1H, CH_2Ph), 4.61 – 4.58 (m, 2H, H-6a, H-1), 4.55 (dd, J = 12.0 Hz, 5.1 Hz, 1H, H-6b), 3.58 – 3.51 (m, 2H, H-4, H-5), 3.35 (dd, J = 7.6 Hz, 9.9 Hz, 1H, H-2), 3.25 (dd, J = 9.9, 8.0 Hz, 1H, H-3), 2.74 (bs, 1H, OH), 0.93 (s, 9H, CH_{3TBS}), 0.16 and 0.15 (2s, 6H, CH_{3TBS}); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 166.9, 138.1, 133.3, 130.0, 129.9, 129.9,

129.8, 128.8, 128.8, 128.5, 128.5, 128.4, 128.3, 128.2, 97.5 (C-1), 82.2 (C-3), 75.3 (CH_2Ph), 74.0 (C-5), 70.4 (C-4), 68.3 (C-2), 64.0 (C-6), 25.7 (CH_{3TBS}), 18.1 (Cq_{TBS}), -4.2 (CH_{3TBS}), -5.1 (CH_{3TBS}); HRMS (ESI) *m/z* calcd for $C_{26}H_{35}N_3O_6Si$ $[M+Na]^+$ 536.2187, found 536.2214.

2-Azido-6-*O*-benzoyl-3-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy-4-*O*-levulinoyl-β-D-glucopyranose (64): The reaction was carried out according to general procedure A using compound **63** (9.81 g, 19.09 mmol), levulinic acid (3.32 g, 28.6 mmol), EDC·HCl (5.5 g, 28.6 mmol) and a catalytic amount of DMAP (50 mg, 0.44 mmol) in dry CH_2Cl_2 (5 mL). The residue was purified by column chromatography (hexane/EtOAc, 9/1) to obtain compound **64** (9.96 g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.06 – 8.02 (m, 2H, aromatic), 7.58 – 7.53 (m, 1H, aromatic), 7.46 – 7.40 (m, 2H, aromatic), 7.38 – 7.26 (m, 5H, aromatic), 5.09 – 5.02 (m, 1H, H-4), 4.82 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.67 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.57 (m, 1H, H-1), 4.49 (dd, *J* = 12.1, 2.4 Hz, 1H, H-6), 4.28 (dd, *J* = 12.1, 6.8 Hz, 1H, H-6), 3.73 – 3.68 (m, 1H, H-5), 3.44 – 3.40 (m, 2H, H-2, H-3), 2.77 – 2.58 (m, 2H, CH_{2Lev}), 2.54 – 2.46 (m, 1H, CH_{2Lev}), 2.42 – 2.34 (m, 1H, CH_{2Lev}), 2.12 (s, 3H, CH_{3Lev}), 0.89 (s, 9H, TBS), 0.11 (s, 6H, TBS); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.28, 171.80, 166.22, 137.96, 133.22, 129.94, 129.89, 128.56, 128.44, 128.12, 127.99, 97.34 (C-1), 80.01 (C-3), 75.01 (C_{Bn}), 72.43 (C-5), 70.72 (C-4), 68.46 (C-2), 63.36 (C-6), 37.95 (CH_{2Lev}), 29.83 (CH_{3Lev}), 27.99 (CH_{2Lev}), 25.66 (CH_{3TBS}), 18.06 (Cq_{TBS}), -4.21 (CH_{3TBS}), -5.14 (CH_{3TBS}) ppm; HRMS (ESI) *m/z* calcd for $C_{31}H_{41}N_3O_8Si$ $[M+Na]^+$ 634.2561, found 634.2565.

2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-O-levulinoyl-α-D-glucopyranosyl trichloroacetimidate (65): To a cooled (0 °C) solution of compound **64** (2.0 g, 3.26 mmol) in dry THF (16 mL), AcOH (0.20 mL) and TBAF (1.38 mL, 3.59 mmol) were added. After 3h, water was added and the mixture was diluted with EtOAc and washed with water. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated to dryness. The residue was concentrated and use in the next reaction without further purification. The hemiacetal (1.62 g, 3.27 mmol) and trichloroacetonitrile (4.9 mL, 49 mmol) were dissolved in anhydrous CH_2Cl_2 (32 mL) with activated 4 Å molecular sieves. After 30 minutes of stirring, the solution was cooled to 0 °C and DBU (48 µL, 0.33 mmol) was added. After 2 h, TLC (hexane/ EtOAc, 3/1) indicated complete conversion of the starting material.

The reaction mixture was concentrated under reduced pressure. The title compound **65** was obtained after column chromatography on silica (hexane/ EtOAc, 1:0 to 2:1) as a colorless solid (1.78 g, 85%). $[\alpha]_D^{20}$ = + 64.9° (*c* = 1.0, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.75 (s, 1H, OCN*H*Cl_3), 8.01 (m, 2H, aromatic), 7.55 (m, 1H, aromatic), 7.42 (m, 2H, aromatic), 7.33 (m, 2H, aromatic), 6.45 (d, *J* = 3.5 Hz, 1H, H-1), 5.30 (dd, *J* = 9.6, 9.3 Hz, 1H, H-4), 4.86 (d, *J* = 11.0 Hz, 1H, CH_2Ph), 4.77 (d, *J* = 11.0 Hz, 1H, CH_2Ph), 4.50 (dd, *J* = 12.3, 2.1 Hz, 1H, H-6a), 4.32 (dd, *J* = 12.3, 5.1 Hz, 1H, H-6b), 4.26 (ddd, *J* = 9.3, 5.1, 2.1 Hz, 1H, H-5), 4.08 (dd, *J* = 10.1, 9.6 Hz, 1H, H-3), 3.78 (dd, *J* = 10.1, 3.5 Hz, 1H, H-2), 2.77 – 2.62 (m, 2H, CH_{2Lev}), 2.54 – 2.50 (m, 1H, CH_{2Lev}), 2.45 (m, 1H, CH_{2Lev}), 2.12 (s, 3H, CH_{3Lev}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.1 (OCNH), 171.5 (C_q), 166.0 (C_q), 160.4 (C_q), 137.2 - 128.0 (C_{arom}), 94.2 (C-1), 77.6 (C-3), 77.2 (CCl_3), 75.1 (C_{Bn}), 70.8 (C-5), 70.0 (C-4), 62.6 (C-2), 62.2 (C-6), 37.7 (CH_{2Lev}), 29.6 (CH_{3Lev}), 27.8 (CH_{2Lev}) ppm, HRMS (ESI) *m/z* calcd for $C_{27}H_{27}Cl_3N_4O_8$ $[M+Na]^+$ 663,0787, found 663.0784.

2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-O-levulinoyl-α/β-D-glucopyranosyl *N*-phenyl trifluoroacetimidate (66): To a cooled (0°C) solution of compound **64** (773 mg, 1.26 mmol) in dry THF (6 mL) , AcOH (79 µL) and TBAF (1.38 mL, 1.26 mmol) were added. After 3h, water was added and the mixture was diluted with EtOAc and washed with water. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated to dryness. The residue was concentrated and use in the next reaction without further purification. To a solution of hemiacetal (636 mg, 1.28 mmol) in acetone (10 mL), potassium carbonate (350 mg, 2.56 mmol) and (*N*-phenyl) trifluoroacetimidoyl chloride[7] (600 mg, 2.89 mmol) were added. The reaction was stirred overnight and the solvent was concentrated. Flash chromatography on silica gel (hexane hexane/ EtOAc, 1/0 to 2:1 containing 1% of triethylamine) afforded a mixture (1:1) of **66**α and **66**β (765 mg, 91%). α-anomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 – 8.03 (m, 2H, aromatic), 7.59 – 7.56 (m, 1H, aromatic), 7.45 – 7.42 (m, 2H, aromatic), 7.37 – 7.31 (m, 5H, aromatic), 7.26 – 7.22 (m, 2H, aromatic), 7.11 – 7.08 (m, 1H, aromatic), 6.72 (d, 2H, aromatic), 6.42 (bs, 1H, H-1), 5.27 (t, *J* = 9.8 Hz, 1H, H-4), 4.88 (d, *J* = 11.0 Hz, CH_2Ph), 4.77 (d, *J* = 11.0 Hz, CH_2Ph), 4.51 (dd, *J* = 12.4 Hz, 2.3 Hz, 1H, H-6_a), 4.36 (dd, *J* = 12.4, 5.4 Hz, 1H, H-6_b), 4.19 – 4.17 (m, 1H, H-5), 4.05 (t, *J* = 9.7 Hz, 1H, H-3), 3.76 – 3.74 (m, 1H, H-2), 2.67 – 2.64 (m, 2H, CH_{2Lev}), 2.6 – 2.53 (m, 1H, CH_{2Lev}), 2.46 – 2.40 (m, 1H, CH_{2Lev}), 2.13 (s,

3H, CH_{3Lev}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.2 (OCNPh), 171.7 (Cq), 166.2, 143.1, 137.4, 133.3, 129.9, 129.0, 128.7, 128.6, 128.2, 124.7, 119.4, 92.7 (C-1), 78.0 (C-3), 75.5 (C_{Bn}), 70.9 (C-5), 70.2 (C-4), 62.7 (C-2), 62.4 (C-6), 37.9 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.0 (CH_{2Lev}) ppm. β-anomer: ^1H NMR (500 MHz, $CDCl_3$) δ = 8.02 – 8.00 (m, 2H, aromatic), 7.56 – 7.53 (m, 1H, aromatic), 7.39 – 7.30 (m, 7H, aromatic), 7.25 – 7.22 (m, 2H, aromatic), 7.12 – 7.09 (m, 1H, aromatic), 6.75 – 6.74 (m, 2H, aromatic), 5.60 (bs, 1H, H-1), 5.17 (t, *J* = 9.7 Hz, 1H, H-4), 4.85 (d, *J* = 11.3 Hz, 1H, CH_2Ph), 4.74 (d, *J* = 11.3 Hz, 1H, CH_2Ph), 4.49 (dd, *J* = 12.4, 2.2 Hz, 1H, H-6_a), 4.32 (dd, *J* = 12.3, 6.2 Hz, 1H, H-6_b), 3.79 – 3.75 (m, 2H, H-2, H-5), 3.60 – 3.56 (m, 1H, H-3), 2.74 – 2.62 (m, 2H, CH_{2Lev}), 2.54 – 2.48 (m, 1H, CH_{2Lev}), 2.44 – 2.38 (m, 1H, CH_{2Lev}), 2.12 (s, 3H, CH_{3Lev}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.2, 171.7, 166.2, 143.1, 137.5, 133.2, 129.9, 129.8, 128.9, 128.8, 128.6, 128.5, 128.26, 128.2, 124.6, 119.3, 95.5 (C-1), 80.3 (C-3), 75.4 (C_{Bn}), 73.3 (CF_3), 70.0 (C-4), 65.1 (C-5, C-2), 62.7 (C-6), 37.9 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.0 (CH_{2Lev}) ppm.

5.6.3.1 Synthesis of the glucosamine non-reducing end building block

2-Azido-3,4-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy -β-D-glucopyranose (67): The compound **61** (2.91 g, 5.92 mmol) was dissolved in 1M borane in THF (30 mL) and catalytic amount of copper triflate was added (214 mg, 0.59 mmol). The reaction was stirred for 2h at room temperature and was cooled and quenched by addition of triethylamine and MeOH. The crude was concentrated and purified by column chromatography on silica gel (hexane/EtOAc; 9/1 to 8/2) to afford compound **67** (2.5 g, 85%). $[\alpha]_D^{20}$ = -22.3° (*c* = 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.47 – 7.27 (m, 10H, aromatic), 4.92 (d, *J* = 10.8 Hz, 1H, CH_2Ph), 4.88 (d, *J* = 11.0 Hz, 1H, CH_2Ph), 4.83 (d, *J* = 10.8 Hz, 1H, CH_2Ph), 4.68 (d, *J* = 11.0 Hz, 1H, CH_2Ph), 4.58 (d, *J* = 7.7 Hz, 1H, H-1), 3.85 (dd, *J* = 11.9, 2.8 Hz, 1H, H-6a), 3.76 – 3.68 (m, 1H, H-6b), 3.60 (t, *J* = 9.3 Hz, 1H, H-4), 3.45 (t, *J* = 9.4 Hz, 1H, H-3), 3.39 – 3.31 (m, 2H, H-2, H-5), 1.94 – 1.84 (bs, 1H, OH), 0.98 (s, 9H, CH_{3TBS}), 0.20 (s, 3H, CH_{3TBS}), 0.19 (s, 3H, CH_{3TBS}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 138.15, 137.96, 128.62, 128.57, 128.12, 128.08, 127.96, 97.21 (C-1), 82.95 (C-3), 77.63 (C-4), 75.56 (C-5), 75.49 (C_{Bn}), 75.13 (C_{Bn}), 68.97 (C-2), 62.11 (C-6), 25.71 (CH_{3TBS}), 18.07 (Cq), -4.13 (CH_{3TBS}), -5.00 (CH_{3TBS}) ppm.

2-Azido-6-*O*-benzoyl-3,4-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy-β-D-glucopyranose (68): The compound **67** (1.35 g, 2.70 mmol) was dissolved in dry CH_2Cl_2 (27 mL) and benzoyl chloride (0.62 mL, 5.4 mmol), pyrdine (3 mL) and DMAP (270 mg, 0.27 mmol) were added at 0 °C. The reaction was stirred for 2 hours and was quenched by addittion of MeOH. The reaction crude was dissolved with CH_2Cl_2 and was washed with 1M HCl, $NaHCO_3$ solution and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated. The crude was purified by column chromatography (hexane/EtOAc, 1:0 to 9:1) to afford compound **68** (1.63 g, quant). $[\alpha]_D^{20}$ = +6.5° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 – 7.97 (m, 2H, aromatic), 7.58 (ddt, *J* = 8.8, 7.2, 1.3 Hz, 1H, aromatic), 7.48 – 7.26 (m, 12H, aromatic), 4.95 (d, *J* = 10.7 Hz, 1H, CH_2Ph), 4.90 (d, *J* = 10.9 Hz, 1H, CH_2Ph), 4.82 (d, *J* = 10.7 Hz, 1H, CH_2Ph), 4.67 – 4.60 (m, 3H, CH_2Ph, H-6), 4.59 (d, J = 7.6 Hz, H-1), 4.40 (dd, *J* = 11.8, 5.9 Hz, 1H, H-6), 3.65 (ddd, *J* = 9.8, 5.9, 2.1 Hz, 1H, H-5), 3.59 (dd, *J* = 9.8, 8.4 Hz, 1H, H-4), 3.47 (dd, *J* = 10.0, 8.4 Hz, 1H, H-3), 3.40 (dd, *J* = 9.9, 7.6 Hz, 1H, H-2), 0.92 (s, 9H, CH_{3TBS}), 0.15 (s, 3H, CH_{3TBS}), 0.13 (s, 3H, CH_{3TBS}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 166.26, 138.05, 137.66, 133.20, 130.04, 129.83, 128.65, 128.46, 128.24, 128.18, 128.16, 128.08, 97.33 (C-1), 83.15 (C-3), 77.90 (C-4), 75.72 (CBn), 75.24 (CBn), 73.50 (C-5), 68.94 (C-2), 63.67 (C-6), 25.71 (CH_{3TBS}), 18.08 (Cq), -4.18 (CH_{3TBS}), -5.06 (CH_{3TBS}) ppm.

2-Azido-6-*O*-benzoyl-3,4-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy–α/β-D-glucopyranosyl trichloroacetimidate (70): The compound **68** (181 mg, 0.299 mmol) was dissolved in dry THF (1.5 mL), TBAF (0.33 mL) and acetic acid (18 µL) were added at 0 °C. After 3h, the reaction was diluted with EtOAc and washed with saturated $NaHCO_3$ aq solution, water and brine. The organic phase was dried under anhydrous $MgSO_4$ and concentrated. The crude product was used in the next reaction without further purification. The hemiacetal **69** was dissolved in dry CH_2Cl_2 (3 mL), trichloroacetonitrile (0.36 mL) and DBU (9 µL) were added at 0 °C. The reaction mixture was stirred overnight at room temperature, concentrated and purified by column chromatography (hexane/EtOAc; 9/1 to 7/3 containing 5% of triethylamine) to obtain compound **70** (120 mg, 0.190 mmol) as an α/β (7/3) mixture . α-anomer ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (s, 1H, NH), 7.99 (dd, *J* = 8.3, 1.4 Hz, 2H, aromatic), 7.61 – 7.54 (m, 1H, aromatic), 7.48 – 7.21 (m, 12H, aromatic), 6.45 (d, *J* = 3.5 Hz, 1H, H-1), 4.97

(s, 2H, CH_2Ph), 4.91 (d, *J* = 10.7 Hz, 1H, CH_2Ph), 4.66 (d, *J* = 10.7 Hz, 1H, CH_2Ph), 4.57 (dd, *J* = 12.2, 2.2 Hz, 1H, H-6a), 4.49 (dd, *J* = 12.2, 4.1 Hz, 1H, H-6b), 4.20 (ddd, *J* = 10.1, 4.0, 2.1 Hz, 1H, H-4), 4.11 (dd, *J* = 10.3, 8.9 Hz, 1H, H-3), 3.81 (dd, *J* = 10.2, 8.9 Hz, 1H, H-5), 3.74 (dd, *J* = 10.2, 3.6 Hz, 1H, H-2) ppm.^{13}C NMR (126 MHz, $CDCl_3$) δ = 166.13, 161.11, 160.86, 137.69, 137.55, 137.25, 133.32, 133.25, 130.01, 129.90, 129.86, 129.80, 128.77, 128.74, 128.72, 128.70, 128.55, 128.52, 128.40, 128.37, 128.34, 128.30, 128.26, 128.24, 96.89, 94.74, 83.33, 80.46, 77.78, 77.26, 75.99, 75.94, 75.66, 75.35, 74.22, 72.15, 65.97, 63.36, 62.98, 62.78 ppm.

4.6.4 Synthesis of Galactosamine Building Block

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-*p*-methoxyphenylimino-D-galactopyranose (72): To a solution of D-galactosamine hydrochloride (10 g, 46.4 mmol) in NaOH 1M (47mL), p-anisaldehyde (5.6 mL) was added. After stirred for 30 min (a white solid was formed) and was stored in the freezer for 2h. The solid was filtered and was washed with cold water and diethyl ether and dried in high vacuum overnight. The white solid was dissolved in pyridine (75 mL) and acetic anhydride (42 mL) was added at 0 °C and was stirred for 24 h. The reaction mixture was poured into ice-water mixture. The solid formed was filtered, washed with cold water and diethyl ether and dried to obtain **72** as a white solid (9.6 g, 44 % in 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H, imine), 7.67 – 7.65 (m, 2H, aromatic), 6.92 – 6.91 (m, 2H, aromatic), 5.93 – 5.91 (d, *J* = 8.2Hz, H-1), 5.46 – 5.45 (m, 1H, H-4), 5.26– 5.23 (dd, *J* = 9.9, 3.2 Hz, H-3), 4.22 – 4.14 (m, H-6, H-5), 3.84 (s, 3H, CH_3-PMP), 3.62 – 3.59 (m, 1H, H-2), 2.17 (s, 3H, CH_{3Ac}), 2.05 (s, 3H, CH_{3Ac}), 2.02 (s, 3H, CH_{3Ac}), 1.88 (s, 3H, CH_{3Ac}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.52, 170.21, 169.77, 168.83, 164.56, 162.34, 130.29, 128.56, 114.14, 93.63, 71.87, 71.67, 68.93, 66.07, 61.44, 55.51, 20.88, 20.81, 20.79, 20.62 ppm. HRMS (ESI): *m/z* calcd. for $C_{22}H_{27}NO_{10}$ $[M+H]^+$ 466.1708, found 466.1692.

1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-D-galactopyranose (73): The compound **72** (5.0 g, 10.8 mmol) was dissolved in hot acetone (57 mL) and HCl 5N (2.4 mL) was added dropwise. A white solid was formed and the reaction was kept at 0 °C for 2h. The solid was filtered and washed with cold diethyl ether to obtain the compound **73** (3.5 g, 94%). $[\alpha]_D^{20}$ = + 14.6° (*c* = 0.5, MeOH), ^{1}H NMR (500 MHz, CD_3OD) δ 5.92 – 5.90 (d, *J* = 8.6 Hz, 1H, H-1), 5.44 – 5.43 (m, 1H, H-4), 5.31 – 5.28 (dd, *J* = 11.1, 2.5 Hz, H-3),

4.29 – 4.27 (t, *J* = 3.4 Hz, 1H, H-5), 4.19 – 4.10 (m, 2H, H-6), 3.65 – 3.61 (dd, *J* = 8.8, 11.1 Hz, H-2), 2.21 (s, 3H, CH_{3Ac}), 2.15 (s, 3H, CH_{3Ac}), 2.07 (s, 3H, CH_{3Ac}), 2.02 (s, 3H, CH_{3Ac}) ppm; ^{13}C NMR (126 MHz, CD_3OD) δ 171.98, 171.67, 171.15, 170.07, 91.90, 73.08, 70.64, 67.22, 62.24, 51.45, 20.70, 20.60, 20.49, 20.36 ppm. HRMS (ESI): *m/z* calcd. for $C_{14}H_{21}NO_9$ $[M+H]^+$ 348.1289, found 348.1279.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-trichloroacetamidate-D-galactopyranose (74): The compound **73** (4.55 g, 13.1 mmol) was dissolved in dry CH_2Cl_2 (56 mL), triethylamine (3.65 mL, 26.2 mmol) and trichloroacetyl chloride (1.9 mL, 17.03 mmol) were added at 0 °C. After 3h of stirring at room temperature, the solution was diluted with CH_2Cl_2 and was washed with water and saturated $NaHCO_3$ aq solution. The organic extracts were dried over anhydrous $MgSO_4$, filtered, concentrated and purified by column chromatography using hexane: EtOAc (9: 1 to 7: 3) to obtain the compound **74** (5.9 g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.94 – 6.89 (d, *J* = 9.5 Hz, 1H, N*H*TCA), 5.87 – 5.83 (d, *J* = 8.8 Hz, 1H, H-1), 5.42 – 5.39 (dd, *J* = 3.4, 1.2 Hz, 1H, H-4), 5.28 – 5.23 (dd, *J* = 11.3, 3.3 Hz, 1H, H-3), 4.47 – 4.40 (m, 1H, H-5), 4.21 – 4.11 (m, 2H, H-6), 4.07 – 4.03 (m, 1H, H-2), 2.21 – 2.18 (s, 3H), 2.14 – 2.12 (s, 3H, CH_{3Ac}), 2.07 – 2.05 (s, 3H, CH_{3Ac}), 2.03 – 2.00 (s, 3H, CH_{3Ac}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.80, 170.61, 170.20, 169.60, 162.51, 92.51, 72.30, 69.91, 66.45, 61.41, 51.87, 20.90, 20.81, 20.76, 20.65 ppm. HRMS (ESI): *m/z* calcd. for $C_{16}H_{20}Cl_3NO_{10}$ $[M+Na]^+$ 514.0045, found 514.0070.

1-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranose (75): Compound **74** (8.33 g, 16.90 mmol) was dissolved in DMF (180 mL) and hydrazine acetate (1.90 g, 20.63 mmol) was added. After stirring for 3 h at room temperature, the reaction mixture was diluted with EtOAc (400 mL) and washed with 1M HCl aq solution (50 mL). The aqueous phase was extracted with EtOAc (2x100 mL) and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated to give hemiacetal as an oil. Without further purification this intermediate compound was dissolved in DMF (57 mL), imidazole (2.30 g, 33.72 mmol) and *tert*-butyldimethylsilyl chloride (3.05 g, 20.23 mmol) were added at room temperature. After the mixture was stirred for 3 h, water (60 mL) was added and the solution was diluted with EtOAc (300 mL). The aqueous phase was extracted with EtOAc (2x200 mL) and

the combined organic extracts were washed with 1M HCl, water, saturated $NaHCO_3$ aq solution and brine. The organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude residue (8.20 g, 14.52 mmol) was dissolved in MeOH (35 mL) and 3.8 mL of a 0.25M methanolic sodium methoxide solution was added at room temperature. After 2 h, the reaction mixture was diluted with methanol (50 mL) and neutralized by addition of Amberlite® IR-120(H). The solution was filtered and concentrated to yield **75** (6.35 g, 86 %) as a colorless foam. $[\alpha]_D^{20}$ + 11.1 (*c* 0.13, MeOH), ^{1}H NMR (500 MHz, CD_3OD) δ 4.80 (d, *J* = 8.1 Hz, H-1), 3.94 – 3.90 (m, 1H, H-2), 3.88 (d, *J* = 2.97 Hz,1H, H-4), 3.80 - 3.76 (m, 2H, H-6, H-3), 3.71 (dd, *J* = 6.2, 11.1 Hz, 1H, H-6), 3.51 (t, *J* = 6.2 Hz, 1H, H-5), 0.89 (s, 9H, CH_{3TBS}), 0.14 (s, 3H, $SiCH_{3TBS}$), 0.12 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, CD_3OD) δ 97.47, 76.60, 71.96, 69.65, 62.11, 58.50, 26.24, -3.83, -4.98 ppm. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd. for $C_{14}H_{26}Cl_3NO_6SiNa$ 460.0493, Found 460.0497.

4,6-Benzylidene-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranose (76): A solution of **75** (2.88 g, 6.57 mmol) in acetonitrile (45 mL) was treated with benzaldehyde dimethyl acetal (2.6 mL, 17.75 mmol) and catalytic amount of 10-camphorsulfonic acid (305 mg, 1.31 mmol). After stirring for1 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ aq solution, water and brine (100 mL each). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash silica gel chromatography (hexane/EtOAc, 7/3 to 1/1) to yield **76** (2.56 g, 4.56 mmol, 93%). $[\alpha]_D^{20}$ +5.4 (*c* 1, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 – 7.49 (m, 2H, aromatic), 7.41 – 7.35 (m, 2H, aromatic), 6.83 (d, *J* = 7.6 Hz, 1H, NH), 5.57 (s, 1H, H_{acetal}), 4.97 (dd, *J* = 7.9, 1.7 Hz, 1H, H-1), 4.28 (dd, *J* = 12.4, 1.3 Hz, 1H, H-6), 4.21 (d, *J* = 3.3 Hz, 1H, H-4), 4.10 – 4.03 (m, 2H, H-6, H-3), 3.86 – 3.78 (m, 1H, H-2), 3.52 – 3.49 (m, 1H, H-5), 0.91 (s, 9H, CH_{3TBS}), 0.19 (s, 3H, $SiCH_{3TBS}$), 0.13 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.6, 137.6, 129.5, 128.5, 126.6, 101.5, 95.4, 75.1, 69.8, 69.4, 66.8, 58.7, 58.6, 25.9, 18.1, -3.8, -4.5 ppm. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd. for $C_{21}H_{30}Cl_3NO_6Na$ 548.0800; Found 548.0797.

4,6-Benzylidene-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (77): To a solution of **76** (2.56 g, 4.58 mmol) in dry CH_2Cl_2 (7.3 mL), EDC·HCl (1.39 g, 7.28 mmol), DMAP (415 mg, 3.39

mmol) and levulinic acid (0.74 mL, 7.28 mmol) were added at 0 °C and after stirring for 10 min was allowed to warm up to room temperature. After 3h, TLC analysis indicated complete conversion of the starting material. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ aq solution, water and brine (50 mL each). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude was purified by flash chromatography (hexane/EtOAc, 6/4) to obtain **77** (2.8 g, 92%). $[\alpha]_D^{20}$= +21.0 (*c* 1, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 –7.36 (m, 5H, aromatic), 6.69 (d, *J* = 8.5Hz, NH), 5.53 (s, 1H, PhCH), 5.28 (dd, *J* = 3.5Hz, 11.3Hz, 1H, H-3), 5.02 (d, *J* = 7.8Hz, 1H, H-1), 4.29 (m, 2H, H-4, H-6), 4.17 (m, 1H, H-2), 4.06 (dd, *J* = 1.6, 12.4Hz, 1H, H-6), 3.53 (d, *J* = 1Hz, 1H, H-5), 2.71 – 2.56 (m, 4H, CH_{2Lev}), 2.05 (s, 3H, CH_{3Lev}), 0.89 (s, 9H, CH_{3TBS}), 0.18 (s, 3H, $SiCH_{3TBS}$), 0.13 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.5, 172.6, 161.8, 137.8, 129.3, 128.4, 126. 6, 101.2, 95.8, 77.4, 77.2, 76.9, 73.3, 70.3, 69.4, 66.6, 55.0, 37.9, 29.8, 28.2, 25.9, 18.1, -3.8, -4.5 ppm. HRMS (ESI) *m/z*: $[M+NH_4]^+$ Calcd. for $C_{26}H_{36}Cl_3NO_8NH_4$ 641.1614; Found 641.1652.

6-*O*-Benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (78): To a solution of **77** (6.0 g, 9.6 mmol) in dry CH_2Cl_2 (35 mL) with 4 Å molecular sieves, triethylsilane (7.6 mL, 48 mmol) and trifluoroacetic acid (3.7 mL, 48 mmol) were added at 0 °C and was stirred for 2h. The reaction mixture was then allowed to warm up to room temperature and stirred for 1h. The reaction was quenched by addition of triethylamine (6.7 mL) and concentrated. The oily residue was purified by flash column chromatography (toluene/ EtOAc, 8/2 to 6/4) to obtain the desired product **78** as a colorless solid (4.2 g, 69%). $[\alpha]_D^{20}$= -0.8 (*c* 0.5, $CHCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H, aromatic), 6.67 (d, *J* = 8.9 Hz, 1H, NH), 5. 08 (dd, *J* = 11.2, 3.0 Hz, 1H, H-3), 4.88 (d, *J* = 7.9 Hz, 1H, H-1), 4.58 (s, 2H, CH_2Ph), 4.19 – 4.11 (m, 2H, H-5, H-2), 3.81 – 3.76 (m, 1H, H-6), 3.75 – 3.69 (m, 2H, H-6, H-4), 2.77 – 2.72 (m, 2H, CH_{2Lev}), 2.65 – 2.51 (m, 2H, CH_{2Lev}), 2.19 (s, 3H, CH_{3Lev}), 0.87 (s, 9H, CH_{3TBS}), 0.14 (s, 3H, $SiCH_{3TBS}$), 0.10 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.6 (Cq), 172.4 (Cq), 161.9 (Cq), 137.9 (Cq), 128.6, 128.0, 127.8 (Caromatic), 96.1 (C-1), 92.7(Cq), 73.9 (CH_2Ph), 73.5 (C-4), 72.7 (C-3), 69.6 (C-6), 67.3 (C-5), 55.0 (C-2), 38.2 (CH_{2Lev}), 30.0 (Me), 28.3 (CH_{2Lev}), 25.8 (CH_{3TBS}), 18.0 (Cq), -3.9 (CH_{3TBS}), -5.0 (CH_{3TBS}) ppm. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd. for $C_{26}H_{38}Cl_3NO_8Na$ 648.1324; Found 648.1330.

4-*O*-Benzoyl-6-*O*-benzyl-1-*O*-*tert.*-butyldimethylsilyl-2-deoxy-3-*O*-levulinyl-2-trichloroacetamido-D-galactopyranose (79): To a solution of **78** (3.76 g, 6.01 mmol) in dry CH_2Cl_2 (18 mL), benzoyl chloride (0.84 mL, 6.01 mmol) and pyridine (1.67 mL, 1.64 mmol) were added at 0 °C. The reaction was stirred for 3h and was diluted with CH_2Cl_2 and was washed with HCl 1M solution, water, saturated $NaHCO_3$ aq solution, water, dried over anhydrous $MgSO_4$, and conentrated. The crude was purified by column chromatography (hexane/ EtOAc, 8/2) to obtain (4.2 g, 96%). $[\alpha]_D^{20}$ = +74.3 ° (*c* = 0.5, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 – 8.07 (m, 2H, aromatic), 7.63 – 7.58 (m, 1H, aromatic), 7.51 – 7.47 (m, 2H, aromatic), 7.23 – 7.18 (m, 5H, aromatic), 6.80 (d, *J* = 8.9 Hz, 1H, NH), 5.72 (dd, *J* = 3.4, 1.2 Hz, 1H, H-4), 5.34 (dd, *J* = 11.4, 3.4 Hz, 1H, H-3), 5.00 (d, *J* = 7.9 Hz, 1H, H-1), 4.49 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.39 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.20 – 4.11 (ddd, *J* = 11.3, 9.0, 7.9 Hz, 1H, H-2), 4.01 (ddd, *J* = 6.4, 6.3, 1.3 Hz, 1H, H-5), 3.59 (m, 2H, H-6), 2.70 – 2.38 (m, 4H, CH_2Lev), 2.04 (s, 3H, CH_3Lev), 0.91 (s, 9H, CH_{3TBS}), 0.19 (s, 3H, $SiCH_{3TBS}$), 0.15 (s, 3H, $SiCH_{3TBS}$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.31, 172.24, 165.80, 162.04, 137.66, 133.62 (C aromatic), 130.07 (C aromatic), 129.41 (C aromatic), 128.73, 128.44 (C aromatic), 127.78 (C aromatic), 96.23 (C-1), 92.58 (Cq), 73.68 (CH_2Ph), 72.72 (C-5), 70.35 (C-3), 68.07 (C-6), 67.93 (C-4), 55.61 (C-2), 37.89 (CH_2Lev), 29.68 (CH_3Lev), 28.05 (CH_2Lev), 25.79 (TBS), 18.04 (Cq), -3.86 (TBS), -4.79 (TBS). HRMS (ESI): *m/z* calcd. for $C_{21}H_{30}Cl_3NO_{10}$ $[M+Na]^+$ 752.1587, found 752.1612.

4-*O*-Acetyl-6-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (80): A solution of compound **78** (136 mg, 0.22 mmol) in pyridine (0.5 mL, 6.12 mmol) was cooled to 0 ºC and acetic anhydride (0.29 mL, 3.07 mmol) was added dropwise. The reaction was allowed to warm up to room temperature and stirred overnight. The solution was diluted with EtOAc (30 mL) and washed with 1M HCl solution, water, saturated $CuSO_4$ aq solution, and water (10 mL each). The organic phase was dried over anhydrous $MgSO_4$, concentrated and purified by flash chromatography (hexane/EtOAc, 8/2 to 6/4) to obtain **80** (92 mg, 82%). $[\alpha]_D^{20}$ -9.3 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.25 (m, 5H, aromatic), 6.68 (d, *J* = 8.9 Hz, 1H, NH), 5.46 (dd, *J* = 3.1, 1.3 Hz, 1H, H-4), 5.20 (dd, *J* = 11.4, 3.3 Hz, 1H, H-3), 4.88 (d, *J* = 7.9 Hz, 1H, H-1), 4.54 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.43 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.14 – 3.98 (m, 1H, H-2), 3.91 – 3.79 (m, 1H, H-5), 3.61 – 3.42 (m, 2H, H-6), 2.83 – 2.70 (m, 1H, CH_{2Lev}), 2.67 – 2.37 (m, 3H,

CH_{2Lev}), 2.16 (s, 3H, CH_{3Lev}), 2.08 (s, 3H, CH_{3Ac}), 0.88 (s, 9H, CH_{3TBS}), 0.14 (s, 3H, $SiCH_{3TBS}$), 0.11 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.2 (Cq), 172.4 (Cq), 170.3 (Cq), 162.0 (Cq), 137.7 (Cq), 128.6, 128.0, 96.2 (C-1), 92.6 (Cq, TCA), 73.7 (C-6), 72.5 (C-5), 70.2 (C-3), 67.9 (C-6), 67.3 (C-4), 55.3 (C-2), 37.9 (CH_{2Lev}), 29.9 (CH_{3Lev}), 27.9 (CH_{2Lev}), 25.8 (TBS), 20.9 (CH_{3Ac}), 18.0 (TBS), -3.9 (TBS), -5.0 (TBS) ppm. HRMS (ESI) *m/z*: $[M+NH_4]^+$ calcd. for $C_{28}H_{40}Cl_3NO_9Si\ NH_4$ 685.1876; Found 685.1887.

4-*O*-Benzoyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-α-D-galactopyranosyl trichloroacetimidate (81): To a solution of **79** (310 mg, 0.42 mmol) in THF (2.1 mL), acetic acid (26 µL, 0.46 mmol) and TBAF (0.46 mL of 1M THF solution, 0.46 mmol) were added at 0 °C. The reaction was allowed to warm up to room temperature and stirred until TLC analysis indicated disappearance of starting material (2h). Then, the crude was diluted with EtOAc (30 mL) and was washed with saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous phase was extracted with EtOAc (3x10 mL), the combined organic phases were washed with water, brine, dried over $MgSO_4$ and concentrated to use in the next reaction without further purification. The crude was dissolved in CH_2Cl_2 (4 mL), trichloroacetonitrile (0.63 mL, 6.3 mmol) and DBU (6 µL, 0.042 mmol) were added at 0 °C. After 2h, TLC analysis indicated the complete conversion of the starting material. The reaction crude was concentrated and purified by column chromatography (hexane/EtOAc, 7/3containing 5% of triethylamine) to obtain compound **6** (249 mg, 78%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.82 (s, 1H, NH), 8.12 – 8.05 (m, 2H, aromatic), 7.66 – 7.60 (m, 1H, aromatic), 7.53 – 7.46 (m, 2H, aromatic), 7.23 – 7.13 (m, 5H, aromatic), 7.01 (d, *J* = 8.6 Hz, 1H, N*H*TCA),6.62 (d, *J* = 3.5 Hz, 1H, H-1), 5.90 – 5.86 (m, 1H, H-4), 5.53 (dd, *J* = 11.4, 3.1 Hz, 1H, H-3), 4.78 (ddd, *J* = 11.8, 8.6, 3.5 Hz, 1H, H-2), 4.49 – 4.44 (m, 2H, H-5, CH_2Ph), 4.36 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 3.64 – 3.53 (m, 2H, H-6), 2.77 – 2.67 (m, 1H, CH_{2Lev}), 2.64 – 2.41 (m, 3H, CH_{2Lev}), 2.10 (s, 3H, CH_{3Lev}) ppm.^{13}C NMR (126 MHz, $CDCl_3$) from HSQC ed. experiment δ 133.9 (C aromatic), 129.9 (C aromatic), 128.7, 128.0 (C aromatic), 94.5 (C-1), 73.6 (CH_2Ph), 70.8 (C-5), 68.5 (C-3), 67.5 (C-4), 67.2 (C-6), 50.3 (C-2), 37.7 (CH_{2Lev}), 29.6 (CH_{3Lev}), 28.0 (CH_{2Lev}) ppm.

4-*O*-Acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-α-D-galactopyranosyl trichloroacetimidate (82): Compound **80** (4.55 g, 6.8 mmol) was dissolved in THF (34 mL), acetic acid (0.42 mL, 7.48 mmol) and TBAF (7.5 mL of 1M THF solution, 7.50 mmol) were added at 0 °C. The reaction mixture was allowed to warm up to room temperature and was stirred overnight. The solution was partitioned between EtOAc (50 mL) and saturated $NaHCO_3$ aq solution. The aqueous phase was extracted with EtOAc (3x30 mL), the combined organic phases were washed with water, brine and dried over anhydrous $MgSO_4$. The reaction crude was concentrated and purified by column chromatography (hexane/ EtOAc, 8/2 to 1/1) to obtain the corresponding hemiacetal (3.4 g, 90%).^{1}H NMR (500 MHz, $CDCl_3$) as a mixture α/β (8.5/1.5) δ 7.37 – 7.26 (m, 5H, aromatic), 7.09 (d, J = 8.5 Hz, 0.15H, NHβ), 6.92 (d, *J* = 9.3 Hz, 0.85H, NHα), 5.44 – 5.42 (m, 0.15H, H-4β), 5.41 – 5.39 (m, 0.85H, H-4α), 5.38 (d, *J* = 2.7 Hz, 0.85H, H-1, H-1α), 5.29 (dd, *J* = 11.2, 3.2 Hz, 0.85H, H-3α), 5.11 (dd, J = 11.2, 3.3 Hz, 0.15H, H-3β), 4.70 (d, *J* = 8.2Hz, 0.15H, H-1β), 4.55 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.50 – 4.42 (m, 1.85H, CH_2Ph, H-2, H-2α), 4.41 – 4.36 (m, 0.85H, H-5α), 4.09 – 4.04 (m, 0.2H, H-2β), 3.82 – 3.80 (m, 0.15H, H-5β), 3.58 (dd, *J* = 9.5, 6.2 Hz, H-6_aβ), 3.54 – 3.48 (m, 1H, H-6_bβ, H-6_aα), 3.45 (dd, *J* = 9.8, 4.9 Hz, 0.85H, H-6_bα), 2.77 – 2.69 (m, 1H, CH_{2Lev}), 2.67 – 2.51 (m, 2H, CH_{2Lev}), 2.48 – 2.40 (m, 1H, CH_{2Lev}), 2.16 (s, 0.47H, $CH_{3Lev\beta}$), 2.15(s, 2.24H, $CH_{3Lev\alpha}$), 2.11 (s, 2.24H, $CH_{3Ac\alpha}$) 2.09 (s, 0.47H, $CH_{3Ac\beta}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.5, 172.5, 170.4, 162.2, 137.2, 128.6, 128.2, 128.1, 92.3, 91.6 (C-1), 73.7 (CH_2Ph), 68.7 (C-3), 68.6 (C-6), 68.1, 68.0, 50.3 (C-2), 37.8 (CH_{2Lev}), 29.8 (CH_{3Lev}), 27.9 (CH_{2Lev}), 27.9 (CH_{2Lev}), 20.8 (CH_{3Ac}) ppm. The hemiacetal (1.5 g, 2.7 mmol) was dissolved in dry CH_2Cl_2 (27 mL), trichloroacetonitrile (4.06 mL, 40.55 mmol) and catalytic amount of DBU (40 µL) were added at 0 °C. The reaction was gradually warmed up to room temperatureand stirred for 2 h until TLC analysis showed complete consumption of the starting material. The reaction mixture was concentrated and purified by column chromatography (hexane/EtOAc, 9/1 to 7/3containing 5% of triethylamine) to obtain compound **82** (1.6 g, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 1H, $OCNHCl_3$), 7.35 – 7.24 (m, 5H, aromatic), 6.91 (d, *J* = 8.7 Hz, 1H, NH), 6.50 (d, *J* = 3.5 Hz, 1H, H-1), 5.61 (dd, *J* = 3.1, 1.4 Hz, 1H, H-4), 5.40 (dd, *J* = 11.4, 3.1 Hz, 1H, H-3), 4.71 – 4.65 (m, 1H, H-2), 4.53 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.40 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.36 – 4.31 (m, 1H, H-5), 3.56 (dd, *J* = 9.6, 5.6 Hz, 1H, H-6_a), 3.48 (dd, *J* = 9.6, 7.4 Hz, 1H, H-6_b), 2.81 – 2.73 (m, 1H, CH_{2Lev}), 2.68 – 2.55 (m, 2H, CH_{2Lev}), 2.52 – 2.44 (m, 1H, CH_{2Lev}), 2.17 (s, 3H,

CH_{3Lev}), 2.10 (s, 3H, CH_{3Ac}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.1, 172.9, 170.0, 162.4, 160.3, 137.4, 128.6, 128.1, 128.0, 94.6, 92.0, 90.8, 73.6, 70.7, 68.5, 67.1, 67.1, 50.1, 37.8, 29.8, 27.9, 20.8 ppm. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd. for $C_{24}H_{26}Cl_6N_2O_9Na$ 718.9667; Found 718.9703.

5.7 EVALUATION OF GLYCOSYL DONORS FOR THE SYNTHESIS OF HS OLIGOSACCHARIDES IN SOLUTION

5.7.1 Glycosylation of the linker

4-((Benzyl(5-hydroxypentyl)carbamoyloxy)methyl)benzyl benzoate (83): To a cooled solution (0ºC) of **7** (958 mg, 1.91 mmol) in dry CH_2Cl_2 (6 mL), pyridine (0.46 mL, 5.73 mmol) and benzoyl chloride (0.33 mL, 2.87 mmol) were added and the solution was stirred overnight. The mixture was diluted with EtOAc and washed with 1M HCl, saturated $NaHCO_3$ aq solution and water. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (CH_2Cl_2/hexane, 1/1) to afford the benzoylated intermediate (1.09 g, 95%). ^1H NMR (500 MHz, $CDCl_3$, 323K) δ 8.10-8.09 (2H, d, J = 7.6Hz, aromatic), 7.57-7.24 (12H, m, aromatic), 5.38 (2H, s, CH_2), 5.21 (2H, s, CH_2), 4.52 (2H, s, CH_2), 3.57 (2H, s, CH_2), 1.67 - 1.50 (5H, m, CH_2, TDS), 1.32 (2H, m, CH_2), 0.91 (3H, s), 0.90 (3H, s), 0.86 (6H, s, TDS), 0.09 (6H, s, TDS); ^{13}C NMR (126 MHz, $CDCl_3$, 323K) δ 166.28, 156.42, 138.02, 137.09, 135.84, 132.91, 130.28, 129.67, 128.47, 128.31, 128.21, 128.00, 127.64, 127.22, 66.74, 66.31, 62.57, 50.49, 47.27, 46.41, 34.30, 32.44, 27.75, 25.19, 23.18, 20.39, 18.49, -3.39; HRMS (ESI): Calcd for $C_{36}H_{49}NO_5SiNa$ $[M+Na]^+$ 626.3278, found 626.3289. HF·pyridine complex (5 mL) was added to a cooled solution (0ºC) of the benzoylated intermediate (1.3 g, 2.15 mmol) in dry THF (5 mL). The mixture was stirred overnight then diluted with EtOAc and solid $NaHCO_3$ was added. The reaction mixture was then filtered and washed with saturated $NaHCO_3$ aq solution and water. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/EtOAc, 7/3) to afford **83** (675 mg, 68%). ^1H NMR (500 MHz, $CDCl_3$, 323K) δ 8.07-8.06 (2H, d, *J* = 7.6Hz, aromatic), 7.52 - 7.20 (12H, m, aromatic), 5.34 (2H, ps), 5.18 (2H, ps), 4.49 (2H, ps), 3.54 (2H, ps), 3.26 (2H, ps), 2.44 (1H, s), 1.52 (4H, ps), 1.30 (2H, ps); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.05 (qC), 156.12 (qC), 137.68 (qC), 136.71, 135.64, 132.71,

129.94 (qC), 129.38, 128.83, 128.24, 128.08, 127.94, 127.79, 127.00, 66.54, 66.04, 62.06, 50.24, 46.92, 32.02, 27.44, 22.75; HRMS (ESI): Calcd for $C_{28}H_{31}NO_5Na$ $[M+Na]^+$ 484.2100, found 484.2061.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(3-*O*-benzyl-2-*O*-benzoyl-4-*O*-levulinoyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (84): The reaction was carried out according to general procedure B using idopyranosyl donor **25** (106 mg, 0.132 mmol), linker acceptor **83** (50 mg, 0.108 mmol), and trifluoromethanesulfonic acid (TfOH) (2.83 µL, 0.03 mmol) were added and the reaction mixture was allowed to warm to room temperature. The residue was purified by column chromatography (hexane/EtOAc, 95/5 to 1/1) to afford **84** (100 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 - 7.14 (34H, m, aromatic) , 5.35 (2H, ps), 5.19- .18 (3H, m, H-2, CH_2), 5.09 (1H, ps, H-4), 4.97 (1H, ps, H-1), 4.81 (1H, d, *J* = 11.8Hz, CH_2Ph), 4.72 (1H, d, *J* = 11.8Hz, CH_2Ph), 4.48 - 4.41 (m,3H, H-5, CH_2), 3.91 (1H, t, H-3), 3.83 - 3.72 (3H, m, H-6, CH_2), 3.39 - 3.37(1H, m), 3.21 - 3.14 (2H, m), 2.56 - 2.53 (2H; m, CH_2(Lev)), 2.44 - 2.41 (2H; m, CH_2(Lev)), 2.04 (3H, s, CH_3(Lev)), 1.68 - 1.49 (5H, m), 1.33 - 1.25 (2H, m), 1.05 (9H, s, $(CH_3)_3C$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.73, 171.96, 166.35, 165.23, 156.60, 156.03 (qC), 137.90, 137.01, 136.88, 135.58, 135.54, 133.33, 133.14, 133.01, 130.04, 129.76, 129.69, 129.66, 129.06, 128.50, 128.38, 128.34, 128.26, 128.19, 127.99, 127.70, 127.65, 127.55, 127.42, 127.20, 127.06, 97.90 (C-1), 73.28 (C-3), 72.13 (CH_2Ph), 68.29 (C-2), 67.80, 67.56 (C-4), 66.72, 66.35 (C-5), 62.66 (C-6), 50.51, 50.20, 47.20, 46.20, 37.76, 29.59, 29.07, 27.98, 27.86, 27.52, 26.72, 23.37, 19.10; HRMS (ESI): Calcd for $C_{69}H_{75}NO_{13}SiNa$ $[M+Na]^+$ 1176.4906, found 1176.4854: $[\alpha]_D^{20}$ = -1.07º (c = 0.8).

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)pentyl) carbamate (85): The reaction was carried out according to general procedure B using linker acceptor **83** (100 mg, 0.217 mmol), thiophenyl donor **26** (230 mg, 0.326 mmol) and trimethylsilyl triflate (TMSOTf) (0.25 eq, 2.83 µL, 0.03 mmol). The product was obtained as a colourless syrup (183 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 8.10 – 8.04 (m, 4H, aromatic), 7.59 – 7.52 (m, 2H, aromatic), 7.46 – 7.09 (m, 18H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.20 – 5.13 (m, 3H, H-2, CH_2-Ph_{Carba}), 5.04 – 5.00 (m, 1H, H-4), 4.95 (bs, 1H, H-1), 4.81 – 4.75 (m, 1H, CH_2Ph), 4.70 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.47 (d, *J* =

14.1 Hz, 2H, CH_2-PhN), 4.36 – 4.29 (m, 1H, H-5), 3.89 – 3.86 (m, 1H, H-3), 3.80 – 3.67 (m, 3H, H-6, $OCH_{2Linker}$), 3.46 – 3.34 (m, 1H, $OCH_{2Linker}$), 3.25 – 3.11 (m, 2H, $NCH_{2Linker}$), 2.68 – 2.53 (m, 3H, CH_{2Lev}), 2.50 – 2.42 (m, 1H, CH_{2Lev}), 2.08 (s, 3H, CH_{3Lev}), 1.66 – 1.46 (m, 5H, $CH_{2Linker}$, CH_{thexyl}), 1.37 – 1.25 (m, 2H, $CH_{2Linker}$), 0.86 (d, *J* = 7.0 Hz, 6H, $CH_{3thexyl}$), 0.83 (s, 6H, $CH_{3thexyl}$), 0.11 (s, 3H, CH_3Si), 0.09 (s, 3H, CH_3Si).ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.0, 172.2, 166.5, 165.4, 156.2, 138.1, 137.1, 135.9, 135.8, 133.5, 133.2, 130.2, 129.9, 129.8, 128.7, 128.55, 128.5, 128.4, 128.3, 128.2, 127.9, 127.7, 127.6, 127.2, 98.1 (C-1), 73.5 (C-3), 72.3 (C_{Bn}), 68.5 (C-2), 67.9, 67.8 (C-4, $OCH_{2Linker}$), 66.9 (CH_2-Ph_{Carba}), 66.6 (C-5), 66.5 (CH_2-Ph_{Bz}), 61.8 (CH_{thexyl}), 50.4 (CH_2-PhN), 46.4 ($NCH_{2Linker}$), 37.9 (CH_{2Lev}), 34.2 (CH_{thexyl}), 29.8 (CH_{3Lev}), 29.3 ($CH_{2Linker}$), 28.0 (CH_{2Lev}), 25.2 (Cq_{thexyl}), 23.6 ($CH_{2Linker}$), 20.4, 20.3, 18.7, 18.6 ($CH_{3thexyl}$), -3.4, -3.5 (CH_3Si); HRMS (ESI): *m/z* calcd. for $C_{61}H_{75}NO_{13}Si$ $[M+Na]^+$ 1080.4900, found: 1080.4887.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (86): The reaction was carried out according to general procedure B using linker acceptor **83** (100 mg, 0.217 mmol), thiophenyl donor **27** (218 mg, 0.326 mmol) and trimethylsilyl triflate (TMSOTf) (0.25 eq., 2.83 µL, 0.03 mmol). The product was obtained as colourless syrup (175 mg, 79%). ^1H NMR (500 MHz, $CDCl_3$) *δ* 8.12 – 8.00 (m, 4H, aromatic), 7.60 – 7.52 (m, 2H, aromatic), 7.48 – 7.33 (m, 9H, aromatic), 7.30 – 7.10 (m, 9H, aromatic), 6.85 – 6.76 (m, 4H, $aromatic_{PMP}$), 5.34 (s, 2H, CH_2-Ph_{Bz}), 5.21 – 5.12 (m, 3H, H-2, CH_2-Ph_{Carba}), 5.09 (s, 1H, H-4), 4.98 (d, *J* = 9.1 Hz, 1H, H-1), 4.85 – 4.79 (m, 1H, CH_2Ph), 4.72 – 4.62 (m, 2H, H-5, CH_2Ph), 4.46 (d, *J* = 16.2 Hz, 2H, CH_2-PhN), 4.11 (dd, *J* = 11.9, 4.7 Hz, 1H, H-6a), 4.02 (d, *J* = 4.0 Hz, 1H, H-6b), 3.87 (s, 1H, H-3), 3.80 – 3.74 (m, 4H, CH_{3PMP}, $OCH_{2Linker}$), 3.49 – 3.36 (m, 1H, $OCH_{2Linker}$), 3.21 – 3.15 (m, 2H, $NCH_{2Linker}$), 2.63 – 2.49 (m, 3H, CH_{2Lev}), 2.43 – 2.39 (m, 1H, CH_{2Lev}), 2.07 (s, 3H, CH_{3Lev}), 1.72 – 1.52 (m, 4H, $CH_{2Linker}$), 1.38 – 1.27 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) *δ* = 205.9, 172.1, 166.5, 165.1, 156.1, 154.1, 152.7, 133.2 – 127.1 (C_{arom}), 116.6 – 114.5 ($C_{aromPMP}$), 98.1 (C-1), 73.0 (C-3), 72.1 (C_{Bn}), 68.7 (C-4), 67.9 ($OCH_{2Linker}$), 67.7 (C-6), 67.5 (C-2), 66.7 (CH_2Ph_{Carba}), 66.4 (CH_2Ph_{Bz}), 64.5 (C-5), 54.7 (CH_{3PMP}), 50.4 (CH_2-PhN), 46.2 ($NCH_{2Linker}$), 37.8 (CH_{2Lev}), 29.8 (CH_{3Lev}), 29.1 – 23.5 (CH_{2Lev}, $CH_{2Linker}$) ppm; HRMS (ESI): *m/z* calcd. for $C_{60}H_{63}NO_{14}$ $[M+Na]^+$: 1044.4141, found: 1044.4147.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)pentyl) carbamate (87): The reaction was carried out according to general procedure B using linker acceptor **83** (100 mg, 0.217 mmol) and thiophenyl donor **35** (213 mg, 0.326 mmol). NIS (1.5 eq, 73 mg, 0.33 mmol) and TMSOTf (0.25 eq, 9.8 µL, 0.054 mmol) were added at -20 °C. The product was obtained as colourless syrup (179 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.09 – 8.05 (m, 4H, aromatic), 7.59 – 7.52 (m, 2H, aromatic), 7.49 – 7.11 (m, 23H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.20 – 5.14 (m, 3H, H-2, CH_2-Ph_{carba}), 5.04 – 5.01 (m, 1H, H-4), 4.98 – 4.93 (m, 1H, H-1), 4.83 – 4.76 (m, 1H, CH_2Ph), 4.68 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.58 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.54 – 4.49 (m, 2H, H-5, CH_2Ph), 4.46 (d, *J* = 15.0 Hz, 2H, CH_2-PhN), 3.85 – 3.82 (m, 1H, H-3), 3.80 – 3.70 (m, 1H, $OCH_{2Linker}$), 3.65 (dd, *J* = 10.0, 6.7 Hz, 1H, H-6a), 3.60 (dd, *J* = 10.0, 5.6 Hz, H-6b), 3.47 – 3.36 (m, 1H, $OCH_{2Linker}$), 3.21 – 3.10 (m, 2H, $NCH_{2Linker}$), 2.63 – 2.49 (m, 3H, CH_{2Lev}), 2.43 – 2.39 (m, 1H, CH_{2Lev}), 2.07 (s, 3H, CH_{3Lev}), 1.67 – 1.42 (m, 4H, $CH_{2Linker}$), 1.36 – 1.28 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.1, 172.1, 166.5, 165.4, 156.8, 156.2, 138.2, 138.0, 137.2, 135.8, 133.5, 133.2, 130.2, 130.0, 129.9, 129.8, 129.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 127.9, 127.9, 127.8, 127.7, 127.6, 127.4, 127.2, 98.2 (C-1), 73.5 (C_{Bn}), 73.3 (C-3), 72.2 (C_{Bn}), 69.2 (C-6), 68.0 ($OCH_{2Linker}$), 67.9 (C-2, C-4), 66.9 (CH_2-Ph_{Carba}), 66.5 (CH_2-Ph_{Bz}), 65.0 (C-5), 50.6, 50.3 (CH_2-PhN), 47.4, 46.3 ($NCH_{2Linker}$), 37.9 (CH_{2Lev}), 29.8 (CH_{3Lev}), 29.3 ($CH_{2Linker}$), 28.0 (CH_{2Lev}), 27.6, 23.6 ($CH_{2Linker}$) ppm; MALDI-TOF: *m/z* calcd for $C_{60}H_{63}NO_{13}$ $[M+Na]^+$ 1028.42, found 1028.39, HRMS (ESI): *m/z* calcd. for $C_{60}H_{63}NO_{13}$ $[M+Na]^+$: 1028.4192, found: 1028.4195.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxybenzyl-α-L-idopyranosyloxy)pentyl) carbamate (88): The reaction was carried out according to general procedure B using linker acceptor **83** (100 mg, 0.217 mmol) and thiophenyl donor **36** (222 mg, 0.326 mmol). NIS (1.5 eq., 73 mg, 0.33 mmol) and TMSOTf (0.25 eq., 2.83 µL, 0.03 mmol) were added at -20 °C. The product was obtained as colourless syrup (157 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 – 8.03 (m, 4H, aromatic), 7.58 – 7.53 (m, 2H, aromatic), 7.46 – 7.21 (m, 20H, aromatic), 6.85 (d, *J* = 8.1 Hz, 2H, $aromatic_{PMB}$), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.22 – 5.11 (m, 3H, H-2, CH_2-Ph_{Carba}), 5.03 – 5.00 (m, 1H, H-4), 4.94 (d, *J* = 6.2 Hz, 1H, H-1), 4.79

(d, J = 11.6 Hz, 1H, CH_2Ph), 4.67 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.53 – 4.39 (m, 5H,CH_{2PMB}, CH_2-PhN, H-5), 3.82 (td, J = 2.8, 1.3 Hz, 1H, H-3), 3.77 (s, 3H, CH_{3PMB}), 3.76 – 3.69 (m, 1H, $OCH_{2Linker}$), 3.64 – 3.54 (m, 2H, H-6), 3.47 – 3.35 (m, 1H, $OCH_{2Linker}$), 3.24 – 3.08 (m, 2H, $NCH_{2Linker}$), 2.66 – 2.43 (m, 3H, CH_{2Lev}), 2.42 – 2.32 (m, 1H, CH_{2Lev}), 2.08 (s, 3H, CH_{3Lev}), 1.58 – 1.45 (m, 4H, $CH_{2Linker}$), 1.36 – 1.25 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.1, 172.1, 166.5, 165.4, 159.3, 156.8, 156.2, 138.0, 137.1, 135.9, 133.5, 133.2, 130.3, 130.2, 130.0, 129.9, 129.8, 129.5, 128.8, 128.7, 128.6, 128.5, 128.4, 128.4, 128.2, 128.2, 127.9, 127.7, 127.6, 127.4, 127.2, 113.8 ($C_{aromaticPMB}$), 98.2 (C-1), 73.2, 72.1, 68.7, 68.0, 67.9, 66.9, 66.5, 64.9, 55.4 (CH_{3PMB}), 50.7, 50.3 (CH_2-PhN), 47.4, 46.4 ($NCH_{2Linker}$), 37.9 (CH_{2Lev}), 29.8 (CH_{3Lev}), 29.3 ($CH_{2Linker}$), 28.0 (CH_{2Lev}), 27.6 ($CH_{2Linker}$), 23.6 ($CH_{2Linker}$) ppm. HRMS (ESI): *m/z* calcd. for $C_{61}H_{65}NO_{14}$ $[M+NH_4]^+$: 1053.4743 found: 1053.4774.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(6-*O*-acetyl-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)pentyl) carbamate (89): The reaction was carried out according to general procedure B using linker acceptor **83** (20 mg, 0.041 mmol) and thiophenyl donor **37** (30 mg, 0.050 mmol). NIS (17 mg, 0.075 mmol) and TfOH (41 µL of 0.1 M solution in CH_2Cl_2) were added at -20 °C. The residue was purified by preparative thin layer chromatography (hexane/EtOAc, 6/4) to afford **43** (21 mg, 52%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.14 – 8.02 (m, 4H, aromatic), 7.62 – 7.08 (m, 20H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.24 – 5.08 (m, 3H, H-2, CH_2-Ph_{Carba}), 4.99 – 4.90 (m, 2H, H-1, H-4), 4.87 – 4.74 (m, 1H, CH_2Ph), 4.67 (d, J = 11.6 Hz, 1H, CH_2Ph), 4.56 – 4.40 (m, 3H, H-5, CH_2-PhN), 4.30 – 4.08 (m, 2H, H-6), 3.86 – 3.80 (m, 1H, H-3), 3.79 – 3.64 (m, 1H, $OCH_{2Linker}$), 3.50 – 3.34 (m, 1H, $OCH_{2Linker}$), 3.30 – 3.10 (m, 2H, $NCH_{2Linker}$), 2.70 – 2.51 (m, 3H, CH_{2Lev}), 2.50 – 2.39 (m, 1H, CH_{2Lev}), 2.09 (s, 3H, CH_{3Lev}), 2.07 – 1.99 (m, 3H, CH_{3Ac}), 1.70 – 1.45 (m, 4H, $CH_{2Linker}$), 1.40 – 1.20 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 205.8, 172.0, 170.5, 166.4, 165.2, 156.6, 137.9, 137.7, 137.0, 136.9, 135.7, 133.5, 133.0, 130.1, 129.8, 129.7, 129.5, 128.5, 128.4, 128.2, 128.0, 127.7, 127.5, 127.3, 127.2, 127.1, 97.9 (C-1), 72.8 (C-3), 72.1 (C_{Bn}), 67.9 ($CH_{2Linker}$), 67.4 (C-4), 67.2 (C-2), 66.7 (CH_2-Ph_{carba}), 66.3 (CH_2-Ph_{Bz}), 63.7 (C-5), 62.8 (C-6), 50.5, 50.2 (CH_2-PhN), 47.2, 46.2 ($NCH_{2Linker}$), 37.8 (CH_{2Lev}), 29.6 (CH_{3Lev}), 29.1 ($CH_{2Linker}$), 27.9 (CH_{2Lev}), 27.5 ($CH_{2Linker}$), 23.5 ($CH_{2Linker}$), 20.7 (CH_{3Ac}) ppm; LRMS (MALDI-TOF): Calcd for $C_{55}H_{59}NO_{14}$ $[M+Na]^+$ 981.04, found 980.85. HRMS (ESI): *m/z* calcd for $C_{55}H_{59}NO_{14}$ $[M+Na]^+$ 980.3828, found 980.3755.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-tri-*iso*-propylsiloxymethyl-α-L-idopyranosyloxy)pentyl) carbamate (90): The reaction was carried out according to general procedure B using linker acceptor **83** (75 mg, 0.16 mmol) and thiophenyl donor **38** (100 mg, 0.133 mmol). NIS (45 mg, 0.20 mmol) and TfOH (1.1 µL, 0.013 mmol) were added at -20 °C. The residue was purified by preparative TLC (hexane/EtOAc, 7/3) to afford **42** (131 mg, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.12 – 8.02 (m, 4H, aromatic), 7.61 – 7.10 (m, 20H, aromatic), 5.39 – 5.32 (s, 2H, CH_2-Ph_{Bz}), 5.23 – 5.12 (m, 3H, H-2, CH_2-Ph_{Carba}), 5.02 – 4.97 (m, 1H, H-4), 4.96 – 4.88 (m, 3H, H-1, CH_{2TOM}), 4.83 – 4.74 (m, 1H, CH_2Ph), 4.68 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.53 – 4.40 (m, 3H, H-5, CH_2-PhN), 3.87 – 3.80 (m, 1H, H-3), 3.80 – 3.68 (m, 3H, H-6, $OCH_{2Linker}$), 3.46 – 3.33 (m, 1H, $OCH_{2Linker}$), 3.28 – 3.10 (m, 2H, $NCH_{2Linker}$), 2.69 – 2.52 (m, 3H, CH_{2Lev}), 2.51 – 2.40 (m, 1H, CH_{2Lev}), 2.08 (s, 3H, CH_{3Lev}), 1.70 – 1.40 (m, 4H, $CH_{2Linker}$), 1.40 – 1.20 (m, 2H, $CH_{2Linker}$), 1.15 - 1.00 (m, 21H, $6CH_{3TOM}$, $3CH_{TOM}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 205.8, 172.0, 166.4, 165.3, 156.6, 137.9, 137.8, 137.0, 136.9, 135.7, 133.4, 133.0, 130.1, 129.8, 129.7, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 97.9 (C-1), 90.1 (CH_{2TOM}), 73.0 (C-3), 71.9 (C_{Bn}), 67.8 (C-4, $CH_{2Linker}$), 67.7 (C-2), 66.7 (CH_2-Ph_{Carba}), 66.6 (C-6), 66.3 (CH_2-Ph_{Bz}), 64.8 (C-5), 50.5, 50.2 (CH_2-PhN), 47.2, 46.2 ($NCH_{2Linker}$), 37.8 (CH_{2Lev}), 29.6 (CH_{3Lev}), 29.3, 29.1 ($CH_{2Linker}$), 27.9 (CH_{2Lev}), 27.5 ($CH_{2Linker}$), 23.4 ($CH_{2Linker}$), 17.8 (CH_{3TOM}), 11.9 (CH_{TOM}) ppm. LRMS (MALDI-TOF): Calcd for $C_{63}H_{79}NO_{14}Si$ $[M+Na]^+$ 1125.37, found 1125.00. HRMS (ESI): *m/z* calcd for $C_{63}H_{79}NO_{14}Si$ $[M+Na]^+$ 1124.5167, found 1124.5140.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (91): . The glycosylation was carried out according to general procedure B using:

Conditions 1. Linker acceptor **83** (35 mg, 0.076 mmol) and thiophenyl donor **43** (55 mg, 0.093 mmol). NIS (21 mg, 0.093 mmol) and TMSOTf (1.6 µL, 0.008 mmol) were added at 0 °C. The crude was purified by column chromatography using hexane/EtOAc (7/3) to obtain compound **91** as a white solid (40 mg, 55%).

Conditions 2. Linker acceptor **83** (30 mg, 0.051 mmol) and thiophenyl donor **43** (19 mg, 0.042 mmol). NIS (28 mg, 0.123 mmol) and TMSOTf (1.5 µL, 0.008 mmol) were

added at room temperature. The crude was purified by column chromatography using hexane/ EtOAc (7/3) to obtain compound **91** (26 mg, 66 %).

Conditions 3. Linker acceptor **83** (35 mg, 0.073 mmol) and *n*-pentenyl donor thiophenyl donor **57** (50 mg, 0.088 mmol), NIS (49 mg, 0.219 mmol) and TMSOTf (2.3 µL, 0.015 mmol) were added at 0 °C. The crude was purified by column chromatography using hexane/EtOAc (7/3) to obtain compound **91** (47 mg, 69 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 – 7.19 (m, 24H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.26 (m, 1H, H-4), 5.19 – 5.15 (m, 3H, H-2, CH_2-Ph_{Carba}), 5.12 (bs, 1H, H-1), 4.93 (bs, 1H, H-5), 4.82 (d, *J* =11.8 Hz, 1H, CH_2Ph), 4.72 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.47 (d, *J* = 12.4 Hz, 2H, CH_2-PhN), 3.89 (m, 1H, H-3), 3.79 (s, 3H, CH_3), 3.79 – 3.73 (m, 1H, $OCH_{2Linker}$), 3.50 – 3.40 (m, 1H, $OCH_{2Linker}$), 3.25 – 3.12 (m, 2H, $NCH_{2Linker}$), 2.63 – 2.61 (m, 2H, CH_{2Lev}), 2.50 – 2.37 (m, 2H, CH_{2Lev}), 2.08 (s, 3H, CH_{3Lev}), 1.60 – 1.51 (m, 4H, $CH_{2Linker}$), 1.30 – 1.26 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.9, 171.6, 169.1, 166.5, 165.3, 156.7, 156.2, 138.0, 137.6, 137.1, 137.0, 135.9, 133.6, 133.1, 130.2, 129.9, 129.8, 129.6, 128.6, 128.5, 128.4, 128.1, 127.8, 127.6, 127.3, 127.2, 98.5 (C-1), 72.5 (C-3), 72.2 (CH_2Ph), 68.7 ($OCH_{2Linker}$), 68.2 (C-4), 67.0 (C-2), 66.9 (CH_2-Ph_{Carba}), 66.5 (CH_2-Ph_{Bz}), 66.0 (C-5), 52.6 (CH_{3COOMe}), 50.6, 50.3 (CH_2-PhN), 47.3, 46.3 ($NCH_{2Linker}$), 37.8 (CH_{2Lev}), 29.7 (CH_{3Lev}), 29.2 ($CH_{2Linker}$), 28.0 (CH_{2Lev}), 27.62 ($CH_{2Linker}$), 23.4 ($CH_{2Linker}$) ppm; HRMS (ESI): *m/z* calcd for $C_{54}H_{57}NO_{14}$ $[M+Na]^+$ 966.3677, found 966.3693.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-((3-*O*-benzyl-2-*O*-benzoyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (92): To a cooled solution (0 ºC) of **15** (76 mg, 0.065 mmol) in dry THF (3 mL), HF·pyridine complex (0.3 mL) was added and the solution was stirred overnight. The mixture was diluted with EtOAc and solid $NaHCO_3$ was added, filtered and washed with saturated $NaHCO_3$ aq solution and water. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. The crude was used in the next step without purification. To a solution of the crude intermediate in acetonitrile/water (1/1 v/v, 1 mL), TEMPO (2 mg, 0.013 mmol) and BAIB (46 mg, 0.143 mmol) were added and the reaction mixture was stirred for 4h. The reaction mixture was quenched by the addition of 1M Na_2SO_3 (0.7 mL). The aqueous layer was acidified with 1M HCl, and extracted with CH_2Cl_2 (3X). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (hexane/ EtOAc/HOAc 19/80/1

to 0/99/1) to obtain compound **20** as oil (51 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 - 7.13 (m, 24H, aromatic), 5.35 – 5.32 (3H, m, H-4, CH_2 linker), 5.18 - 5.09 (4H, m, H-1, H-2, CH_2 linker), 4.97 - 4.94 (1H, m, H-5), 4.81 - 4.44 (6H, m), 3.91 (1H, s, H-3), 3.74 - 3.71 (1H, m), 3.52 - 3.46 (1H, m), 3.21 - 3.15 (2H, m), 2.67 - 2.62 (2H, m), 2.51 - 2.42 (2H, m), 2.09 (3H, s), 1.63 - 1.49 (4H, m), 1.32 - 1.27 (2H, m); ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.70, 171.52, 170.10, 166.44, 165.14, 156.68 (C carbamate), 156.18 (C carbamate), 137.75, 137.39, 136.75, 135.73, 133.56, 133.06, 130.01, 129.79, 129.67, 129.32, 128.52, 128.44, 128.36, 128.27, 128.04, 127.76, 127.48, 127.27, 127.08, 98.46 (C-1), 72.28 (C-3), 72.14 (CH_2Ph), 68.72, 67.85 (C-4), 66.86, 66.76, 66.38 (C-2), 65.71 (C-5), 50.53, 50.22, 47.11, 46.15, 37.76, 29.55, 28.96, 27.88, 27.32, 23.29; LRMS (ESI): Calcd for $C_{53}H_{55}NO_{14}Na$ $[M+Na]^+$ 952.36, found 952.32; $[\alpha]_D^{20}$ = +2.9º (c = 1.15).

5.7.2 Disaccharide synthesis

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (101):. The compound **84** (125 mg, 108 mmol) was delevulinated using hydrazine acetate (19 mg, 216 μmol) in CH_2Cl_2/MeOH (2.7 mL / 0.27 mL). When TLC (hexane/EtOAc, 2/1) showed complete conversion, the reaction was diluted with CH_2Cl_2 (50 mL) and washed twice with 1M HCl (100 mL), saturated $NaHCO_3$ aq solution (100 mL), and brine (100 mL). The organic phase was dried over $MgSO_4$ and concentrated. The crude was purified by column chromatography using hexane/EtOAc (8/2) to obtain compound **93** (97 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.13 – 7.20 (m, 34H, aromatic), 5.40 (s, 2H, CH_2-Ph_{Bz}), 5.31 – 5.22 (m, 3H, H-2, CH_2-Ph_{Carba}), 5.02 (s, 1H, H-1), 4.89 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.69 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.54 – 4.51 (m, 2H, CH_2-PhN), 4.39 (m, 1H, H-5), 3.99 – 3.98 (m, 2H, H-6), 3.90 – 3.83 (m, 3H, H-3, H-4, $OCH_{2Linker}$), 3.46 – 3.44 (m, 1H, $OCH_{2Linker}$), 3.28 – 3.21 (m, 2H, $NCH_{2Linker}$), 2.79 (bs, 1H, OH), 1.68 – 1.56 (m, 4H, $CH_{2Linker}$), 1.37 – 1.29 (m, 2H, $CH_{2Linker}$), 1.13 (9H, s, $(CH_3)_3C$) ppm. The glycosylation reaction was carried out according to general procedure C using idose acceptor **93** (20 mg, 0.019 mmol), azido glucose donor **65** (17 mg, 0.026 mmol) and TMSOTf (0.25 eq., 47 μL of 0.1 M solution). The reaction

mixture was purified by column chromatography using hexane/ EtOAc (8/2) to obtain compound **101** (12 mg, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.14 – 8.06 (m, 5H, aromatic), 7.97 – 7.94 (m, 2H, aromatic), 7.75 – 7.68 (m, 4H, aromatic), 7.51 – 7.13 (m, 34H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.20 – 5.12 (m, 3H, H-2, CH_2-Ph_{Carba}) , 5.09 – 5.03 (m, 2H, H-1, H-4′), 4.87 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.81 (d, *J* = 3.7 Hz, 1H, H-1′), 4.74 (d, *J* = 11.5 Hz, CH_2Ph), 4.48 – 4.42 (m, 2H, CH_2-PhN), 4.35 – 4.28 (m, 3H, H-5, CH_2Ph), 4.18 – 4.14 (m, 1H, H-3), 4.07 (d, *J* = 3.0 Hz, 2H, H-6′), 3.96 – 3.87 (m, 3H, H-6, H-5′), 3.84 – 3.80 (m, 1H, H-4), 3.75 – 3.69 (m, 1H, $OCH_{2Linker}$), 3.66 (t, *J* = 9.7Hz, 1H, H-3′), 3.35 (dd, *J* = 10.0, 3.8 Hz, 2H, H-2′, $OCH_{2Linker}$) , 3.21 – 3.10 (m, 2H, $NCH_{2Linker}$), 2.64 - 2.60 (m, 2H, CH_{2Lev}) , 2.47 – 2.40 (m, 1H, CH_{2Lev}), 2.36 – 2.30 (m, 1H, CH_{2Lev}), 2.09 (s, 3H, CH_{3Lev}), 1.59 – 1.46 (m, 4H, $CH_{2Linker}$), 1.30 – 1.19 (m, 2H, $CH_{2Linker}$), 1.06 (s, 9H, $(CH_3)_3C$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.0, 171.4, 166.5, 166.1, 165.8, 156.7, 156.2, 138.0, 137.5, 135.9, 135.8, 135.7, 133.3, 133.2, 133.1, 133.0, 130.2, 130.1, 130.0, 130.0, 129.9, 129.8, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.2, 98.5 (C-1), 97.6 (C-1′), 78.6 (C-3′), 75.1 (C_{Bn}), 74.3 (C-4), 73.5 (C-3), 72.6 (C_{Bn}), 70.4 (C-4′), 69.9 (C-2), 68.9 (C-5′), 68.5 (C-5), 67.9 ($OCH_{2Linker}$), 66.9 (CH_2-Ph_{Carba}), 66.5 (CH_2-Ph_{Bz}), 63.7 (C-6), 63.6 (C-2′), 62.3 (C-6′), 50.6, 50.3 (CH_2-PhN), 47.4, 46.4 ($NCH_{2Linker}$), 37.9 (CH_{2Lev}), 37.8 (CH_{2Lev}), 29.8 (CH_{3Lev}), 29.5 ($CH_{2Linker}$), 29.2 ($CH_{2Linker}$), 28.2, 28.1, 27.9 (CH_{2Lev}), 27.7 ($CH_{2Linker}$), 27.0, 26.9 (CH_{3Lev}), 23.6, 23.5 ($CH_{2Linker}$), 19.3, 19.2 ppm (CH_3)$_3$) ppm; HRMS (ESI): *m/z* calcd for $C_{89}H_{94}N_4O_{18}Si$ $[M+Na]^+$ 1558.6261, found 1558.6282.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**102**): The compound **85** (150 mg, 142 µmol) was delevulinated using hydrazine acetate (20 mg, 212 µmol) in CH_2Cl_2/methanol (4:1, 2.5 mL). When TLC (hexane / EtOAc, 2:1) showed completed conversion, the mixture was concentrated and the residue was purified by column chromatography (hexane EtOAc 1/0 to 7/3) to afford the glycosyl acceptor **94** (125 mg, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 – 7.98 (m, 4H, aromatic), 7.61 – 7.52 (m, 2H, aromatic), 7.48 – 7.11 (m, 18H, aromatic), 5.36 (s, 2H, CH_2-Ph_{Bz}), 5.25 – 5.12 (m, 3H, H-2, CH_2-Ph_{Carba}), 4.95 – 4.90 (bs, 1H, H-1), 4.82 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.63 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.48 (d, *J* = 11.3 Hz, 2H, CH_2-PhN), 4.28 – 4.20 (m, 1H, H-5), 3.88 – 3.80 (m, 4H, H-6, H-3, H-4), 3.80 – 3.72 (m, 1H, $OCH_{2Linker}$), 3.48 – 3.35 (m,

1H, $OCH_{2Linker}$), 3.28 – 3.13 (m, 2H, $NCH_{2Linker}$), 2.86 (bs, 1H, OH), 1.68 – 1.47 (m, 5H, CH_{thexyl}, $CH_{2Linker}$), 1.42 – 1.24 (m, 2H, $CH_{2Linker}$), 0.89 (d, J = 7.0 Hz, 6H, $CH_{3thexyl}$), 0.87 (s, 6H, $CH_{3thexyl}$), 0.14, 0.13 (2s, CH_3Si); δ^{13}C(126 MHz, $CDCl_3$): 166.5, 165.3, 156.7, 156.2, 138.1, 137.0, 135.8, 133.5, 133.2, 130.2, 129.9, 129.8, 129.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.1, 128.1, 127.9, 127.8, 127.7, 127.4, 127.2, 98.5, 75.6, 71.9, 68.5, 67.9, 67.6, 66.9, 66.5, 63.2, 50.7, 50.4, 47.4, 46.3, 34.2, 29.3, 28.1, 27.7, 25.2, 23.6, 20.4, 18.7, 18.6 ppm. HRMS (ESI): *m/z* calcd. for $C_{56}H_{73}N_2O_{11}Si$ $[M+NH_4]^+$ 977.4978, found 977.5001. The glycosylation reaction was carried out according to general procedure C using idose acceptor **94** (46 mg, 48 µmol) and azido glucose donor **65** (43 mg, 67 µmol) and TMSOTf (0.25 eq., 2.16 µL, 12 µmol). The product was obtained as colourless syrup **102** (35 mg, 51%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 – 7.97 (m, 6H, aromatic), 7.62 – 7.05 (m, 28H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.25 – 5.10 (m, 4H, H-2, H-4′, CH_2-Ph_{Carba}), 5.04 (bs, 1H, H-1), 4.89 (d, J = 3.7 Hz, 1H, H-1′), 4.85 – 4.79 (m, 1H, CH_2Ph), 4.72 (d, J = 11.6 Hz, 1H, CH_2Ph), 4.51 – 4.34 (m, 5H, CH_2-PhN, CH_2Ph, H-6a′), 4.30 (dd, J = 12.3, 4.6 Hz, 1H, H-6b′), 4.25 – 4.10 (m, 3H, H-3, H-5, H-5′), 3.94 – 3.84 (m, 3H, H-6, H-4), 3.83 – 3.70 (m, H-3′, $OCH_{2Linker}$), 3.47 – 3.33 (m, 2H, H-2′, $OCH_{2Linker}$), 3.26 – 3.06 (m, 2H, OCH_2-Linker), 2.77 – 2.58 (m, 2H, CH_{2Lev}), 2.57 – 2.46 (m, 1H, CH_{2Lev}), 2.43 – 2.31 (m, 1H, CH_{2Lev}), 2.11 (s, 3H, CH_{3Lev}), 1.68 – 1.46 (m, 5H, CH_{thexyl}, $CH_{2Linker}$), 1.34 – 1.23 (m, 2H, $CH_{2Linker}$), 0.97 – 0.81 (m, 12H, $CH_{3thexyl}$), 0.16 (s, 3H, CH_3-Si), 0.14 (s, 3H, CH_3-Si) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 207.1, 206.1, 171.6, 166.5, 166.2, 165.7, 156.8, 156.2, 138.0, 137.5, 137.1, 135.9, 133.3, 133.3, 133.2, 130.2, 130.1, 129.9, 129.8, 128.7, 128.5, 128.4, 128.4, 128.1, 128.0, 127.9, 127.8, 127.4, 127.2, 98.5 (C-1), 97.5 (C-1′), 78.3 (C-3′), 75.1 (C_{Bn}), 73.7 (C-4), 73.6 (C-3), 72.7 (C_{Bn}), 70.7, 70.3 (C-2, C-4′), 69.0, 68.9 (C-5, C-5′), 68.1 ($OCH_{2Linker}$), 66.9 (CH_2-Ph_{Bz}), 66.5 (CH_2-Ph_{Bz}), 63.6 (C-2′), 62.7 (C-6′), 62.5 (C-6), 50.7, 50.4 (CH_2-PhN), 47.4, 46.4 ($NCH_{2Linker}$), 37.9 (CH_{2Lev}), 34.2 (CH_{thexyl}), 29.8 (CH_{3Lev}), 29.3, 28.2 ($CH_{2Linker}$), 28.0, 27.7 (CH_{2Lev}, $CH_{2Linker}$), 25.3 (Cq_{thexyl}), 23.6 ($CH_{2Linker}$), 20.5, 20.5, 18.7 ($CH_{3thexyl}$), -3.1, -3.3 (CH_3-Si) ppm; HRMS (ESI): *m/z* calcd. for $C_{81}H_{94}N_4O_{18}Si$ $[M+Na]^+$: 1461.6225, found: 1461.6293.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (103): The compound **86** (110 mg, 107 µmol) was delevulinated with hydrazine acetate (20 mg, 212 µmol) in CH_2Cl_2/methanol (4:1, 2.5 mL). When TLC (hexane/EtOAc, 2/1) showed complete conversion, the reaction was diluted with CH_2Cl_2 (50 mL) and washed twice with 1M HCl (100 mL), saturated $NaHCO_3$ aq solution (100 mL), and brine (100 mL). The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (hexane/EtOAc 1/0 to 7/3) to afford the glycosyl acceptor **95** (88 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 – 7.97 (m, 4H, aromatic), 7.70 – 7.52 (m, 2H, aromatic), 7.49 – 7.11 (m, 18H, aromatic), 6.91 – 6.76 (m, 4H, aromatic$_{PMP}$), 5.34 (s, 2H, CH_2-Ph$_{Bz}$), 5.25 (s, 1H, H-2), 5.16 (d, *J* = 18.7 Hz, 2H, CH_2-Ph$_{Carba}$), 4.96 (d, *J* = 7.6 Hz, 1H, H-1), 4.84 (dd, *J* = 11.3, 2.4 Hz, 1H, CH_2Ph), 4.62 (d, *J* = 13.2 Hz, 2H, CH_2Ph, H-5), 4.47 (d, *J* = 12.8 Hz, 2H, CH_2-PhN), 4.16 (s, 2H, H-6), 3.92 – 3.76 (m, 3H, H-4, H-3, OCH$_{2Linker}$), 3.75 (s, 3H, CH$_{3PMP}$), 3.55 – 3.36 (m, 1H, OCH$_{2Linker}$), 3.30 – 3.11 (m, 2H, NCH$_{2Linker}$), 2.61 (bs, 1H, OH), 1.59 (s, 4H, CH$_{2Linker}$), 1.33 (s, 2H CH$_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): 166.6, 165.2, 154.2, 153.0, 138.1, 138.1, 138.0, 137.2, 137.1, 135.9, 133.8, 133.2, 130.2, 129.9, 129.5, 129.4, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.2, 127.9, 127.8, 127.4, 127.4, 127.2, 115.7, 114.8, 98.5, 75.1, 71.9, 68.6, 68.1, 68.0, 67.6, 66.9, 66.5, 66.0, 55.8, 50.3, 47.3, 46.3, 29.3, 23.6 ppm. HRMS (ESI): *m/z* calcd. for $C_{55}H_{57}NO_{12}$ $[M+NH_4]^+$: 941.4219, found: 941.4220. The glycosylation reaction was carried out according to general procedure C using idose acceptor **95** (60 mg, 65 µmol), azido glucose donor **65** (58 mg, 91 µmol) and TMSOTf (0.25 eq., 2.89 µL, 16 µmol). The product **103** was obtained as colourless syrup (60 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 8.23 – 8.16 (m, 2H, aromatic), 8.08 – 8.05 (m, 2H, aromatic), 8.03 – 7.97 (m, 2H, aromatic), 7.63 – 7.10 (m, 28H, aromatic), 6.92 – 6.75 (m, 4H, aromatic$_{PMP}$), 5.35 (s, 2H, CH_2-Ph$_{Bz}$), 5.25 – 5.11 (m, 3H, CH_2-Ph$_{Carba}$, H-2), 5.05 – 4.99 (m, 2H, H-1, H-4′), 4.88 (d, *J* = 11.8 Hz, CH_2Ph), 4.82 (d, *J* = 3.2 Hz, 1H, H-1′), 4.69 (d, *J* = 12.6Hz, CH_2Ph), 4.65 – 4.58 (m, 1H, H-5), 4.47 (d, *J* = 12.2 Hz, 2H, CH_2-PhN), 4.35 – 4.23 (m, 4H, H-6a′, H-6a, CH_2Ph), 4.19 – 4.04 (m, 3H, H-6b′, H-6b, H-3), 4.08 – 4.03 (m, 1H, H-5′), 3.97 – 3.93 (m, 1H, H-4), 3.81 – 3.68 (m, 5H, H-3′, OCH$_{2Linker}$, CH$_{3PMP}$), 3.47 – 3.38 (m, 1H, OCH$_{2Linker}$), 3.34 (dd, *J* = 10.1, 3.5 Hz, 1H, H-2′), 3.25 – 3.11 (m, 2H, NCH$_{2Linker}$), 2.67 – 2.53 (m, 2H, CH$_{2Lev}$), 2.40 – 2.23 (m, 2H, CH$_{2Lev}$), 2.09 (s, 3H, CH$_{3Lev}$), 1.59 – 1.46 (m, 4H, CH$_{2Linker}$), 1.37 – 1.24 (m, 2H,

$CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.1, 171.6, 166.5, 166.2, 165.9, 154.3, 152.4, 137.8, 137.5, 135.9, 133.5, 133.2, 133.2, 130.2, 130.0, 129.9, 129.9, 129.8, 128.7, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.2, 115.4, 114.9 ($C_{aromPMP}$), 98.6 (C-1), 96.7 (C-1′), 78.3 (C-3′), 75.1 (C_{Bn}), 72.2 (C_{Bn}), 72.1 (C-4), 71.8 (C-3), 70.6 (C-4′), 69.0 (C-2, C-5′), 68.1 ($OCH_{2Linker}$), 66.9 (CH_2-Ph_{Carba}), 66.7 (CH_2-Ph_{Bz}), 66.5 (C-6), 65.4 (C-5), 63.5 (C-2′), 62.6 (C-6′), 55.8 (CH_{3PMP}), 50.7, 50.4 (CH_2-PhN), 47.4, 46.4 ($NCH_{2Linker}$), 37.9 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.1, 27.9 ($CH_{2Linker}$), 27.7 (CH_{2Lev}), 23.6 ($CH_{2Linker}$) ppm; HRMS (ESI): *m/z* calcd. for $C_{80}H_{82}N_4O_{19}$ $[M+NH_4]^+$: 1420.5912, found: 1420.5927.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3,6-di-*O*-benzyl-a-L-idopyranosyloxy)pentyl) carbamate (**104**): The compound **87** (145 mg, 0.144 mmol) was delevulinated with hydrazine acetate (20 mg, 212 µmol) in CH_2Cl_2/MeOH (4:1, 2.5 mL). When TLC (hexane/EtOAc, 2/1) showed completed conversion, the mixture was concentrated and the residue was purified by column chromatography (hexane/EtOAc, 1/0 to 7/3) to afford the glycosyl acceptor **96** (119 mg, 91%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.09 – 8.05 (m, 2H, aromatic), 8.04 – 7.98 (m, 2H, aromatic), 7.61 – 7.52 (m, 2H, aromatic), 7.48 – 7.11 (m, 23H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.22 (bs, 1H, H-2), 5.20 – 5.13 (d, *J* = 16.2 Hz, 2H, CH_2-Ph_{Carba}), 4.95 (d, *J* = 6.7 Hz, 1H, H-1), 4.86 – 4.78 (m, 1H, CH_2Ph), 4.61 (m, 3H, CH_2Ph), 4.51 – 4.35 (m, 3H, CH_2-PhN, H-5), 3.85 – 3.70 (m, 4H, H-3, H-4, H-6), 3.46 – 3.36 (m, 1H, $OCH_{2Linker}$), 3.27 – 3.09 (m, 2H, $NCH_{2Linker}$), 2.77 (bs, 1H, OH), 1.67 – 1.47 (m, 4H, $CH_{2Linker}$), 1.38 – 1.28 (m, 2H, $CH_{2Linker}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 166.5, 165.3, 138.2, 138.0, 135.9, 133.6, 133.2, 130.2, 129.9, 129.8, 129.6, 128.7, 128.7, 128.6, 128.5, 128.4, 128.2, 127.9, 127.8, 127.8, 127.8, 127.7, 127.4, 127.2, 98.6, 75.3, 73.7, 71.9, 70.5, 68.2, 68.1, 66.9, 66.5, 66.3, 53.6, 50.7, 50.3, 47.4, 46.4, 29.3, 28.1, 27.6, 23.6 ppm. HRMS (ESI): *m/z* calcd. for $C_{55}H_{57}NO_{11}$ $[M+NH_4]^+$: 925.4270, found 925.4261. The glycosylation reaction was carried out according to general procedure C using idose acceptor **96** (57 mg, 63 µmol), azido glucose donor **65** (58 mg, 88 µmol) and TMSOTf (0.25 eq., 2.84 µL, 16 µmol). The product **104** was obtained as colourless syrup (45 mg, 51%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.17 – 8.13 (m, 2H, aromatic), 8.09 – 8.05 (m, 2H, aromatic), 8.02 – 7.98 (m, 2H, aromatic), 7.59 – 7.09 (m, 33H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.23 – 5.12 (m, 3H, CH_2-Ph_{Carba}, H-2), 5.08 (t, *J* = 9.7 Hz, 1H, H-4′), 5.00

(bs, 1H, H-1), 4.85 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.81 (d, *J* = 3.6 Hz, 1H, H-1′), 4.69 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.52 (s, 2H, CH_2Ph), 4.49 – 4.39 (m, 3H, CH_2-PhN, H-5), 4.34 (dd, *J* = 12.2, 2.4 Hz, 1H, H-6a′), 4.31 (d, *J* = 11.0 Hz, 1H, CH_2Ph), 4.27 (d, *J* = 10.9 Hz, 1H, CH_2Ph), 4.21 (dd, *J* = 12.3, 5.0 Hz, 1H, H-6b′), 4.17 – 4.08 (m, 2H, H-5, H-3), 3.82 – 3.79 (m, 1H, H-4), 3.79 – 3.70 (m, 4H, H-6ab, H-3′, $OCH_{2Linker}$), 3.46 – 3.38 (m, 1H, $OCH_{2Linker}$), 3.36 (dd, *J* = 10.1, 3.5 Hz, 1H, H-2′), 3.25 – 3.10 (m, 2H, $NCH_{2Linker}$), 2.74 – 2.57 (m, 2H, CH_{2Lev}), 2.53 – 2.45 (m, 1H, CH_{2Lev}), 2.41 – 2.32 (m, 1H, CH_{2Lev}), 2.12 (s, 3H, CH_{3Lev}), 1.58 – 1.43 (m, 4H, $CH_{2Linker}$), 1.35 – 1.24 (m, 2H, $CH_{2Linker}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.1, 171.6, 166.5, 166.2, 165.8, 156.8, 156.2, 138.1, 138.0, 137.9, 137.5, 137.0, 135.9, 133.4, 133.2, 130.2, 130.1, 130.0, 129.9, 129.8, 128.7, 128.5, 128.4, 128.4, 128.2, 128.0, 128.0, 128.0, 127.8, 127.8, 127.6, 127.4, 127.2, 98.5 (C-1), 97.7 (C-1′), 78.4 (C-3′), 75.0 (C_{Bn}), 74.1 (C-4), 73.4 (C_{Bn}), 72.8 (C-3), 72.4 (C_{Bn}), 70.7 (C-4′), 69.4 (C-6), 69.3 (C-2), 69.1 (C-5), 68.1 ($OCH_{2Linker}$), 66.9 (CH_2-Ph_{Carba}), 66.5 (CH_2-Ph_{Bz}), 66.3 (C-5), 63.6 (C-2′), 62.7 (C-6′), 50.7, 50.4 (CH_2-PhN), 47.4, 46.3 ($NCH_{2Linker}$), 37.9 (CH_{2Lev}), 29.8 (CH_{3Lev}), 29.3 ($CH_{2Linker}$), 28.0 (CH_{2Lev}), 27.7, 23.6 ($CH_{2Linker}$). HRMS (ESI): *m/z* calcd. for $C_{80}H_{82}N_4O_{18}$ $[M+Na]^+$: 1409.5516, found: 1409.5499.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-6-*O*-*p*-methoxybenzyl-α-L-idopyranosyloxy)pentyl) carbamate (**105**): The compound **88** (110 mg, 106 µmol) was delevulinated using hydrazine acetate (20 mg, 212 µmol) in CH_2Cl_2/methanol (4:1, 2.5 mL). When TLC (hexane/EtOAc, 2/1) showed complete conversion, the reaction was diluted with CH_2Cl_2 (50 mL) and washed twice with 1M HCl (100 mL), saturated $NaHCO_3$ aq solution (100 mL), and brine (100 mL). The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (hexane/EtOAc, 1/0 to 7/3) to afford the glycosyl acceptor **97** (85 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 – 8.04 (m, 2H, aromatic), 8.03 – 7.99 (m, 2H, aromatic), 7.65 – 7.50 (m, 2H, aromatic), 7.49 – 7.36 (m, 7H, aromatic), 7.36 – 7.19 (m, 12H, aromatic), 7.14 (d, *J* = 7.1 Hz, 1H, aromatic), 6.86 (d, *J* = 8.0 Hz, 2H, $aromatic_{PMB}$), 5.34 (s, 2H, CH_2-Ph_{Bz}), 5.21 (s, 1H, H-2), 5.17 (d, *J* = 17.2 Hz, 2H, CH_2-Ph_{Carba}), 4.94 (d, *J* = 6.3 Hz, 1H, H-1), 4.81 (d, *J* = 11.0 Hz, 1H, CH_2Ph), 4.60 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.52 (s, 2H, CH_{2PMB}), 4.50 – 4.43 (m, 2H, CH_2-PhN), 4.39 (bs, 1H, H-5), 3.82 – 3.75 (m, 5H, H-3, H-4, CH_{3PMB}), 3.71 (d, *J* = 5.4 Hz, 2H, H-6ab), 3.41

(d, J = 19.8 Hz, 1H, $OCH_{2Linker}$), 3.28 – 3.10 (m, 2H, $NCH_{2Linker}$), 2.80 (bs, 1H, OH), 1.54 (m, 4H, $CH_{2Linker}$), 1.32 (m, 2H, $CH_{2Linker}$). ^{13}C NMR (126 MHz, $CDCl_3$): 166.6, 165.3, 159.4, 138.1, 133.6, 133.2, 130.2, 130.2, 129.9, 129.8, 129.4, 128.7, 128.5, 128.4, 128.2, 127.9, 127.8, 127.8, 127.5, 127.3, 113.9, 98.6, 75.4, 73.4, 71.9, 70.2, 68.2, 68.0, 66.9, 66.5, 66.3, 55.4, 50.7, 50.4, 47.4, 46.3, 29.3, 23.6. HRMS (ESI): *m/z* calcd. for $C_{56}H_{59}NO_{12}$ $[M+NH_4]^+$: 960.3929, found: 960.3662. The glycosylation reaction was carried out according to general procedure C using idose acceptor **97** (62 mg, 66 µmol), azido glucose donor **65** (59 mg, 92 µmol) and TMSOTf (0.25 eq., 3.0 µL, 16.5 µmol). The product **105** was obtained as colourless syrup (57 mg, 61%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.21 – 8.13 (m, 2H, aromatic), 8.09 – 8.05 (m, 2H, aromatic), 8.03 – 7.99 (m, 2H, aromatic), 6.84 (d, J = 8.3 Hz, 1H, $aromatic_{PMB}$), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.22 – 5.13 (m, 3H, H-2, CH_2-Ph_{Carba}), 5.09 (t, J = 9.7 Hz, 1H, H-4′), 4.99 (bs, 1H, H-1), 4.85 (d, J = 12.0 Hz, 1H, CH_2Ph), 4.82 (d, J = 3.6 Hz, 1H, H-1′), 4.69 (d, J = 11.7 Hz, 1H, CH_2Ph), 4.50 – 4.38 (m, 5H, CH_2-PhN, H-5, CH_{2PMB}), 4.38 – 4.21 (m, 4H, H-6ab′, CH_2Ph), 4.17 – 4.13 (m, 1H, H-5′), 4.11 (t, J = 3.3 Hz, 1H, H-3), 3.82 – 3.79 (m, 1H, H-4), 3.78 – 3.68 (m, 7H, H-6ab, H-3′, CH_{3PMB}, $OCH_{2Linker}$), 3.47 – 3.38 (m, 1H, $OCH_{2Linker}$), 3.36 (dd, J = 10.1, 3.6 Hz, 1H, H-2′), 3.26 – 3.10 (m, 2H, $NCH_{2Linker}$), 2.75 – 2.59 (m, 2H, CH_{2Lev}), 2.56 – 2.47 (m, 1H, CH_{2Lev}), 2.42 – 2.33 (m, 1H, CH_{2Lev}), 2.12 (s, 3H, CH_{3Lev}), 1.60 – 1.45 (m, 4H, $CH_{2Linker}$), 1.37 – 1.28 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 206.2, 171.6, 166.5, 166.2, 165.8, 159.3, 156.7, 156.2, 137.9, 137.5, 135.9, 133.4, 133.2, 130.2, 130.0, 130.0, 129.9, 129.8, 129.8, 129.3, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.2, 128.0, 128.0, 127.8, 127.4, 127.2, 113.9 ($C_{aromaticPMB}$), 98.5 (C-1), 97.6 (C-1′), 78.4 (C-3′), 75.0 (C_{Bn}), 74.0 (C-4), 73.1 (CH_{2PMB}), 72.8 (C-3), 72.3 (C_{Bn}), 70.7 (C-4′), 69.3 (C-6, C-2), 69.1 (C-5′), 68.0 ($OCH_{2Linker}$), 66.9 (CH_2-Ph_{Carba}), 66.5 (CH_2-Ph_{Bz}), 66.3 (C-5), 63.6 (C-2′), 62.7 (C-6′), 55.3 (CH_{3PMB}), 50.6, 50.4 (CH_2-PhN), 47.4, 46.3 ($NCH_{2Linker}$), 37.9 (CH_{2Lev}), 29.8 (CH_{3Lev}), 29.2 ($CH_{2Linker}$), 28.0 (CH_{2Lev}), 27.6, 23.5 ($CH_{2Linker}$) ppm; HRMS (ESI): *m/z* calcd. for $C_{81}H_{84}N_4O_{19}$ $[M+NH_4]^+$: 1439.5622, found: 1439.5650.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-tri-*iso*-propylsiloxymethyl-α-L-idopyranosyloxy)pentyl) carbamate (**106**): The compound **89** (31 mg, 0.028 mmol) was delevulinated with hydrazine acetate (5 mg, 57 µmol) in CH_2Cl_2/MeOH (9:1, 1 mL). When TLC (hexane / EtOAc, 2:1) showed

completed conversion (2h), the mixture was concentrated and the residue was purified by column chromatography (hexane/EtOAc 1/0 to 7/3) to afford the idose acceptor **98** (20 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 – 7.98 (m, 4H), 7.64 – 7.10 (m, 20H), 5.35 (s, 2H), 5.24 – 5.12 (m, 3H), 4.99 – 4.88 (m, 3H), 4.85 – 4.77 (m, 1H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.53 – 4.43 (m, 2H), 4.43 – 4.36 (m, 1H), 3.91 – 3.68 (m, 5H), 3.47 – 3.32 (m, 1H), 3.28 – 3.10 (m, 2H), 2.75 (d, *J* = 8.6 Hz, 1H), 1.72 – 1.44 (m, 4H), 1.40 – 1.20 (m, 2H), 1.17 – 0.99 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 165.2, 156.1, 137.9, 137.1, 136.9, 135.7, 133.4, 133.0, 130.1, 129.8, 129.7, 129.4, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.6, 127.5, 127.3, 127.2, 127.1, 98.5, 90.1, 75.2, 71.7, 68.1, 67.8, 67.6, 66.7, 66.4, 66.1, 50.5, 50.2, 47.2, 46.2, 29.7, 29.1, 28.0, 27.5, 23.4, 17.8, 12.0. HRMS (ESI): *m/z* calcd for $C_{58}H_{73}NO_{12}Si$ $[M+Na]^+$ 1026.4800, found 1026.4738. The glycosylation reaction was carried out according to general procedure C using idose acceptor **98** (20 mg, 20 μmol), azido glucose donor **65** (16 mg, 24 μmol) and TMSOTf (0.10 eq., 20 μL of 0.1 M solution). The residue was purified by preparative TLC (hexane/ EtOAc, 6/4) to afford **106** (15 mg, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 – 7.96 (m, 6H, aromatic), 7.64 – 7.06 (m, 28H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.24 – 5.08 (m, 4H, CH_2-Ph_{Carba}, H-2, H-4′), 5.00 – 4.89 (m, 3H, CH_{2TOM}, H-1), 4.85 – 4.80 (m, 2H, H-1′, CH_2Ph), 4.69 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.52 – 4.43 (m, 2H, CH_2-PhN), 4.42 – 4.21 (m, 2H, H-6′, H-5, CH_2Ph), 4.19 – 4.13 (m, 1H, H-5′), 4.11 – 4.06 (m, 1H, H-3), 3.94 – 3.80 (m, 3H, H-6, H-4), 3.80 – 3.68 (m, 2H, H-3′, $OCH_{2Linker}$), 3.45 – 3.32 (m, 2H, H-2′, $OCH_{2Linker}$), 3.28 – 3.06 (m, 2H, $NCH_{2Linker}$), 2.73 – 2.57 (m, 2H, CH_{2Lev}), 2.55 – 2.43 (m, 1H, CH_{2Lev}), 2.42 – 2.28 (m, 1H, CH_{2Lev}), 2.10 (s, 3H, CH_{3Lev}), 1.75 – 1.40 (m, 4H, $CH_{2Linker}$), 1.40 – 1.20 (m, 2H, $CH_{2Linker}$), 1.15 – 0.95 (m, 21H, $6CH_{3TOM}$, $3CH_{TOM}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.9, 171.4, 166.4, 166.1, 165.7, 156.6, 156.1, 137.8, 137.4, 136.9, 135.7, 133.2, 133.0, 130.1, 129.8, 129.8, 129.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.2, 127.1, 98.3 (C-1), 97.3 (C-1′), 89.9 (CH_{2TOM}), 78.3 (C-3′), 74.9 (C_{Bn}), 73.5 (C-4), 72.8 (C-3), 72.2 (C_{Bn}), 70.5, 69.4 (C-2, C-4′), 68.9 (C-5′), 67.9 ($OCH_{2Linker}$), 66.7 (CH_2-Ph_{Carba}), 66.4 (C-5, CH_2-Ph_{Bz}), 66.3 (C-6), 63.4 (C-2′), 62.5 (C-6′), 50.5, 50.2 (CH_2-PhN), 47.2, 46.2 ($NCH_{2Linker}$), 37.8 (CH_{2Lev}), 29.7 (CH_{3Lev}) , 29.1 – 27.5 ($CH_{2Lev,}$ $CH_{2Linker}$), 23.4 ($CH_{2Linker}$), 18.0, 17.8 (CH_{3TOM}), 11.9 (CH_{TOM}) ppm; LRMS (MALDI-TOF): *m/z* calcd for $C_{83}H_{98}N_4O_{19}Si$ $[M+Na]^+$ 1506.65, found 1505.33, HRMS (ESI): *m/z* calcd. for $C_{83}H_{98}N_4O_{19}Si$ $[M+Na]^+$: 1506.6487, found: 1506.6523.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-6-*O*-acetyl-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)pentyl) carbamate (**107**): The compound **90** (31 mg, 0.028 mmol) was delevulinated with hydrazine acetate (5 mg, 57 µmol) in CH_2Cl_2/MeOH (9:1, 1 mL). When TLC (hexane / EtOAc, 2/1) showed completed conversion (2h), the mixture was concentrated and the residue was purified by column chromatography (hexane/EtOAc, 1/0 to 6/4) to afford the idose acceptor **99** (20 mg, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 – 7.94 (m, 4H, aromatic), 7.66 – 7.06 (m, 20H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.26 – 5.10 (m, 3H, H-2, CH_2-Ph_{Carba}), 4.96 – 4.88 (d, *J* = 7.2 Hz, 1H, H-1), 4.87 – 4.78 (m, 1H, CH_2Ph), 4.62 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.54 – 4.39 (m, 3H, H-5, CH_2-PhN), 4.35 (dd, *J* = 11.6, 7.5 Hz, 1H, H-6a), 4.27 (dd, *J* = 11.3, 4.5 Hz, 1H, H-6b), 3.85 – 3.79 (bs, 1H, H-3), 3.79 – 3.65 (m, 2H, H-4, $OCH_{2Linker}$), 3.51 – 3.34 (m, 1H, $OCH_{2Linker}$), 3.31 – 3.10 (m, 2H, $NCH_{2Linker}$), 2.08 – 2.00 (m, 3H, CH_{3Ac}), 1.72 – 1.44 (m, 4H, $CH_{2Linker}$), 1.44 – 1.16 (m, 2H, $CH_{2Linker}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 166.4, 165.0, 156.1, 137.9, 137.7, 137.0, 136.9, 135.8, 133.6, 133.0, 130.1, 129.7, 129.1, 128.6, 128.5, 128.4, 128.3, 128.3, 128.0, 127.8, 127.6, 127.3, 127.3, 127.1, 98.2 (C-1), 74.8 (C-3), 71.8 (C_{Bn}), 67.9 ($OCH_{2Linker}$), 67.7 (C-2), 67.1 (C-4), 66.8 (CH_2-Ph_{Carba}) , 66.3 (CH_2-Ph_{Bz}), 65.4 (C-5), 63.7 (C-6), 50.5, 50.2 (CH_2-PhN), 47.2, 46.2 ($NCH_{2Linker}$), 29.1, 28.0, 27.5, 23.5 ($CH_{2Linker}$), 20.8 (CH_{3Ac}). The glycosylation reaction was carried out according to general procedure C using idose acceptor **99** (123 mg, 128 µmol), azido glucose donor **65** (115 mg, 179 µmol) and TMSOTf (7 µL, 32 µmol). The product **107** was obtained as α/β mixture (25 mg, 15%,). Selected characteristic NMR signals: ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 5.29 – 5.25 (m, 3H, H-1´α, CH_2-Ph_{Carba}), 4.98 (d, *J* = 3.5 Hz, 1H, H-1β), 4.91 – 4.83 (m, 2H, H-1α), 4.67 – 4.57 (m, 3 H, H-1´β, CH_2-Bn) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 97.9 (C-1α, $J_{C,H}$ = 171 Hz), 97.8 (C-1α, $J_{C,H}$ = 172 Hz), 96.3 (C-1´β, $J_{C,H}$ = 162 Hz), 91.9 (C-1´α, $J_{C,H}$ = 171 Hz); HRMS (ESI): *m/z* calcd for $C_{75}H_{78}N_4O_{19}$ $[M+Na]^+$ 1361.5152, found 1361.5129.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**108**): The compound **91** (169 mg, 0.179 mmol) was delevulinated using hydrazine acetate (24 mg, 0.27 mmol) in dry CH_2Cl_2/MeOH (4.5 mL/0.45 mL). The crude was purified by columm chromatography

(hexane/EtOAc, 6/4) to afford the idose acceptor **100** (128 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.12 – 7.95 (m, 4H, aromatic), 7.64 – 7.10 (m, 20H, aromatic), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.24 – 5.16 (m, 3H, H-2, CH_2-Ph_{Carba}), 5.12 (bs, 1H, H-1), 4.90 (m, 1H, H-5), 4.82 (m, 1H, CH_2Ph), 4.64 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.47 (d, J = 11.4Hz, 2H, CH_2-PhN), 4.11 (bs, 1H, H-4), 3.87 (m, 1H, H-3), 3.82 (s, 3H, CH_{3COOMe}), 3.78 – 3.73 (m, 1H, $CH_{2Linker}$), 3.51 – 3.45 (m, 1H, $CH_{2Linker}$), 3.24 – 3.14 (m, 2H, $CH_{2Linker}$), 2.80 (bs, 1H, OH), 1.64 – 1.43 (m, 4H, $CH_{2Linker}$), 1.40 – 1.24 (m, 2H, $CH_{2Linker}$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.0, 166.3, 165.0, 156.0, 137.8, 137.5, 137.0, 135.7, 133.7, 133.0, 130.0, 129.7, 129.7, 129.0, 128.6, 128.5, 128.4, 128.3, 128.0, 127.8, 127.7, 127.6, 127.3, 127.2, 127.1, 98.7, 74.3, 71.8, 68.7, 68.2, 67.6, 67.3, 66.8, 66.3, 52.4, 50.5, 50.2, 47.2, 46.1, 29.7, 29.1, 27.9, 27.5, 23.3 ppm. The glycosylation reaction was carried out according to general procedure C using:

Conditions 1: idose acceptor **100** (56 mg, 0.066 mmol) and azido glucose donor **66** (62 mg, 0.093 mmol). TMSOTf (0.6 µL, 0.003 mmol) was added at 0 °C. The reaction mixture was purified by flash column chromatography using hexane: EtOAc (8:2) to obtain compound **52** (26 mg, 32%).

Conditions 2: The glycosylation reaction was carried out according to general procedure C using acceptor **100** (40 mg, 0.047 mmol) and azido glucose donor **65** (42 mg, 0.065 mmol). TMSOTf (0.6 µL, 0.003 mmol) was added at 0 °C. The reaction mixture was purified by flash column chromatography using toluene/EtOAc (6/4), following by preparative TLC eluting with hexane/EtOAc (6/4) to obtain compound **108** (30 mg, 48%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 – 8.17 (m, 2H, aromatic), 8.10 – 8.02 (m, 4H, aromatic), 7.11 (m, 28H, aromatic), 5.36 (s, 2H, CH_2-Ph_{Bz}), 5.21 – 5.13 (m, 5H, H-1, H-2, H-4′, CH_2-Ph_{Carba}), 4.92 (d, J = 11.5Hz, CH_2Ph), 4.87 (s, 1H, H-5), 4.77 (d, J = 3.10 Hz, H-1′), 4.74 (d, J = 11.5 Hz, CH_2Ph), 4.61 (dd, J = 1.8, 12.4Hz, 1H, H-6′a), 4.48 (d, J = 10.8 Hz, 2H, CH_2-PhN), 4.28 (dd, J = 1.8, 12.4 Hz, 1H, H-6′b), 4.20 – 4.17 (m, 1H, H-4), 4.16 – 4.11 (m, 1H, H-5′), 4.09 – 4.06 (m, 2H, H-3, CH_2Ph), 4.02 (d, J = 10.8 Hz, 1H, CH_2Ph), 3.82 (s, 3H, CH_{3COOMe}), 3.77 – 3.74 (m, 1H, $OCH_{2Linker}$), 3.62 (t, J = 9.4 Hz, H-3′), 3.55 – 3.42 (m, 1H, $OCH_{2Linker}$), 3.30 (dd, J = 3.1, 9.9 Hz, H-2′), 3.24 – 3.17 (m, 2H, $NCH_{2Linker}$), 2.69 – 2.66 (m, 2H, CH_{2Lev}), 2.57 – 2.42 (m, 2H, CH_{2Lev}), 2.11 (s, 3H, CH_{3Lev}), 1.63 – 1.51 (m, 4H, $CH_{2Linker}$), 1.31 – 1.27 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.9, 171.1, 169.6, 166.3, 166.0, 165.4, 156.5, 156.0, 137.8, 137.7, 137.4, 137.2, 136.9, 136.9, 136.8, 136.8, 135.6, 133.3, 132.9, 132.9,

130.0, 129.8, 129.6, 129.6, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.2, 127.2, 127.0, 99.1 (C-1), 99.0 (C-1), 77.9 (C-3′), 76.0 (C-4), 74.5 (CH_2Ph), 72.6 (C-3), 72.2 (CH_2Ph), 70.1 (C-4′), 68.9 (C-5′), 68.5 ($CH_{2Linker}$), 68.0 (C-2), 67.2 (C-5), 66.7 (CH_2-$Ph_{Carba)}$, 66.3 (CH_2-Ph_{Bz}), 63.2 (C-2′), 61.8 (C-6), 52.3 (CH_{3COOMe}), 50.4, 50.1 (CH_2-PhN), 47.1, 46.0 (NCH_2-Linker), 37.7 ($CH_{2Linker}$), 29.6 (CH_{3Lev}), 29.0 ($CH_{2Linker}$), 27.8 (CH_{2Lev}, $CH_{2Linker}$), 27.4 ($CH_{2Linker}$) , 23.3 ($CH_{2Linker}$) ppm; LRMS (MALDI-TOF): Calcd for $C_{74}H_{76}N_4O_{19}$ $[M+Na]^+$ 1348.40, found 1347.78. HRMS (ESI): *m/z* calcd for $C_{74}H_{76}N_4O_{19}$ $[M+Na]^+$ 1347.5001, found 1347.4924.

5.7.2.1 Evaluation of n-pentenyl orthoesters as of L-iduronic acid donors

***tert*-Butyldimethylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranose** (**109**): To a solution of *tert*-butyldimethylsilyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**61**) (180 mg, 0.36 mmol) in dry CH_2Cl_2, triethylsilane (0.34 mL, 2.17 mmol) and trifluoroacetic acid (0.17 mL, 2.17 mmol) were added at 0 °C. After 2 hours, the reaction was quenched with triethylamine and concentrated. The crude was purified by column chromatography (hexane/EtOAc, 9/1 to 7/3) to obtain **53** (140 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 – 7.28 (m, 10H, aromatic), 4.93 (d, J = 11.4 Hz, 1H, CH_2Ph), 4.78 (d, J = 11.4 Hz, 1H, CH_2Ph), 4.64 – 4.52 (m, 3H, CH_2Ph, H-1), 3.73 (d, J = 4.7 Hz, 2H, H-6), 3.65 (dd, J = 8.8, 9.5 Hz, 1H, H-4), 3.46 – 3.39 (m, 1H, H-5), 3.33 (dd, J = 7.6, 10.0 Hz, 1H, H-2), 3.23 (dd, J = 8.7, 9.9 Hz, 1H, H-3), 2.90 – 2.50 (bs, 1H, OH), 0.96 (s, 9H, CH_{3TBS}), 0.19 (s, 6H, CH_{3TBS}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2, 137.8, 128.6, 128.4, 128.0, 127.9, 127.7, 127.6, 97.2 (C-1), 82.3 (C-3), 74.9 (C_{Bn}), 74.0 (C-5), 73.7 (C_{Bn}), 71.9 (C-4), 70.3 (C-6), 68.1 (C-2), 25.6 (CH_{3TBS}), -4.3 ($SiCH_3$), -5.3 ($SiCH_3$) ppm; HRMS (ESI): *m/z* calcd for $C_{26}H_{37}N_3O_5Si$ $[M+Na]^+$ 522.2400, found 522.2388.

Dimethylthexylsilyl 2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-D-glucopyranose (**110**): EtSH (0.25 mL, 3.3 mmol) and catalytic *p*TsOH (35 mg) were added to a solution of dimethylthexylsilyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α,β-D-glucopyranose (350 mg, 0.66 mmol) in dry CH_2Cl_2 (8 mL). After stirring for 3 h under argon, the mixture was neutralized with solid $NaHCO_3$, diluted with CH_2Cl_2, washed with water, dried over anhydrous $MgSO_4$ and concentrated to dryness. The purification of the residue was carried out by column chromatography (hexane/EtOAc, 8/2) to yield

dimethylthexylsilyl 2-azido-3-*O*-benzyl-2-deoxy-α,β-D-glucopyranose (271 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 5H, aromatic), 4.95 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.72 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.55 (d, *J* = 7.5 Hz, 1H, H-1), 3.81 (dd, *J* = 11.8, 3.6 Hz, 1H, H-6a), 3.73 (dd, *J* = 11.8, 4.8 Hz, 1H, H-6b), 3.56 (dd, *J* = 9.7, 8.7 Hz, 1H, H-4), 3.27 (m, 2H, H-2, H-5), 3.21 (m, 1H, H-3), 1.68 (m, 1H, CH_{thexyl}), 0.92 – 0.90 (2s, 12H, $CH_{3thexyl}$), 0.21 and 0.20 (2s, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* = 138.2, 128.8, 128.2, 128.1, 97.2, 82.6, 75.2, 75.1, 70.6 68.6, 62.6, 34.0, 24.9, 20.1, 20.0, 18.60, 18.5, -1.9, -3.1. BzCN (76 mg, 0.58 mmol) and catalytic amount of Et_3N were added to a cooled (-40 °C) solution of dimethylthexylsilyl 2-azido-3-*O*-benzyl-2-deoxy-α,β-D-glucopyranose (250 mg, 0.57 mmol) in dry CH_3CN (11 mL). After 4h, MeOH was added and the mixture was allowed to reach room temperature. The solvent was concentrated, and the residue was dissolved in MeOH and concentrated to dryness. The purification was carried out by flash column chromatography (hexane/ EtOAc, 9/1) to afford **54** (250 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 – 8.03 (m, 2H, aromatic), 7.59 – 7.55 (m, 1H, aromatic), 7.47 – 7.42 (m, 2H, aromatic), 7.40 – 7.30 (m, 5H, aromatic), 4.97 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.74 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.57 – 4.56 (m, 3H, H-6ab, H-1), 3.56 – 3.50 (m, 2H, H-4, H-5), 3.32 (dd, *J* = 9.9, 7.6 Hz, 1H, H-2), 3.26 – 3.22 (m, 1H, H-3), 2.52 (bs, OH), 1.67 – 1.61 (m, 1H, CH_{thexyl}), 0.88 – 0.86 (3s, 12H, $CH_{3thexyl}$), 0.18 – 0.16 (2s, 6H, $Si(CH_3)_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.2, 166.9, 133.4, 129.9, 128.9, 128.5, 128.3, 97.3 (C-1), 82.3 (C-3), 75.3 (C_{Bn}), 74.0, 70.4 (C-4, C-5), 68.5 (C-2), 63.9 (C-6), 34.0 (CH_{thexyl}), 20.1, 20.0, 18.6, 18.5 ($CH_{3thexyl}$), -2.0, -3.1 ($Si(CH_3)_2$); HRMS (ESI): *m/z* calcd for $C_{28}H_{39}N_3O_6Si$ $[M+Na]^+$ 564.2506, found 564.2471.

***tert*-Butyldimethylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl)uronate)-D-glucopyranose (111)**: The reaction was carried out according to general procedure B using acceptor **109** (32 mg, 0.064 mmol) and n-pentenyl donor **49** (222 mg, 0.326 mmol). NIS (22 mg, 0.097 mmol) and TMSOTf (2.3 µL, 0.013 mmol) were added at 0 °C and the mixture was stirred for 2h. The crude was concentrated and analysis by UPLC-MS showed 40% formation of the desired disaccharide (Calcd for $C_{44}H_{57}N_3O_{13}SiNa$: 886.37, found 886.29) and 20 % of a disaccharide lacking one acetyl group (Calcd for $C_{42}H_{55}N_3O_{12}SiNa$: 844.36, found 844.28). The crude was dissolved in dry pyridine (1 mL), acetic anhydride (0.5 mL) was added at 0 °C and the reaction was stirred overnight. EtOH was added and the reaction

mixture was concentrated. The crude was purified by column chromatography hexane/EtOAc (8/2) to obtain **56** (27 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.40 – 7.19 (m, 15H, aromatic), 5.21 (bs, 1H, H-1′), 5.08 – 5.04 (m, 1H), 5.02 (d, *J* = 2.3 Hz, 1H), 4.87 – 4.83 (m, 1H), 4.72 – 4.68 (m, 3H, CH_2Ph), 4.60 (d, *J* = 12.3Hz, 1H, CH_2Ph), 4.53 – 4.47 (m, 3H, CH_2Ph, H-1), 4.00 (t, *J* = 9.5 Hz, 1H), 3.82 – 3.79 (m, 1H), 3.72 (dd, *J* = 11.2, 3.8 Hz, 1H, H-6a), 3.67 (dd, *J* = 11.4, 2.3 Hz, 1H, H-6b), 3.38 (m, 5H, CH_{3COOMe}), 3.21 (t, *J* = 9.6Hz, 1H), 2.01 and 2.00 (2s, 6H, CH_{3Ac}), 0.93 (s, 9H, CH_{3TBS}), 0.15 (s, 3H, $SiCH_3$), 0.14 (s, 3H, $SiCH_3$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2, 169.9, 168.7, 138.3, 138.2, 137.5, 128.6, 128.4, 128.3, 128.2, 128.1, 127.6, 127.6, 127.4, 97.5 (C-1), 97.4 (C-1′), 81.0, 75.4, 74.5 (C_{Bn}), 74.1, 73.3 (C_{Bn}), 72.6 (C_{Bn}), 72.5, 68.9, 68.2 (C-6), 68.0, 67.2, 66.4, 52.2 (CH_{3COOMe}), 25.7 (CH_{3TBS}), 21.1, 20.9 (CH_{3Ac}), 18.1 (Cq_{TBS}), -4.1, -5.1 ($Si(CH_3)_2$) ppm; HRMS (ESI): *m/z* calcd for $C_{44}H_{57}N_3NaO_{13}Si$ $[M+Na]^+$ 886.3553, found 886.3527.

Dimethylthexylsilyl 2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(methyl (3-*O*-benzyl-2,4-di-*O*-levulinoyl-α-L-idopyranosyl)uronate)-D-glucopyranose (**112**): The reaction was carried out according to general procedure B using acceptor **110** (21 mg 0.039 mmol) and *n*-pentenyl donor **50** (29 mg, 0.051 mmol), NIS (12 mg, 0.055 mmol) and TMSOTf (1.33µL, 0.010 mmol) were added at 0 °C and the mixture was stirred for 2h. The crude was concentrated and the conversion to disaccharide **57** was determinated by LCMS to be 30%. (Calcd for $C_{52}H_{67}N_3O_{16}SiNH_4^+$: 1040.43, found 1040.42).

Dimethylthexylsilyl 2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(methyl (2,4-di-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyl)uronate)-D-glucopyranose (**113**): The reaction was carried out according to general procedure B using acceptor **109** (45 mg, 0.083 mmol) and n-pentenyl donor **51** (76 mg, 0.083 mmol). NIS (56 mg, 0.249 mmol) and TMSOTf (3 µL, 0.016 mmol) were added at 0 °C and the mixture was stirred for 2h. The crude was concentrated and was purified by column chromatography hexane/ EtOAc (7/3) to obtain **113** (73 mg, 85%). $[\alpha]_D^{20}$ = -33.6° (*c* = 1, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.06 – 7.95 (m, 4H, aromatic), 7.67 – 7.62 (m, 2H, aromatic), 7.57 – 7.48 (m, 2H, aromatic), 7.44 – 7.17 (m, 15H, aromatic), 7.01 – 7.95 (m, 2H, aromatic), 5.41 – 5.37 (m, 2H, H-1′, H-4′) , 5.22 – 5.15 (m, 2H, H-5′, H-2′), 4.89 – 4.82 (m, 3H, CH_2Ph, H-6), 4.74 (d, *J* = 10.9 Hz, CH_2Ph), 4.70 (d, *J* = 10.9Hz, CH_2Ph), 4.54 (d, *J* = 7.2 Hz, 1H, H-1), 4.42 (dd, *J* = 11.9, 6.1 Hz, 1H, H-6), 4.14 – 4.11 (m, 1H, H-3′), 3.98

(dd, J = 9.8, 8.6 Hz, 1H, H-4), 3.69 – 3.63 (m, 1H, H-5), 3.40 – 3.30 (m, 5H, CH_{3COOMe}, H-2, H-3), 1.64 – 1.58 (m, 1H, CH_{thexyl}), 0.85 – 0.83 (12H, $CH_{3thexyl}$), 0.14 - 0.11 (6H, $Si(CH_3)_2$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 168.8, 166.1, 165.6, 165.4, 138.1, 137.4, 133.6, 133.3, 133.1, 130.1, 130.0, 129.9, 129.4, 128.9, 128.6, 128.4, 128.4, 128.3, 128.2, 127.7, 127.5, 97.8 ($J_{C1'-H1'}$ = 171 Hz, C-1′), 97.2 (J_{C1-H1} = 160 Hz, C-1), 81.1 (C-3), 75.2 (C-4), 74.8 (C_{Bn}), 73.8 (C-5), 73.0 (C_{Bn}), 72.9 (C-3′), 69.1 (C-2), 68.5 (C-4′), 67.7 (C-2′), 67.1 (C-5′), 63.2 (C-6), 52.3 (CH_{3COOMe}), 34.0 (CH_{thexyl}), 20.1, 20.0, 18.6, 18.5 ($CH_{3thexyl}$), -2.0, -3.2 ($Si(CH_3)_2$) ppm; HRMS (ESI): *m/z* calcd for $C_{56}H_{63}N_3O_{14}Si$ $[M+Na]^+$ 1052.3977, found 1052.3912.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**114**): To a solution of **108** (94 mg, 0.071 mmol) in dry CH_2Cl_2/MeOH (25 mL/2.5 mL), hydrazine acetate (9 mg, 0.106 mmol) was added. The reaction was stirred for 3h and the crude was concentrated and purified using hexane/EtOAc (6/4) to obtain **114** as a white solid (70 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 – 8.15 (m, 2H, aromatic), 8.10 – 8.00 (m, 4H, aromatic), 7.58 – 7.15 (m, 28H, aromatic), 5.36 (s, 2H, CH_2-Ph_{Bz}), 5.19 – 5.16 (m, 4H, H-1, H-2, CH_2-Ph_{Carba}), 4.88 – 4.82 (m, 4H, H-5, H-1′, H-6a, CH_2Ph), 4.74 (d, 1H, CH_2Ph), 4.47 – 4.45 (m, 3H, CH_2Ph, H-6b), 4.36 (d, 1H, CH_2Ph), 4.16 – 4.13 (m, 3H, CH_2Ph, H-3, H-4), 4.04 – 4.02 (m, 1H, H-5′), 3.83 (s, 3H, CH_{3COOMe}), 3.78 – 3.75 (m, 1H, $OCH_{2Linker}$), 3.54 – 3.46 (m, 3H, H-3′, H-4′, $OCH_{2Linker}$), 3.23 – 3.13 (m, 3H, H-2′, $NCH_{2Linker}$), 3.04 (bs, 1H, OH), 1.63 – 1.50 (m, 4H, $CH_{2Linker}$), 1.31 – 1.25 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.9, 167.5, 166.5, 165.6, 156.7, 156.2, 137.9, 137.7, 133.5, 133.1, 130.1, 129.9, 129.8, 129.5, 128.8, 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.4, 127.2, 99.4 (C-1′), 99.2 (C-1), 79.3 (C-3′), 75.7 (C-4), 75.0 (CH_2Ph), 73.2 (C-3), 72.4 (CH_2Ph), 71.4 (C-4′), 70.5 (C-5′), 68.7 ($CH_{2Linker}$), 68.0 (C-2), 67.5 (C-5), 66.9 (CH_2-Ph_{Carba}), 66.5 (CH_2-Ph_{Bz}), 63.2 (C-2′), 63.1 (C-6′), 52.4 (CH_{3COOMe}), 50.6, 50.3 (CH_2-PhN), 47.3 ($NCH_{2Linker}$), 46.3 ($CH_{2Linker}$), 29.2 ($CH_{2Linker}$), 27.63 ($CH_{2Linker}$), 23.5 ($CH_{2Linker}$) ppm. HRMS (ESI): *m/z* calcd for $C_{69}H_{70}N_4O_{17}$ $[M+Na]^+$ 1249.4634, found 1249.4623.

4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(methyl (2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl -L-idopyranosyl)uronate)-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**115**): The glycosylation was carried out according to general procedure B using:

Conditions 1. Acceptor **114** (35 mg, 0.028 mmol) and thiophenyl donor **43** (21 mg, 0.034 mmol), NIS (19 mg, 0.085 mmol) and TMSOTf (1.5 µL, 0.006 mmol) were added at room temperature. The crude was purified by column chromatography using hexane/EtOAc (7/3) to obtain compound **115** (16 mg, 34 %).

Conditions 2. To a solution of acceptor **114** (64 mg, 0.052 mmol) and donor **57** (36 mg, 0.062 mmol), NIS (35 mg, 0.156 mmol) and TMSOTf (1.8 µL, 0.01 mmol) were added at 0 °C**.** The crude was purified by column chromatography using hexane/EtOAc (7/3) to obtain compound **115** (40 mg, 45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 – 8.00 (m, 8H, aromatic), 7.57 – 7.13 (m, 36H, aromatic), 5.44 (d, J = 3.6Hz, 1H, H-1′′), 5.35 (s, 2H, CH_2-Ph_{Bz}), 5.20 – 5.16 (m, 5H, H-1, H-2′′, H-4′′, CH_2-Ph_{Carba}), 5.09 (s, 1H, H-2), 4.88 (d, J = 11.4Hz, 1H, CH_2Ph), 4.83 – 4.70 (m, 7H, H-6_a, H-5′′, H-1′, H-5, CH_2Ph), 4.48 – 4.45 (m, 3H, H-6_b, CH_2-PhN), 4.41 (d, J = 10.4Hz, 4H, CH_2Ph), 4.13 – 4.09 (m, 1H, H-3), 4.03 – 3.89 (m, 5H, H-4′, H-5′, H-3′′, H-4, CH_2Ph) , 3.78 – 3.70 (m, 1H, $OCH_{2Linker}$), 3.68 (s, 3H, CH_{3COOMe}), 3.55 – 3.46 (m, 2H, H-3′, $OCH_{2Linker}$), 3.42 (s, 3H, CH_{3COOMe}), 3.27 (dd, J = 10.3, 3.4Hz, 1H, H-2′), 3.24 – 3.11 (m, 2H, $NCH_{2Linker}$), 2.59 (t, J = 6.6 Hz, 2H, CH_{2Lev}), 2.38 (t, J = 6.6 Hz, 2H, CH_{2Lev}), 2.09 (s, 3H, CH_{3Lev}), 1.58 – 1.43 (m, 4H, $CH_{2Linker}$), 1.35 – 1.24 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 205.8, 171.6, 169.8, 168.7, 166.5, 166.1, 165.7, 165.1, 156.7, 156.2, 138.0, 137.7, 137.3, 137.0, 135.8, 133.6, 133.5, 133.1, 133.0, 130.2, 130.0, 129.8, 129.3, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.5, 127.3, 127.2, 99.1 (C-1, $J_{C1\text{-}H1}$ = 171 Hz), 98.9 (C-1′, $J_{C1'\text{-}H1'}$ = 169 Hz), 98.1 (C-1′′, $J_{C1''\text{-}H1''}$ = 171 Hz), 78.6 (H-3′), 75.7, 75.6 (C-4, C-4′), 74.6 (CH_2Ph), 74.3 (C-3), 73.4 (CH_2Ph), 72.8 (C-3′′), 72.4, 70.2 (C-5′), 69.8 (C-2′′), 68.6 ($CH_{2Linker}$), 68.3 (C-2, C-4′′), 67.6 (C-5), 66.9 (CH_2-Ph_{carba}), 66.5 (CH_2-Ph_{Bz}), 63.8 (C-2′), 62.2 (C-6), 52.4 (CH_{3COOMe}), 52.0 (CH_{3COOMe}), 50.6, 50.3 (CH_2-PhN), 47.3 , 46.3 ($NCH_{2Linker}$), 37.7 (CH_{2Lev}), 29.7 (CH_{3Lev}), 29.2 ($CH_{2Linker}$), 28.1 ($CH_{2Linker}$), 27.8 ($CH_{2Linker}$, CH_{3Lev}), 27.6 ($CH_{2Linker}$), 23.5 ($CH_{2Linker}$) ppm; HRMS (ESI): *m/z* calcd for $C_{95}H_{96}N_4O_{26}$ $[M+Na]^+$ 1731.6205, found 1731.6274.

5.8 EVALUATION OF DONORS IN THE SOLID PHASE SYNTHESIS OF HS PRECURSORS

5.8.1 Initial studies: trisaccharide solid phase synthesis using idose TBPDS.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (SP-116): Linker **SP-9** (**SP-9$_{PS0.2}$**, **SP-9$_{PS0.4}$**, **SP-9$_{TENT0.2}$** and **SP-9$_{TENT0.4}$**, 100 mg, 0.22 mmol/g) was glycosylated in one cycle with thioglycoside **25** (2 eq), NIS (2.5 eq) and catalytic amount of TMSOTf according to general procedure G. The conversion was determined by analytical NaOMe cleavage:

1. Resin **SP-116$_{PS0.2}$**: UPLC-MS (ESI): 85% as compound **117**(*m/z:* $C_{49}H_{57}NO_9Si$ $[M+Na]^+$ 854.37 found 854.35).
2. Resin **SP-116$_{PS0.4}$**: UPLC-MS (ESI): 95% as compound **117**(*m/z:* $C_{49}H_{57}NO_9Si$ $[M+Na]^+$ 854.37 found 854.35).
3. Resin**SP-116$_{TENT0.2}$**: No activation
4. Resin **SP-116$_{TENT0.4}$**: UPLC-MS (ESI): 30% as compound **117**(*m/z:* $C_{49}H_{57}NO_9Si$ $[M+Na]^+$ 854.37 found 854.35).

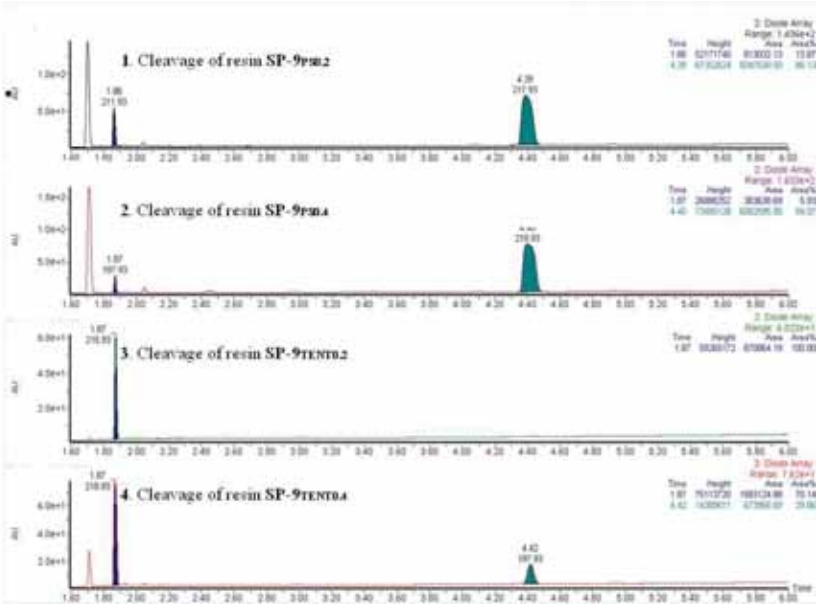


Figure 5.1. UPLC-MS chromatogram of analytical cleavage data conversion of linker **SP-9** (cleaved as **10** retention time (t_r) at 1.9 min., *m/z* calcd for $C_{21}H_{27}NO_4$ $[M+Na]^+$ 380.18 found 380.16) to the monosaccharide **SP-116** (cleaved as **118** (t_r) at 4.4 min., peak **C** (*m/z* calcd for $C_{49}H_{57}NO_9Si$ $[M+Na]^+$ 854.37 found 854.35).

4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (116): Compound **116** was obtained according to cleavage Procedure F. MALDI-TOF MS analysis showed complete retention of all protecting groups. MALDI-TOF MS: Calcd for $C_{62}H_{71}NO_{12}SiNa$ $[M+Na]^+$ 1072.46, found 1072.70.

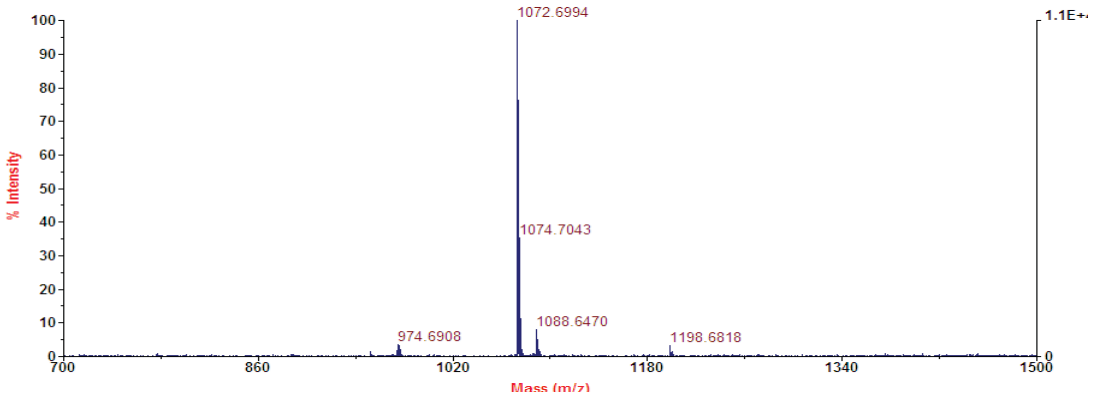


Figure 5.2. Maldi-Tof MS for glycoconjugate **116**.

The resin **SP-116** (100mg/0.22 mmol/g) was transformed to Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-118**) using general procedure H in 3 cycles with 2 eq of hydrazine acetate.

4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (118): Compound **118** was obtained according to cleavage Procedure F. MALDI-TOF MS analysis showed complete cleavage of the levulinyl protecting group. MALDI-TOF MS: Calcd for $C_{57}H_{65}NO_{10}SiNa$ $[M+Na]^+$ 974.43, found 975.25.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-(4-O-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-119): The reaction was performed according to general procedure G using five cycles on resin **SP-118** (0.500 g, 0.1 mmol) with thricloroacetimidate **65** (5 x 5 eq, 320 mg, 0.5 mmol) and

TMSOTf (5 µL, 0.025 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle1) not analyzed, (cycle 2) 54%, (cycle 3) 67%, (cycle 4) 78%, (cycle 5) 82% as compound **120** (*m/z:* $[M+NH_4]^+$ calcd for $C_{62}H_{72}N_4O_{13}Si$ 1126.52 found, 1126.32).

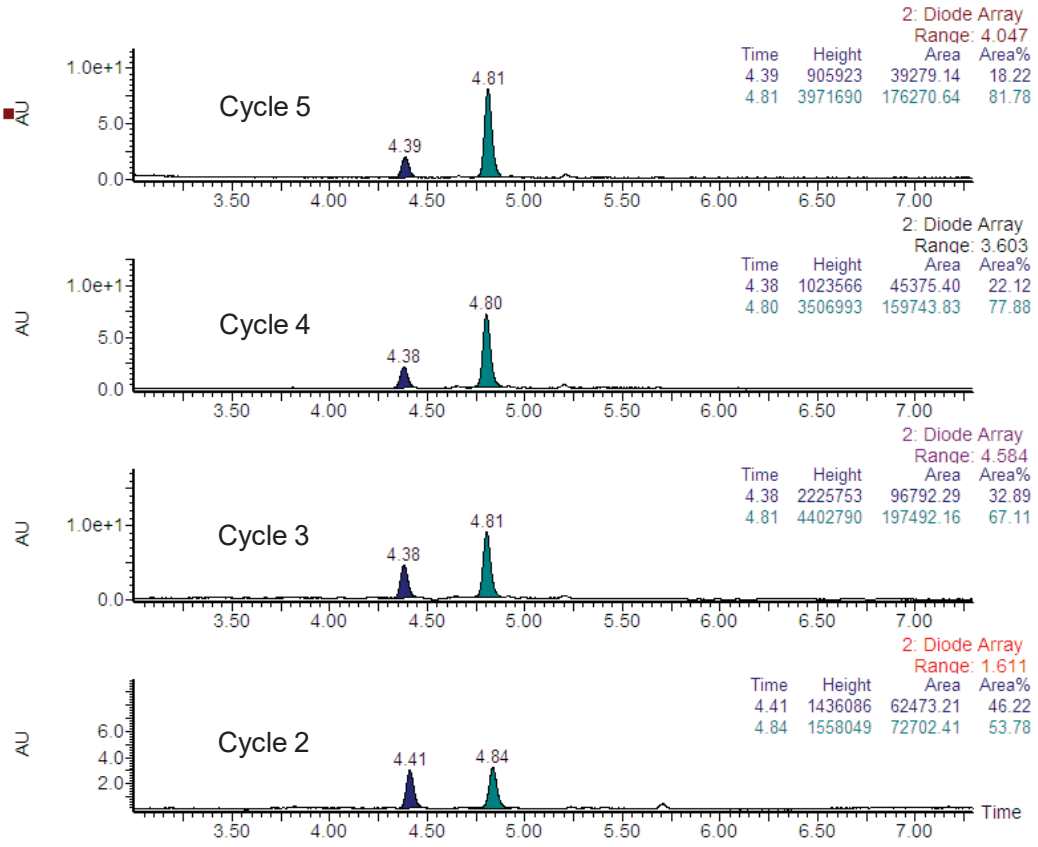


Figure 5.1. UPLC-MS chromatogram of analytical cleavage data for conversion of monosaccharide **SP-118** (average retention time=4.39 min) to the disaccharide **SP-119** (average retention time=4.81 min) derivative.

4-(Acetoxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-acetyl-3-*O*-benzyl-4-*O*-(4,6-*O*-di-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (121): Compound **121** was obtained from **SP-119** in 45% overall yield after preparative cleavage according to Procedure D and purification. The intermediate (6 mg, 0.005 mmol) was taken up in CH_2Cl_2 (1.5 mL) and treated with pyridine (0.1 mL, 1.3 mmol), acetic anhydride (0.1 mL, 1.0 mmol), and a catalytic amount of DMAP. After 12 hours, the reaction mixture was diluted with CH_2Cl_2 and successively washed with saturated $CuSO_4$ aq solution and

water. The organic phase was then dried over anhydrous $MgSO_4$, filtered and concentrated. Preparative TLC (hexane/EtOAc, 7/3) afforded compound **121** as a white solid (6 mg, 93% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77-7.10 (25H, m), 5.22-5.11 (2H, m), 5.08 (2H, s), 5.01 (1H, dd, J=9.7 Hz), 4.96-4.92 (1H, m), 4.91 (1H, d, *J*=3.6 Hz), 4.87-4.82 (1H, m), 4.80 (1H, d, *J*=11.7 Hz), 4.76 (1H, d, *J*=11.1 Hz), 4.65 (1H, d, *J*=11.8 Hz), 4.55 (1H, d, *J*=11.0 Hz), 4.50-4.42 (2H, m), 4.28-4.23 (1H, m), 3.99 (1H, dd, *J*=4.5, 12.4 Hz), 3.92-3.79 (5H, m), 3.78-3.72 (1H, m), 3.69-3.59 (3H, m), 3.40 (1H, dd, *J*=3.6, 10.2 Hz), 3.36-3.26 (1H, m), 3.26-3.10 (2H, m), 2.09 (6H, s), 1.94 (3H, s), 1.88 (3H, s), 1.70-1.45 (4H, m), 1.35-1.20 (2H, m), 1.04 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.84, 170.49, 170.05, 169.27, 156.60, 156.06, 137.75, 137.38, 136.89, 135.62, 135.46, 133.14, 132.97, 129.89, 129.83, 128.53, 128.35, 127.99, 127.79, 127.64, 127.35, 127.28, 127.09, 97.84, 95.91, 77.99, 74.94, 72.25, 72.06, 71.44, 69.71, 68.51, 67.87, 67.59, 66.74, 65.97, 63.34, 63.03, 61.70, 50.51, 50.19, 47.19, 46.19, 29.69, 29.08, 26.79, 23.40, 20.99, 20.82, 20.66, 20.59, 19.14.; HRMS (ESI): Calcd for $C_{71}H_{84}N_4O_{17}SiNa$ $[M+Na]^+$ 1315.5498, found 1315.5516.

The resin **SP-119** (100mg/0.22 mmol/g) was transformed to Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-(4-O-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl] carbamate (**SP-122**) using general procedure H in 3 cycles with 2 equiv. of hydrazine acetate.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-123): The reaction was performed according to general procedure G using four cycles on resin **SP-122** (150 mg, 0.03 mmol) with trichloroacetimidate **28** (5 equiv, 128 mg, 0.15 mmol) and TMSOTf (2 μL, 0.007 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) 41%, (cycle 2) 45%, (cycle 3) 50%, (cycle 4) 53% as compound **124** (*m/z:* $[M+NH_4]^+$ calcd for $C_{90}H_{106}N_5O_{18}Si_2$ 1600.71 found 1600.50.

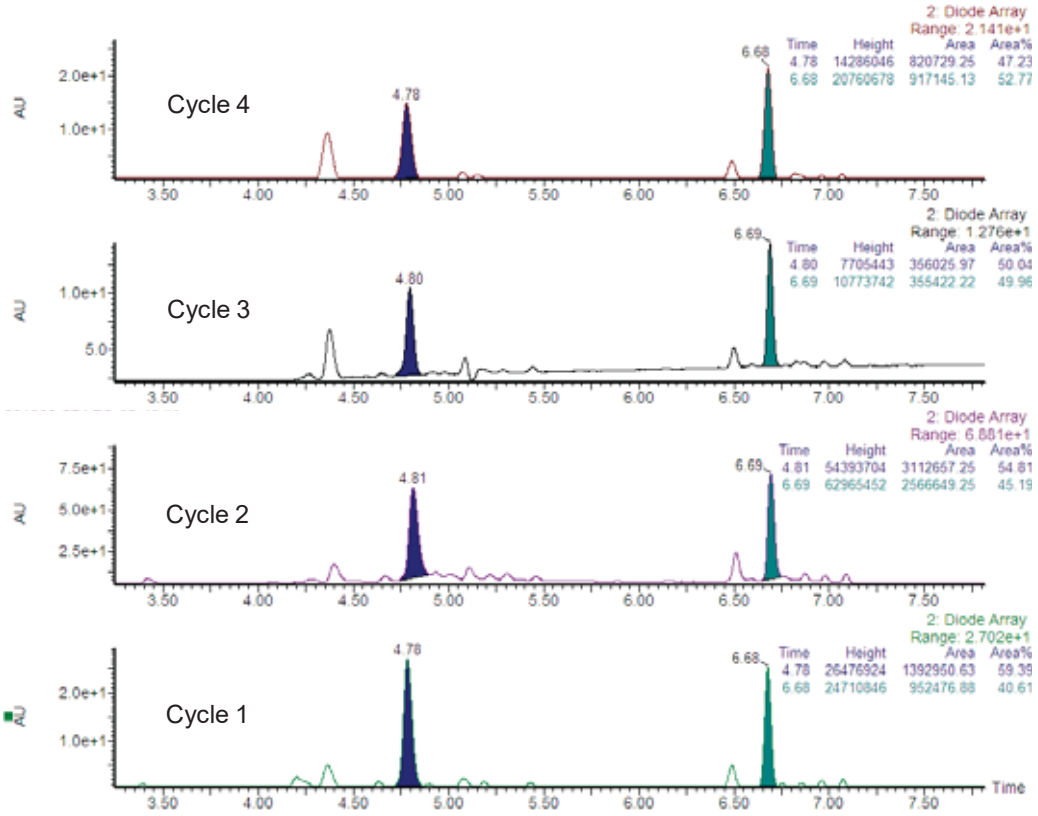


Figure 5.2. UPLC-MS chromatogram of analytical cleavage data for conversion of disaccharide **SP-122** (average retention time t_r=4.39 min) to trisaccharide **SP-123** (average retention time t_r=6.68 min) derivative.

4-(Acetoxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-acetyl-3-*O*-benzyl-4-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-(2,4-di-*O*-acetyl-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyl)-2-deoxy-α-D-glucopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (125): Compound **125** was obtained in 10% overall yield by preparative cleavage (Procedure D) and purification from **SP-123**. The crude product (4 mg, 0.002 mmol) was taken up in CH_2Cl_2 (1.5 mL) and treated with pyridine (0.1 mL, 1.3 mmol), acetic anhydride (0.1 mL, 1.0 mmol), and catalytic amount of DMAP. After 12 hours, the reaction mixture was diluted with CH_2Cl_2 and successively washed with saturated $CuSO_4$ aq solution and water. The organic phase was then dried over anhydrous $MgSO_4$, filtered and concentrated. Preparative TLC (hexane/ EtOAc, 7/3) afforded compound **125** as a white solid (4 mg, 95% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74-7.09 (44H, m), 5.21-5.11 (2H, m), 5.08 (2H, s), 5.02-4.98 (2H, m), 4.95-4.81 (5H, m), 4.79-4.60 (4H, m), 4.55-4.41 (4H, m), 4.22-4.00 (4H,

m), 3.94-3.80 (3H, m), 3.80-3.73 (3H, m), 3.73-3.57 (5H, m), 3.37-3.08 (4H, m), 2.09 (3H, s), 2.03 (3H, s), 1.98 (3H, s), 1.92 (3H, s), 1.90 (3H, s), 1.70-1.40 (4H, m), 1.35-1.20 (2H, m), 1.06 (9H, s), 1.01 (9H, s); ^{13}C NMR shift values taken from HSQC δ 135.56, 135.51, 129.85, 129.69, 128.32, 127.90, 127.85, 127.82, 97.90, 97.77, 96.81, 79.16, 75.40, 75.33, 74.30, 73.23, 72.79, 72.48, 72.34, 69.79, 68.95, 68.51, 68.42, 67.78, 67.55, 66.69, 66.63, 66.57, 65.88, 63.84, 63.74, 63.36, 62.40, 62.16, 61.82, 61.87, 50.33, 47.16, 46.07, 29.57, 29.19, 26.94, 26.87, 23.00, 21.17, 21.12, 21.01; HRMS (ESI): Calcd for $C_{102}H_{120}N_4O_{23}Si_2Na$ $[M+Na]^+$ 1847.7780, found 1847.7733.

4.8.2 Solid phase synthesis of HS precursor trisaccharide using n-pentenyl orthoester of iduronic acid as glycosyl donors

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl-(5-(methyl (2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (SP-126): The reaction was performed according to general procedure G employing:

Conditions 1: Resin **SP-9** (200 mg, 0.22 mmol/g), donor **44** (120 mg, 0.20 mmol), NIS (58 mg, 0.26 mmol) and TMSOTf (1.4 µL, 0.008 mmol) in dry CH_2Cl_2 (2 mL) at room temperature.

Conditions 2: Resin **SP-9** (250 mg, 0.2 mmol/g), donor **43** (200 mg, 0.15 mmol) and TMSOTf (1.1 µL, 0.006 mmol) in dry CH_2Cl_2 (2 mL) at -40 °C.

Conditions 3: Resin **SP-9** (160 mg, 0.2 mmol/g), donor **57** (94 mg, 0.16 mmol) and TMSOTf (1.2 µL, 0.007 mmol) in dry CH_2Cl_2 (2 mL) at 0°C

The conversion of every glycosylation reaction was determined after analytical NaOMe cleavage: 1. 84%, 2. 87%, 3. 85%. LCMS (ESI) m/z: t_r at 5.10 min as compound **127**, calcd for $C_{34}H_{41}NO_{10}$-$[Na]^+$: 646.3, found 646.2; t_r at 5.79 min as compound **127**+Me, calcd for $C_{35}H_{43}NO_{10}$-$[Na]^+$: 660.3, found 660.2.

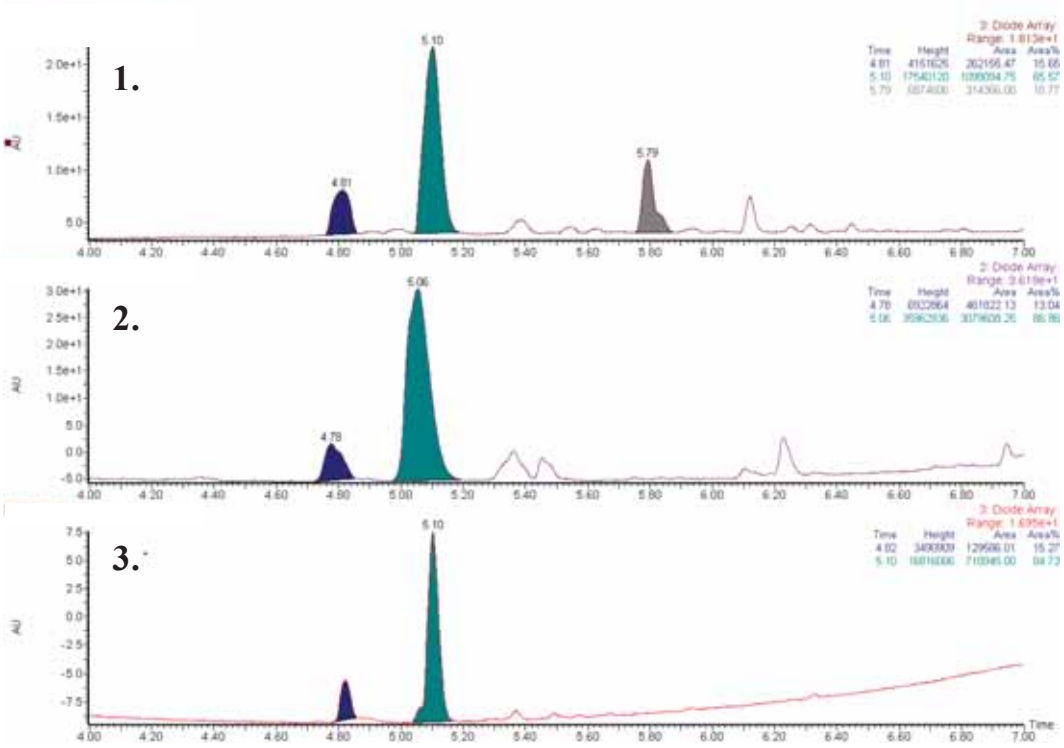


Figure 5.5. UPLC-MS chromatogram of analytical cleavage data for conversion of linker **SP-9** (t_r= 4.81 min) to the monosaccharide **SP-126** (t_r= 5.10 min as **127** and t_r = 5.79 min as **127**+Me derivative).

The resin **SP-126** (94 mg / 0.22 mmol/g) was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**SP-128**) using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**SP-129**): The reaction was performed according to general procedure G using:

Conditions 1: 1 cycle on resin **SP-128** (250 mg / 0.44 mmol/g) with trichloroacetimidate **65** (1 x 3 equiv., 192 mg) and TMSOTf (1.8 µL, 0.01 mmol) in dry CH_2Cl_2 (1.4 mL) at -40 °C. Conversion was determined after analytical sodium methoxide cleavage: 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-idopyranosyloxy)uronate)pentyl) carbamate (**130**). Conversion: 21%, LCMS (ESI) m/z: at retention time 5.86 min calcd for $C_{47}H_{56}N_4O_{14}$-$[NH_4]^+$: 918.38, found: 918.36.

Conditions 2: 4 cycles of glycosylation were performed on resin **SP-128** (155 mg / 0.22 mmol/g) with trichloroacetimidate **65** (3 x 5 eq., 65 mg, 0.16 mmol) and TMSOTf (68 µL of 0.1M solution of TMSOTf) in dry CH_2Cl_2 (1.2 mL) at -20 °C. Conversion was determined after analytical dibutyltin oxide cleavage as 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-idopyranosyloxy)uronate)pentyl) carbamate (**131**). Conversion 84%, LCMS (ESI) m/z: at t_r 11.96 min calcd for $C_{67}H_{72}N_4O_{18}$-$[NH_4]^+$: 1238.4, found 1238.3.

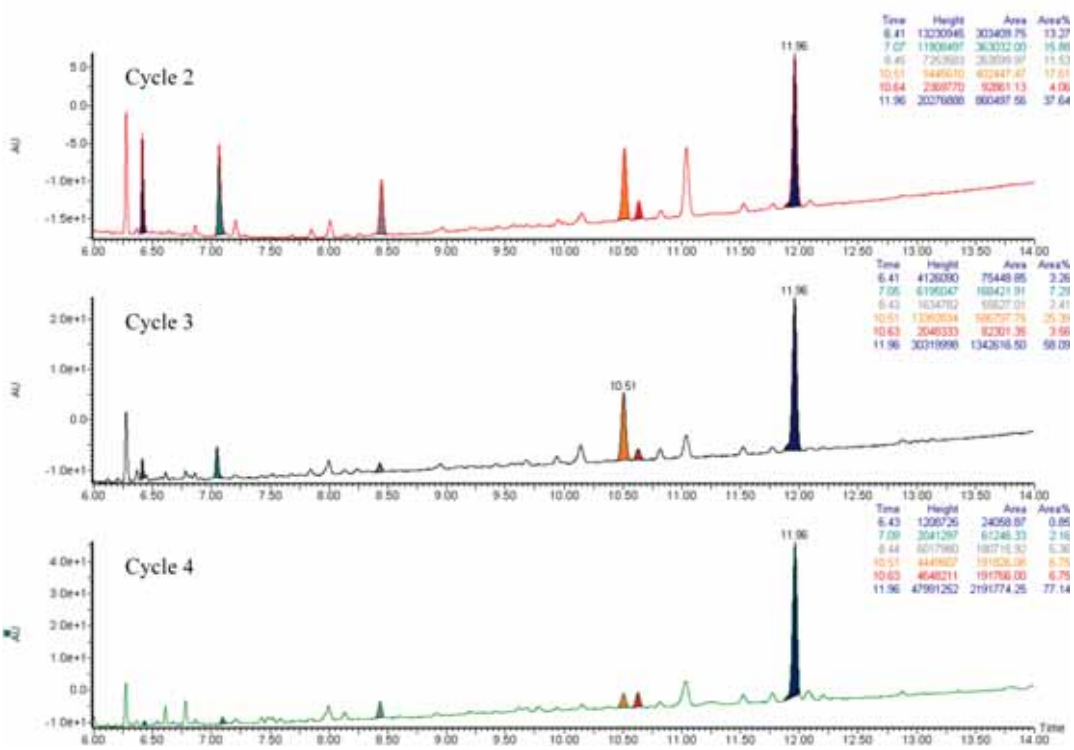


Figure 5.6. UPLC-MS chromatogram of analytical cleavage data for conversion of monosaccharide **SP-128** to the disaccharide **SP-129**. Bu_2SnO cleavage produces minor amounts of partially deproctected intermediates: for monosaccharide acceptor **127** (retention times = 6.41 (**127**), 7.05 (**127**+Me), 8.45 min (**127**+Me+Bz)) and for disaccharide product **131** (11.96 min) and **131**-.

The resin **SP-129** (135 mg/0.22 mmol/g) was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-idopyranosyloxy)uronate)pentyl) carbamate (**SP-132**) using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-(methyl (2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyl)uronate)-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**SP-133**): The reaction was performed according to general procedure G using 3 cycles on resin **SP-132** (127 mg / 0.22 mmol/g) with donor **57** (79 mg, 0.14 mmol) and TMSOTf (56 µL of 0.1M solution of TMSOTf in CH_2Cl_2) in dry CH_2Cl_2 (0.96 mL) at -20 °C. Conversion was determined after analytical dibutyltin oxide mediated cleavage: 4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-(methyl (2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyl)uronate)-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**134**). Conversion: 76%, LCMS (ESI) m/z: at retention time (t_r) 13.35 min, calcd for $C_{88}H_{92}N_4O_{25}$-$[NH_4]^+$: 1622.6, found 1622.4.

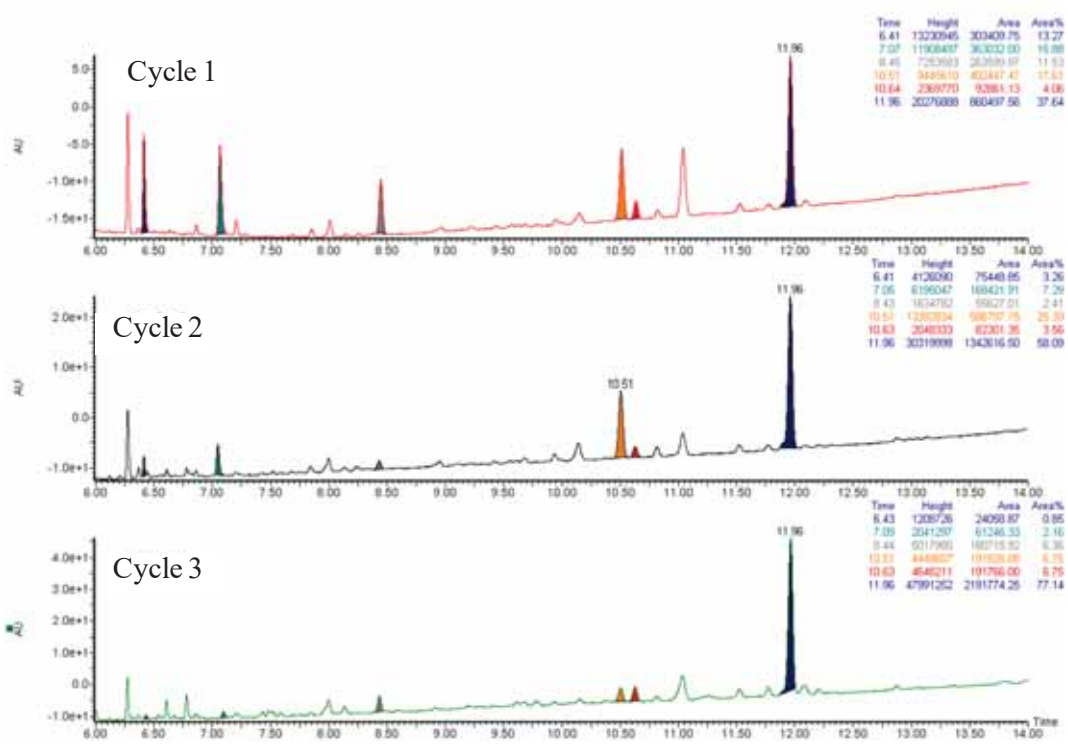


Figure 5.7. UPLC-MS data for conversion disaccharide **SP-132** to the trisaccharide **SP-133.** Bu_2SnO cleavage affords to partially deprotected intermediates: for **acceptor 131**(retention time = 10.50min (**78**-Bz), 11.89 min (**78**)), for **134** only product (retention time = 13.35 min (**134**)).

The resin **SP-133** (125 mg, 0.2 mmol/g) was swollen in dry CH_2Cl_2 (2mL), dry MeOH (1mL) and dibutyltin oxide (123 mg) was added. The reaction mixture was heated in the microwave for 10 min at 120°C. The resin was washed 3 times with a mixture of CH_2Cl_2: MeOH (1:1) and 3 times with MeOH. This cleavage was repeated until no further release of compound from the resin was observed and employing a large excess of dibutyltin oxide (820 mg) in the last cleavage cycle. The crude was dissolved in MeOH and filtered and was taken up in CH_2Cl_2 (0.2 mL) and treated with pyridine (0.9 mL, 0.52 mmol), benzoyl chloride (1.2 mL, 0.26 mmol), and a catalytic amount of DMAP. After 12 hours, the reaction mixture was diluted with CH_2Cl_2 and successively washed with saturated $CuSO_4$ aq solution and water. The organic phase was then dried over anhydrous $MgSO_4$, filtered and concentrated. Column chromatography followed by preparative TLC (hexane/ EtOAc, 7/3) afforded compound **115** as a white solid (3.7 mg, 8% overall yield).

5.8.3 Solid phase synthesis of HS trisaccharide precursor using L- idopyranosyl donors

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(6-*O*-acetyl-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-135**): Reaction was performed according to general procedure G using resin **SP-9** (100 mg / 0.44 mmol/g), thioglycoside **37** (3 eq, 73 mg, 0.12 mmol). NIS (36 mg, 0.16 mmol) and TfOH (44 µL of 0.1 M solution in CH_2Cl_2) were added at -20 °C. Conversion was determined after analytical NaOMe cleavage: 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(3-*O*-benzyl-α-L-idopyranosyloxy)pentyl) carbamate (**136**). Conversion: 96%, UPLC-MS (ESI) m/z: calcd $C_{34}H_{43}N_{43}O_9$-$[NH_4]^+$: 627.29, found 627.30.

The resin **SP-135** (100mg/0.44 mmol/g) was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(6-*O*-acetyl-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-137**) using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(6-*O*-acetyl-4-*O*-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-138**): The reaction was

performed according to general procedure G using 1 cycle on resin **SP-137** (100 mg / 0.44 mmol/g) with trichloroacetimidate **65** (1 x 3 eq., 80 mg) and TMSOTf (1.8 µL, 0.01 mmol) at -40 °C. Conversion was determinate after analytical sodium methoxide cleavage: 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-α-L-idopyranosyloxy)pentyl) carbamate (**139**). Conversion: 22%, LCMS (ESI) m/z: calcd $C_{47}H_{58}N_4O_{13}$-$[NH_4]^+$: 904.40, found 904.37.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-140**): Reaction was performed according to general procedure G using resin **SP-9** (150 mg / 0.44 mmol/g, 66 µmol), thioglycoside **26** (5 eq., 116 mg, 0.165 mmol), NIS (6.5 eq., 48 mg, 0.215 mmol) and TMSOTf (33 µL, 0.1M solution in CH_2Cl_2). Conversion was determined after analytical NaOMe cleavage as 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(3-*O*-benzyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**141**). Conversion: >95%, LCMS (ESI) *m/z*: calcd for $C_{42}H_{61}N_1O_9Si$-$[NH_4]^+$: 769.44, found: 769.25.

The resin **SP-140** (150 mg / 0.44 mmol/g, 66 µmol) was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-142**) using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-143**): Reaction was performed according to general procedure G using 3 cycles on resin **SP-142** (150 mg / 0.44 mmol/g, 66 µmol) and trichloroacetimidate **65** (3 x 3 eq., 64 mg, 0.198 mmol). TMSOTf (33 µL, 0.1 M solution in CH_2Cl_2) was added at -20 °C. Conversion was determined after analytical NaOMe cleavage: 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**144**). Conversion 77%, UPLC-MS (ESI) *m/z*: calcd for $C_{55}H_{76}N_4O_{13}Si$-$[NH_4]^+$: 1046.55, found 1046.69.

The resin **SP-143** (150 mg / 0.44 mmol/g, 66 µmol) was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-

deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-145**) using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-6-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyl)-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-146**): Reaction was performed according to general procedure G using 3 cycles on resin **SP-145** (70 mg / 0.44 mmol/g, 31 μmol), trichloroacetimidate **29** (4 x 3 eq., 70 mg, 92 μmol) and TMSOTf (31 μL, 0.1M solution in CH_2Cl_2). Conversion was determined after analytical NaOMe cleavage 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-4-*O*-(3-*O*-benzyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyl)-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**147**). Conversion 77%, LCMS (ESI): *m/z* calcd for $C_{55}H_{76}N_4O_{13}Si$-$[NH_4]^+$: 1046.55, found 1046.69.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-148**): Reaction was performed according to general procedure G using resin **SP-9** (200 mg / 0.44 mmol/g, 88 μmol), thioglycoside **27** (5 eq., 295 mg, 0.44 mmol), NIS (6.5 eq., 128 mg, 0.572 mmol) and TMSOTf (88 μL, 0.1 M solution in CH_2Cl_2). Conversion was determined after analytical NaOMe cleavage as 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (**149**). Conversion >95%, LCMS (ESI) *m/z*: calcd for $C_{41}H_{49}NO_{10}$-$[NH_4]^+$: 733.37, found 733.26.

The resin **SP-148** (200 mg / 0.44 mmol/g, 88 μmol) was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-150**) using general procedure E.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-O-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (SP-151): The reactions were performed according to general procedure G using:

Conditions 1: 4 cycles of glycosylation were performed on resin **SP-150** (200 mg / 0.44 mmol/g, 88 µmol) with trichloroacetimidate **65** (3 eq., 169 mg, 0.264 mmol) and TMSOTf (44 µL, 0.1 M solution in CH_2Cl_2).
Conditions 2: 2 cycles of glycosylation were performed on resin **SP-150** (120 mg / 0.44 mmol/g, 52.8 µmol) with trichloroacetimidate **65** (6 eq., 203 mg, 0.317 mmol) and TMSOTf (44 µL, 0.1 M solution in CH_2Cl_2).
Conditions 3: 1 cycle of glycosylation were performed on resin **SP-150** (120 mg / 0.44 mmol/g, 52.8 µmol) with trichloroacetimidate **65** (12 eq., 406 mg, 0.633 mmol) and TMSOTf (52 µL, 0.1 M solution in CH_2Cl_2).
Conditions 4: 2 cycles of glycosylation were performed on resin **SP-150** (200 mg / 0.44 mmol/g, 88 µmol) with trifluoroacetimidate **66** (6 eq., 353 mg, 0.528 mmol) and TMSOTf (88 µL, 0.1 M solution in CH_2Cl_2).
Conditions 5: 1 cycle of glycosylation was performed on resin **SP-150** (120 mg / 0.44 mmol/g, 52.8 µmol) with trichloroacetimidate **65** (12 eq., 406 mg, 0.364 mmol) and 1 cycle (6 eq., 203 mg, 0.317 mmol) and TMSOTf (44 µL, 0.1 M solution in CH_2Cl_2).
Conditions 6: 3 cycles of glycosylation were performed on resin **SP-150** (200 mg/0.22 mmol/g, 44 µmol) with trichloroacetimidate **65** (3 x 6 eq., 170 mg, 0.264 mmol) and TMSOTf (44 µL, 0.1 M solution in CH_2Cl_2).

The conversions were determined after analytical NaOMe cleavage: 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-O-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (**152**). Conversions: 1. 80%, 2. 85%, 3. 68%, 4. 71%, 5. 85%, 6. 90%. LCMS (ESI) m/z: calcd for $C_{54}H_{64}N_4O_{14}$-$[Na]^+$: 1015.43, found: 1015.29.

The resin **SP-151** (340 mg / 0.22 mmol/g, 74.8 µmol) was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-O-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-153**) using general procedure H.

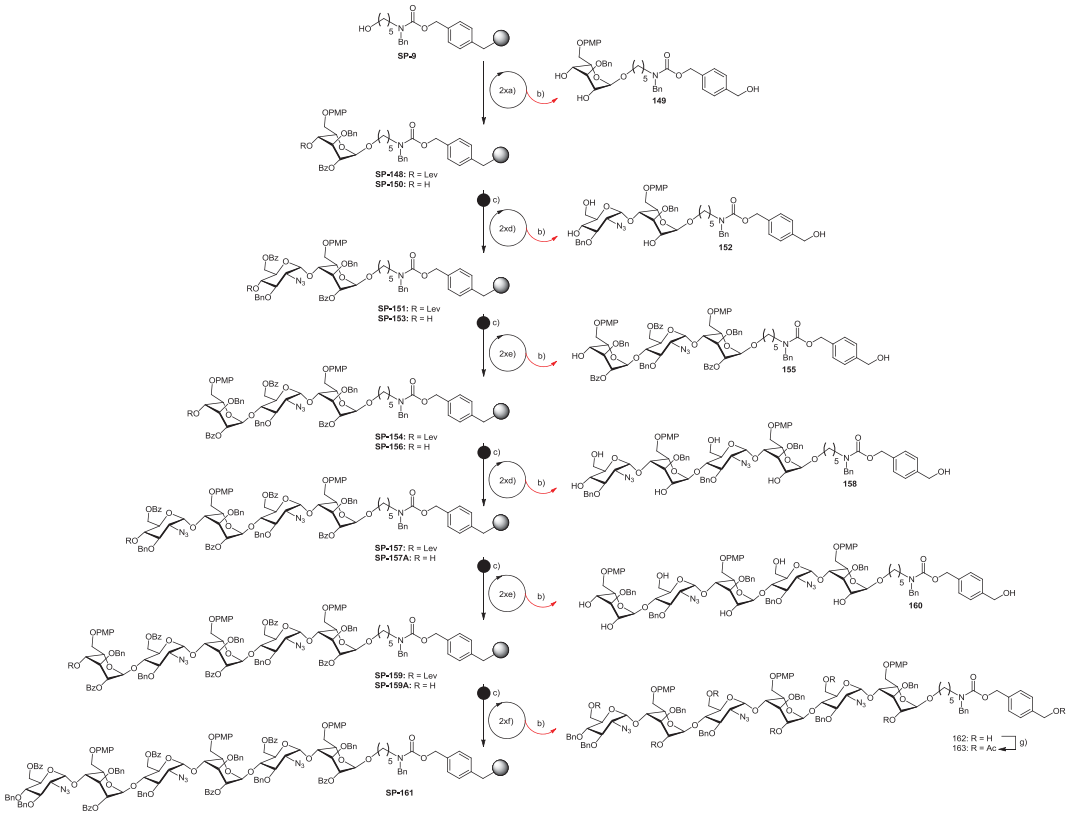
Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-O-(2-azido-4-*O*-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (**SP-154**): The reaction was performed according to general procedure G using 2 cycles on resin **SP-153** (200 mg /

0.44 mmol/g, 88 µmol) with trichloroacetimidate **30** (2 x 6 eq., 388 mg, 528 µmol) and TMSOTf (88 µL, 0.1 M solution in CH_2Cl_2). Conversion was determined after analytical NaOMe cleavage: 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-4-*O*-(3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (**155**). Conversion 94%, LCMS (ESI) *m/z*: calcd for $C_{74}H_{86}N_4O_{20}$-$[NH_4]^+$: 1368.62, found: 1368.20.

4-(Acetoxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-acetyl-4-*O*-(6-*O*-acetyl-2-azido-4-*O*-(2,4-di-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (156): Monosaccharide formation was performed according to general procedure G using resin **SP-9** (1.18 g / 0.22 mmol/g, 260 µmol), thioglycoside **27** (5 eq., 870 mg, 1.29 mmol), NIS (6 eq., 350 mg, 1.56 mmol) and TMSOTf (5 µL, 30 µmol). After capping and delevulination disacccharide formation was performed according to general procedure G using 3 cycles on resin **SP-150** (180 mg (198 mg)/ 0.22 mmol/g, 40 µmol) with trichloroacetimidate **65** (3 x 6 eq., 152 mg, 238 µmol) and TMSOTf (79 µL, 0.1 M solution in CH_2Cl_2). After capping and delevulination disacccharide formation was performed according to general procedure D using 2 cycles on resin **SP-154** (180 mg (198 mg)/ 0.44 mmol/g, 40 µmol) with trichloroacetimidate **30** (2 x 6 eq., 171 mg, 237 µmol) and TMSOTf (79 µL, 0.1 M solution in CH_2Cl_2). The resin **SP-154** (175 mg, resin after cleavage 136 mg, 299 µmmol) was swollen in 4 mL dry CH_2Cl_2 and then treated with 0.25 M NaOMe solution (1 mL) for 5 min at 55 under microwave irradiation. Then, the resin was washed with 2 x 5 mL CH_2Cl_2/MeOH (1:1) and 2 x 5 mL MeOH. This procedure was repeated until TLC control (CH_2Cl_2/MeOH, 98:2) showed no further compound cleavage (8 cycles). The washing solutions were pooled and neutralized with Amberlite® IR-120(H). After concentration, crude **155** was acetylated Ac_2O and a catalytic amount of DMAP in pyridine overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and the organic layer was washed with 1 M HCl (2x), saturated $CuSO_4$ aq solution (50 mL), water and brine. After concentration the crude product was purified by column chromatography on silica gel using hexane/acetone. The product **156** was obtained as colourless syrup (34 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.37 – 7.28 (m, 14H, aromatic), 7.26 – 7.20 (m, 10H, aromatic), 7.14 – 6.95 (m, 6H, aromatic$_{PMP}$),

6.85 – 6.73 (m, 2H, aromatic$_{\text{-PMP}}$), 5.19 – 5.09 (m, 2H, CH_2-Ph_{Carba}), 5.08 (s, 2H, CH_2-Ph_{Ac}), 4.99 – 4.96 (m, 1H, H-2), 4.98 – 4.90 (m, 3H, H-4′′, H-1′, H-1′′), 4.86 – 4.78 (m, 4H, H-2′′, H-1, CH_2Ph), 4.74 – 4.58 (m, 4H, CH_2Ph), 4.57 – 4.51 (m, 2H, H-5, H-5′′), 4.50 – 4.43 (m, 2H, CH_2-PhN), 4.31 (dd, J = 12.3, 2.1 Hz, 1H, H-6′), 4.20 – 4.15 (m, 1H, H-6′′), 4.11 – 4.04 (m, 2H, H-6′′, H-6′), 3.96 – 3.85 (m, 3H, H-3, H-4, H-5′), 3.85 – 3.77 (m, 2H, H-3′, H-4′), 3.76 – 3.65 (m, 10H, H-3′′, H-6, $OCH_{2Linker}$, 2xCH_{3PMP}), 3.45 – 3.34 (m, 1H, $OCH_{2Linker}$), 3.32 (dd, J = 9.6, 3.6 Hz, 1H, H-2′), 3.25 – 3.13 (m, 2H, CH_2N_{Linker}), 2.11 (s, 3H, CH_{3Ac}), 2.09 (s, 3H, CH_{3Ac}), 2.01 (s, 3H, CH_{3Ac}), 1.99 (s, 3H, CH_{3Ac}), 1.97 (s, 3H, CH_{3Ac}), 1.67 – 1.47 (m, 4H, $CH_{2Linker}$), 1.38 – 1.24 (m, 2H, $CH_{2Linker}$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 171.0, 170.6, 170.3, 170.3, 169.6, 154.2, 154.1, 152.6, 152.3, 138.0, 137.8, 137.8, 137.5, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.8, 127.8, 127.7, 127.4, 127.2, 115.4, 115.4, 115.0, 114.6 ($C_{aromaticPMP}$), 98.2 ($J_{C,H}$ = 170 Hz, C-1), 98.0 ($J_{C,H}$ = 169 Hz, C-1′′), 95.9 ($J_{C,H}$ = 171 Hz, C-1′), 78.9 (C-3′), 75.2 (C_{Bn}), 74.7 (C-4′), 73.1 (C-3′′), 72.6 (C_{Bn}), 72.2 (C_{Bn}), 71.5 (C-3), 70.4 (C-4), 69.9 (C-5′), 68.2 (C-2), 68.1 (C-2′′, $OCH_{2Linker}$), 67.6 (C-4′′), 66.9 (CH_2-Ph_{carba}), 66.7 (C-6, C-6′′), 66.1 (CH_2-Ph_{Ac}), 65.4, 65.3 (C-5, C-5′′), 64.0 (C-2′), 62.3 (C-6′), 55.8 (CH_{3PMP}), 55.7 (CH_{3PMP}), 50.6, 50.3 (CH_2-PhN), 47.4, 46.3 ($NCH_{2Linker}$), 29.3, 28.1, 27.6, 23.5 ($CH_{2Linker}$), 21.1, 21.0, 21.0, 20.9 (CH_{3Ac}) ppm; HRMS (ESI): *m/z* calcd. for $C_{84}H_{96}N_4O_{25}$ $[M+Na]^+$ 1583.6256, found: 1583.6265.

5.8.4 Solid phase synthesis of HS hexasaccharide precursor



Scheme 5.1. **Solid-phase assembly of a heparin sulfate oligosaccharide precursor.** a) **24**, 20% TMSOTf, -20 °C to r.t; b) NaOMe (cat), MeOH, MW; c) hydrazine acetate, CH_2Cl_2: MeOH (4:1); d) **65**, 20% TMSOTf, -20 °C to r.t; e) **30**, 20% TMSOTf, -20 °C to r.t; f) **41**, 20% TMSOTf, -20 °C to r.t; g) Ac_2O, pyridine, 0 °C to r.t.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (SP-148): Linker **SP-9** (340 mg, 0.22 mmol/g, 75 µmol) was glycosylated in one cycle with thioglycoside **24** (5 equiv, 251 mg, 0.37 mmol), NIS (6 equiv, 101 mg, 0.45 mmol) and TMSOTf (150 µL of 0.1M solution in dry CH_2Cl_2, 15 µmol) according to general procedure G. The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): 99% as compound **149** (*m/z:* $[M+Na]^+$ calcd for $C_{41}H_{49}NO_{10}Na$ 738.32, found 738.30).

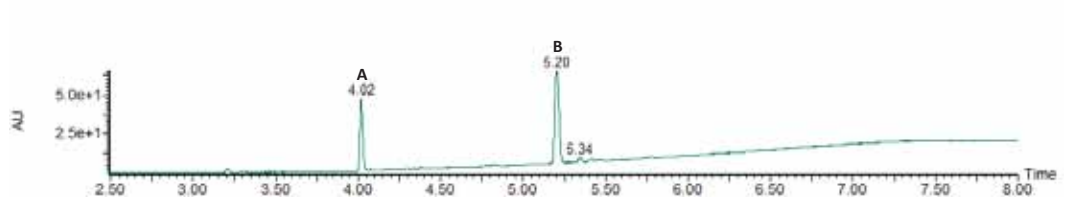


Figure 5.8. UPLC-MS chromatogram of analytical cleavage data conversion of linker **SP-9** to the monosaccharide **SP-148** (as **149**, retention time (t_r) at 5.20 min., peak **B** (*m/z* calcd for $C_{41}H_{49}NO_{10}$ $[M+Na]^+$ 738.32 found 738.30)), unidentified peak: peak **A** (no mass detectable).

SP-150

The resin **SP-148** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate **SP-150** using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-(4-O-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-151): The reaction was performed according to general procedure G using two cycles on resin **SP-150** (340 mg, 0.075 mmol) with thricloroacetimidate **65** (3 x 6 equiv, 289 mg, 0.45 mmol) and TMSOTf (150 µL of 0.1 M solution in dry CH_2Cl_2, 15 µmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle1), n.a, (cycle 2) 85% as compound **152** (*m/z:* $[M+NH_4]^+$ calcd for $C_{54}H_{64}N_4O_{14}Na$ 1010.48 found, 1010.21).

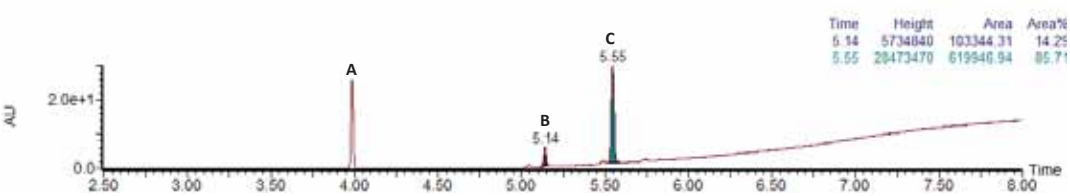


Figure 5.9. UPLC-MS chromatogram of analytical cleavage data conversion of monosaccharide **SP-148** (cleaved as **149** retention time (t_r) at 5.14 min., peak **B** (calcd for $C_{41}H_{49}NO_{10}$ $[M+Na]^+$ 738.32 found 738.29)) to the disaccharide **SP-151** (cleaved as **152** (t_r) at 5.55 min., peak **C** (*m/z* calcd for $C_{54}H_{64}NO_{14}$ $[M+Na]^+$ 1015.43 found), unidentified peak: peak **A** (no mass detectable).

SP-153

The resin **SP-151** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-O-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate **SP-153** using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-154): The reaction was performed according to general procedure G using three cycles on resin **SP-153** (335 mg, 0.074 mmol) with trichloroacetimidate **30** (6 equiv, 320 mg, 0.44 mmol) and TMSOTf (150 μL of 0.1M solution in dry CH_2Cl_2, 15 μmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle1) na, (cycle 2) 93% as compound **155** (*m/z:* $[M+NH_4]^+$ calcd for $C_{73}H_{84}N_4O_{20}NH_4$ 1368.62 found 1368.16).

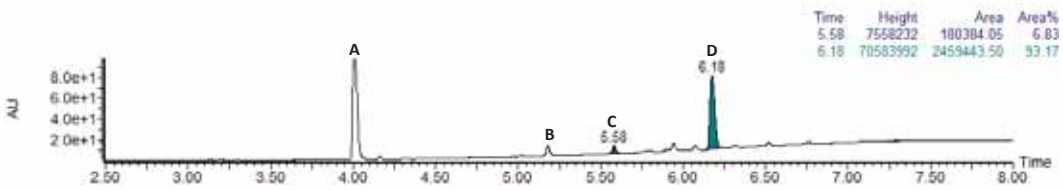


Figure 5.10. UPLC-MS chromatogram of analytical cleavage data conversion of disaccharide **SP-153** (t_r at 5.58 min., peak **C** (as **S-20**, m/z calcd for $C_{54}H_{64}NO_{14}$ $[M+NH_4]^+$ 1010.47 found 1010.69)) to the trisaccharide **SP-154** (as **155**, t_r at 6.18 min., peak **D** (*m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1368.62 found 1368.16)); **A** (no detectable mass), peak **B** (unreacted monosaccharide cleaved as **S-19,** t_r at 5.18 min. (calcd for $C_{41}H_{49}NO_{10}$ $[M+NH_4]^+$ 733.37 found 733.29).

SP-156

The resin **SP-154** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate **SP-156** using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-levulinoyl-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-157): Tetrasaccharide formation was performed according to general procedure G using three cycles on resin **SP-156** (397 mg) with trichloroacetimidate **65** (3 x 6 equiv, 288 mg, 0.45 mmol) and TMSOTf (150 μL of 0.1M solution in dry CH_2Cl_2, 0.015 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) 28%; (cycle 2) 63%; (cycle 3) 78% as compound **158** (*m/z*: $[M+NH_4]^+$ calcd for $C_{73}H_{84}N_4O_{20}$ NH_4 1645.72 found 1645.57).

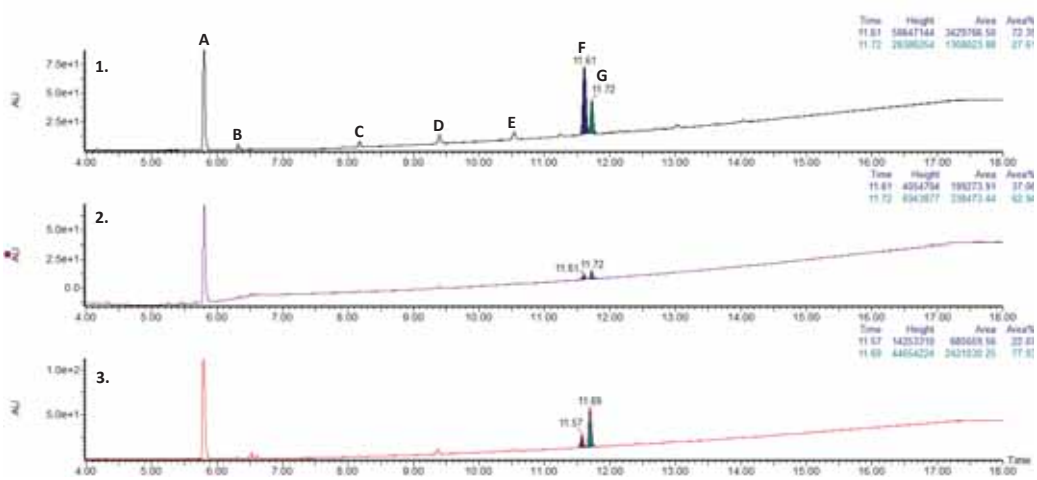


Figure 5.11. UPLC-MS chromatogram of analytical cleavage data conversion of trisaccharide **SP-156** (t_r at 11.61 min., peak **F** (as **155**, *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1368.62 found 1368.42)) to the tetrasaccharide **SP-157** (t_r at 11.72 min., peak **G** (as **158,** *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1645.72 found 1645.57)); **A** (no detectable mass), peak **B** (t_r = 4.17 min., *m/z* calcd for $C_{21}H_{27}NO_4$ $[M+Na]^+$ 380.18 found 380.14)), peak **C** (as **149**, *m/z* calcd for $C_{41}H_{49}NO_{10}$ $[M+NH_4]^+$ 733.37 found 733.25), peak **D** (as **152**, *m/z* calcd for $C_{54}H_{64}NO_{14}$ $[M+Na]^+$ 1015.43 found 1015.34), peak **E** (deletion sequence, t_r at 10.54 min. (*m/z* calcd for $C_{61}H_{71}NO_{16}$ $[M+NH_4]^+$ 1091.51 found 1091.36)). Traces 1-3 correspond to analytical cleavage after 1, 2 and 3 cycles of glycosylation, respectively.

SP-157A

The resin **SP-157** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate **SP-157A** using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-159): Pentasaccharide synthesis was performed according to general procedure G using three cycles on resin **SP-157A** with trichloroacetimidate **30** (3 x 6 equiv, 324 mg, 0.45 mmol) and TMSOTf (150 µL of 0.1M solution in dry CH_2Cl_2, 0.015 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) 62%; (cycle 2) 72%, (cycle 3) 76% as compound **160** (*m/z:* $[M+2NH_4]^{2+}$ calcd for $C_{107}H_{123}N_7O_{30}$ NH_4 1010.94 found 1010.84).

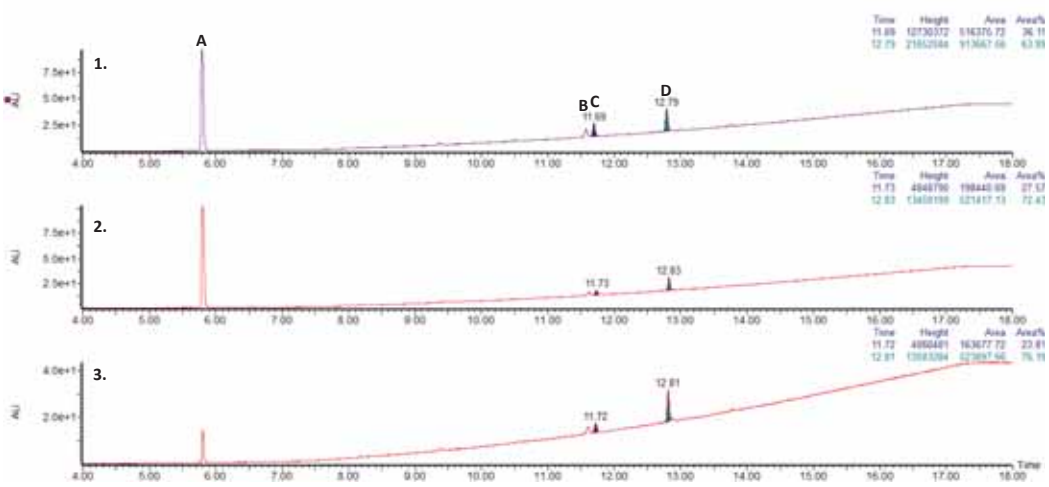


Figure 5.12. UPLC-MS chromatogram of analytical cleavage data conversion of tetrasaccharide **SP-157A** (t_r at 11.69 min., peak **C** (as **158**, *m/z* calcd for $C_{73}H_{84}N_4O_{20}$

$[M+NH_4]^+$ 1645.72 found 1645.52)) to the pentasaccharide **SP-159** (as **160,** t_r at 12.79 min., peak **D** (*m/z* calcd for $C_{107}H_{123}N_7O_{30}$ $[M+2NH_4]^{2+}$ 1010.94 found 1010.84)); **A** (no detectable mass), peak **B** (as **155**, t_r = 11.57 min., *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1368.62 found 1368.42)).

SP-159A

The resin **SP-159** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate **SP-159A** using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-azido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl(-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-161): Hexasaccharide synthesis was performed according to general procedure G using three cycles on resin **SP-159A** with trichloroacetimidate **41** (3 x 6 equiv, 284 mg, 0.45 mmol) and TMSOTf (150 µL of 0.1 M solution in dry CH_2Cl_2, 0.015 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) 46%; (cycle 2) 92%, (cycle 3) 92% as compound **162** (*m/z:* $[M+2NH_4]^{2+}$ calcd for $C_{127}H_{144}N_{10}O_{34}$ NH_4 1194.52, found 1194.48).

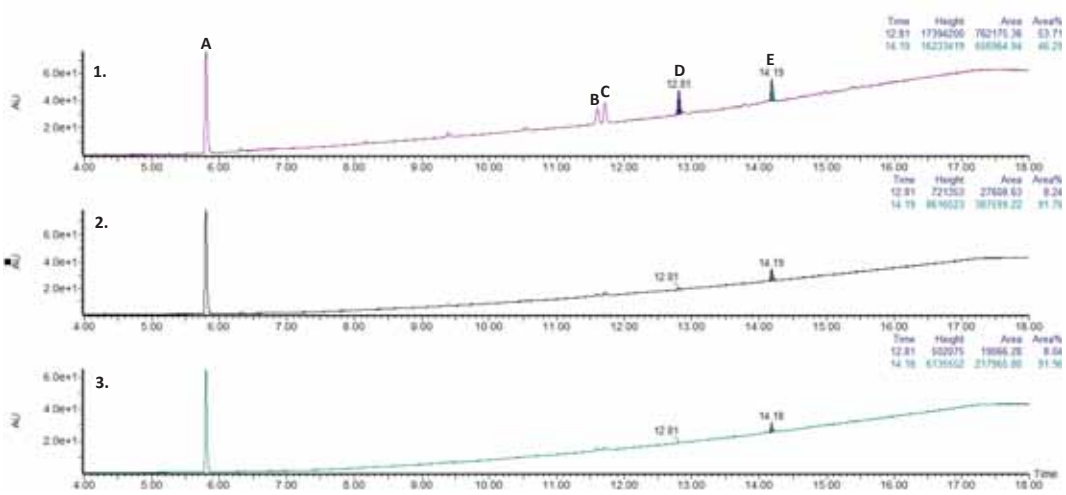


Figure 5.13. UPLC-MS chromatogram of analytical cleavage data conversion of pentasaccharide **SP-159A** (t_r at 12.81 min., peak **D** (as **160**, *m/z* calcd for $C_{107}H_{123}N_7O_{30}$ $[M+2NH_4]^{2+}$ 1010.94 found 1010.92)) to the hexasaccharide **SP-161** (as **162**, t_r at 14.19 min., peak **E** (*m/z* calcd for $C_{127}H_{144}N_{10}O_{34}$ $[M+2NH_4]^{2+}$ 1194.52 found 1194.48)); peak **A** (no detectable mass), peak **B** (as **155**, *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1368.62 found 1368.60)) and peak **C** (as **158**, *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1645.72 found 1645.63).

4-(Acetoxymethyl)benzyl *N*-benzyl *N*-[5-((6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl(-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (163): The resin **SP-161** (392 mg of resin, resin after cleavage 257 mg; 0.056 mmol) was swollen in dry CH_2Cl_2 (4 mL) and quantitative cleavage was performed according to general procedure D using 12 cycles of NaOMe/MeOH (500 µL). The crude was treated with additional amount of 0.25M NaOMe solution until UPLC-MS shows complete deprotection to intermediate **162** (See Figure 4.14). Acetylation was performed overnight at room temperature using acetic anhydride (3 mL) and a catalytic amount of DMAP in pyridine (4 mL). The reaction mixture was diluted with CH_2Cl_2 (50 mL), and the organic layer was washed with 1M HCl (2x), saturated $CuSO_4$ aq solution (50 mL), water and brine. The crude product was purified by column chromatography (hexane/ acetone; 9/1 to 1/1) and by preparative

HPLC (eluents: 20mM NH_4HCO_3/MeCN; gradient: 10% (5 min) - 99% (in 30 min) – 99% (isocratic) was obtained compound **163** (17 mg, 11% over 14 steps, 85% in each step). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 27H, aromatic), 7.26 – 7.19 (m, 7H), 7.19 – 7.08 (m, 10H, aromatic), 6.86 – 6.64 (m, 12H, aromatic), 5.15 (d, J = 20.7 Hz, 1H, CH_2-Ph_{Carba}), 5.08 (s, 2H, CH_2-Ph_{Ac}), 5.00 – 4.87 (m, 7H, 2xH-1_{Ido}, 3xH-1_{Azido}, H-$2_{Ido,}$ CH_2Ph), 4.87 – 4.71 (m, 8H, 2xH-2_{Ido}, H-1_{Ido}, 5xCH_2Ph), 4.70 – 4.57 (m, 6H, 3xCH_2Ph), 4.57 – 4.52 (m, 2H, 1xCH_2Ph, H-5_{Ido}), 4.50 – 4.42 (m, 5H, CH_2Ph, 2xH-5_{Ido}, CH_2-PhN), 4.29 – 4.22 (m, 2H, 2x H-6_{Azido}), 4.19 – 4.14 (m, 1H, H-6_{Ido}), 4.13 – 4.04 (m, 4H, 3x H-6_{Azido} , H-6_{Ido}), 4.01 – 3.94 (m, 3H, 2x H-6_{Ido}, H-6_{Azido}), 3.94 – 3.73 (m, 16H, , 2x H-6_{Ido}, 3xH-5_{Azido}, 3xH-3_{Ido}, 3xH-3_{Azido}, 2xH-4_{Azido}, 3xH-4_{Ido}), 3.73 – 3.67 (m, 10H, CH_2O, 3x CH_{3PMP}), 3.47 (dd, J = 10.0, 8.7 Hz, 1H, H-4_{Azido}), 3.35 (bs, 1H, CH_2O), 3.32 (dd, J = 9.7, 3.7 Hz, 1H, H-2_{Azido}), 3.26 – 3.12 (m, 4H, 2xH-2_{Azido}, CH_2N), 2.11 (s, 3H, CH_{3Ac}), 2.09 (s, 3H, CH_{3Ac}), 2.07 (s, 3H, CH_{3Ac}), 2.04 (s, 3H, CH_{3Ac}), 1.96 (d, J = 1.9 Hz, 9H, CH_{3Ac}) 1.63 – 1.47 (m, 4H, $CH_{2Linker}$), 1.37 – 1.26 (m, 2H, $CH_{2Linker}$).^{13}C NMR (126 MHz, $CDCl_3$) from HSQC experiment δ = 128.2, 128.0, 127. 8, 115.22, 115.15, 114.69, 97.96 (C-1_{Ido}, J_{CH} = 170.0 Hz), 97.95 (C-1_{Ido}, J_{CH} = 170.0 Hz), 97.9 (C-1_{Ido}, J_{CH} = 170.0 Hz), 95.8 (3xC-1_{Azido}, J_{CH} = 170.0 Hz), 81.2, 78.9, 78.4, 77.8, 75.3, 75.12, 75.05, 74.9, 74.8, 72.8, 72.1, 71.7, 70.5, 69.8, 69.6, 68.63 (C-2_{Ido}), 68.56 (C-2_{Ido}), 68.1 (C-2_{Ido}), 67.9(CH_2O_{Linker}), 66.77(CH_2Ph_{Carba}), 66.76 (C-6_{Ido}), 66.5 (C-5_{Ido}), 66.0 (CH_2Ph_{Ac}), 65.99(2xC-6_{Ido}), 65.3 (C-5_{Ido}), 63.7 (C-2_{Azido}), 63.2 (C-2_{Azido}), 62.5 (C-6_{Azido}), 62.2(2xC-6_{Azido}), 55.68 (CH_{3PMP}), 50.42 (CH_2PhN), 46.18 (CH_2N_{Linker}), 29.30($CH_{2Linker}$), 23.02($CH_{2Linker}$), 20.84(CH_{3Ac}), 20.80(CH_{3Ac}), 20.70(CH_{3Ac}); HRMS (ESI) *m/z*: $[M+2NH_4]^{2+}$ calcd. for $C_{141}H_{158}N_{10}O_{41}(NH_4)_2$ 1341.5631, found: 1341.5605.

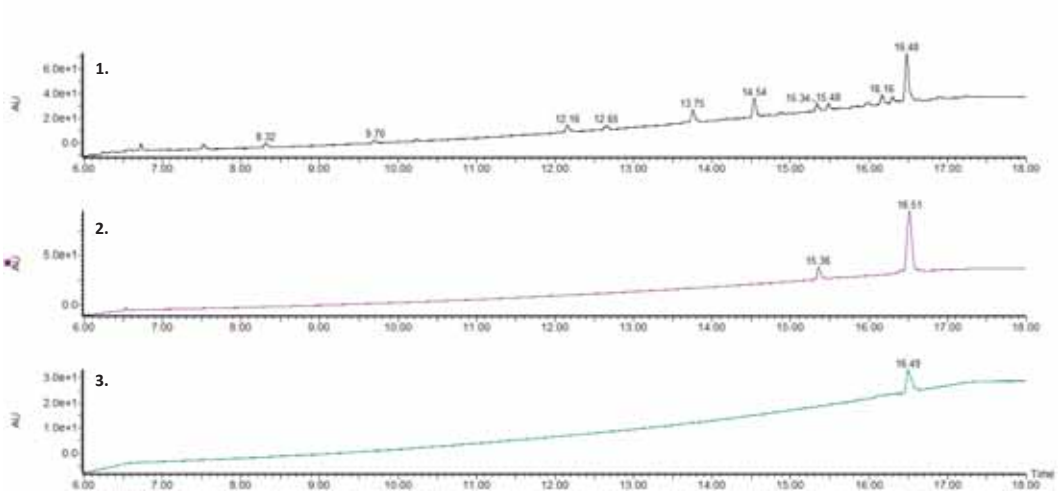


Figure 5.14. UPLC-MS data for the synthesis of hexasaccharide **163**. 1) Chromatogram of the reaction crude after acetylation; 2) chromatogram of **10** after column chromatography (t_r = 15.36 min, *m/z* calcd for $C_{121}H_{137}N_7O_{37}$ $[M+2NH_4]^{2+}$ 1157.98 found 1157.95, t_r = 16.51 min, *m/z* calcd for $C_{141}H_{158}N_{10}O_{41}$ $[M+2NH_4]^{2+}$ 1341.56 found 1341.55); 3) chromatogram of **163** after preparative HPLC purification (t_r = 16.49 min, *m/z* calcd for $C_{141}H_{158}N_{10}O_{41}$ $[M+2NH_4]^{2+}$ 1341.56 found 1341.55).

5.9 TOWARDS SOLID PHASE SYNTHESIS OF DERMATAN SULFATE

5.9.1 Initial attemps on solid phase: disaccharide synthesis.

Resin-Bound 4-(hydroxymethyl)benzyl N-benzyl-N-[5-(4-*O*-benzoyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-163): The reaction was performed according to general procedure G employing one cycle on resin **SP-9** (110 mg, 0.22 mmol/g, 0.024 mmol) with trichloroacetimidate donor **81** (1 x 5 eq., 0.121 mmol) and TMSOTf (3 μL, 0.016 mmol). The conversion was determined after analytical NaOMe cleavage. Conversion: 97%; UPLC-MS (ESI): 67% as compound **165** (*m/z* $[M+H]^+$ Calcd for $C_{36}H_{43}Cl_3N_2O_9H$ 753.20, found 752.91) and 30% as compound **166** (*m/z* $[M+H]^+$ Calcd for $C_{34}H_{44}N_2O_8H$ 609.30, found 609.25). The partial loss of trichloroacetamide could be avoided when cleavage reaction was carried out at 40°C instead of 55°C.

4-(hydroxymethyl)benzyl N-benzyl-N-[5-(3,4-*O*-diacetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (168): The resin **SP-163** (96 mg, 0.018 mmol) was swollen in dry CH_2Cl_2 (3 mL) and quantitative cleavage was performed according to general procedure D using 4 cycles of NaOMe/MeOH (750 µL). The crude (29 mg) was acetylated overnight at room temperature using acetic anhydride and catalytic amount of DMAP in pyridine. The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with 1M HCl, saturated $CuSO_4$ aq solution, water and brine. After concentration the crude product was purified by preparative TLC to obtain compound **168** (14 mg, 88% yield); ^{1}H NMR (500 MHz, $CDCl_3$, 298 K, mixture of rotamers) δ 7.39 – 7.14 (m, 14H, aromatic), 6.90 – 6.82 (d, *J* = 8.5 Hz, 0.5H, $NHTCArot_a$), 6.69 – 6.62 (d, *J* = 8.8 Hz, 0.4H, $NHTCArot_b$), 5.48 – 5.44 (dd, *J* = 3.3 Hz, 1H, H-4), 5.27 (t, *J* = 11.3 Hz, 1H, H-3), 5.17 – 5.04 (m, 4H, 2 CH_2linker), 4.65 (d, J = 8.3 Hz, 0.5H, H-$1rot_a$), 4.60 (d, *J* = 8.3 Hz, 0.5H, H-$1rot_b$), 4.55 (d, J = 11.8Hz, CH_2Ph), 4.47 – 4.42 (m, 3H, CH_2-PhN, CH_2Ph), 4.10 – 4.06 (m, 1H, H-2), 3.92 – 3.82 (m, 2H, OCH_2linker, H-5), 3.60 – 3.56 (dd, *J* = 9.5, 5.9 Hz, 1H, H-6_a), 3.53 – 3.49 (dd, *J* = 9.5, 7.0 Hz, 1H, H-6_b), 3.48 – 3.34 (m, 1H, $OCH_{2Linker}$), 3.29 – 3.11 (m, 2H, $NCH_{2Linker}$), 2.13 – 2.09 (bs, 3H, CH_{3Ac}), 2.07 (s, 3H, CH_{3Ac}), 1.99 (s, 3H, CH_{3Ac}), 1.63 – 1.48 (m, 4H, $CH_{2Linker}$), 1.35 – 1.20 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$, 25 °C, mixture of rotamers) δ 170.50 (Cq), 170.23 (Cq), 162.09 (Cq), 156.31 (Cq), 137.95 (Cq), 137.64 (Cq), 137.01 (Cq), 135.82 (Cq), 128.71, 128.62, 128.47, 128.19, 128.09, 128.05, 127.97, 127.48, 127.30, 101.09 (C-1), 100.94 (C-1), 92.60 (Cq), 73.76 (CH_2Ph), 72.47 (C-5), 70.14 ($OCH_{2Linker}$), 70.00 ($OCH_{2Linker}$), 69.89 (C-3), 67.68 (C-6), 67.30 (C-4), 66.93 ($CH_{2Linker}$), 66.13 ($CH_{2Linker}$), 53.41 (C-2), 50.68 ($CH_{2Linker}$), 50.48 ($CH_{2Linker}$), 47.35 ($CH_{2Linker}$), 46.26 ($CH_{2Linker}$), 29.26, 28.96, 28.00, 27.33, 23.27 ($CH_{2Linker}$), 21.17 (CH_3), 20.82 (CH_3), 20.68 (CH_3) ppm. HRMS (ESI): *m/z* calcd. for $C_{42}H_{49}Cl_3N_2O_{12}$ $[M+Na]^+$ 901.2238, found 901.2220.

SP-167

The resin **SP-163** was transformed to resin-bound 4-(hydroxymethyl)benzyl N-benzyl-N-[5-(4-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyloxy)pentyl]carbamate **SP-167** using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-O-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-169): Disaccharide formation was performed according to general procedure G using 2 cycles on resin **SP-182** (0.390 g, 0.087 mmol) with trichloroacetimidate donor **30** (5 equiv, 0.315 g, 0.44 mmol) and TMSOTf (3 µL, 0.017 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) 74%; (cycle 2) 97% as compound **170** (*m/z* $[M+NH_4]^+$ calcd for $C_{56}H_{65}Cl_3N_2O_{15}$ NH_4 1128.34, found 1127.93.

4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (170):

The resin **SP-169** (203 mg, resin after cleavage 165 mg, 0.036 mmol) was swollen in dry CH_2Cl_2 (2 mL) and quantitative cleavage was performed according to general procedure **F** using 8 cycles of NaOMe/MeOH (200 µL). The crude was purified by columm chromatography CH_2Cl_2/MeOH (94/6) to obtain compound **170** of α/β (9/1) mixture (30 mg, 75% overall yield). α-isomer: ^{1}H NMR (500 MHz, CD_3OD) δ 7.48 – 7.21 (m, 21H, aromatic), 7.19 –7.11 (m, 1H, aromatic), 6.95 – 6.72 (m, 4H, $aromatic_{PMP}$), 5.20 – 5.08 (m, 2H, CH_2-Ph_{Carba}), 4.97 (s, 1H, H-1′), 4.75 – 4.72 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.63 – 4.44 (m, 9H, H-5′, H-1, CH_2-PhN, CH_2Ph, CH_2Ph, 1HCH_2Ph), 4.21 – 4.06 (m, 4H, H-2, H-5, H-6′), 3.90 – 3.79 (m, 4H, H-3, H-4′, H-2′, H-6), 3.72 – 3.67 (m, 6H, H-3, CH_{3PMP}, H-6, H-4, $OCH_{2Linker}$), 3.65 (m, 1H, H-3′), 3.41 (bs, 1H,$OCH_{2Linker}$) , 3.26 – 3.12 (m, 2H, $NCH_{2Linker}$), 1.62 – 1.41 (m, 4H, $CH_{2Linker}$), 1.38 – 1.30 (m, 2H, $CH_{2Linker}$). ^{13}C NMR (126 MHz, CD_3OD, selected from HSQC) δ 164.31, 158.46, 157.93, 155.54, 154.21, 142.73, 139.60, 139.29, 137.06, 129.61, 129.50, 129.39, 129.11, 128.97, 128.88, 128.77, 128.68, 128.36, 128.13, 116.48 (C_{PMP}), 115.70 (C_{PMP}), 104.70 (C-1′), 102.40 (C-1), 94.39 (Cq, TCA), 80.73 (C-4), 78.54 (C-3′), 75.07, 74.44, 73.15, 70.79, 70.51, 69.98, 69.76, 69.35, 69.05, 68.26 (CH_2linker), 68.18 (C-2′), 66.90, 64.91, 56.12 (Me), 54.85 (C-2), 51.55, 51.36, 47.59, 28.96, 28.45, 24.35, 23.53 ppm.

5.9.2 Solution synthesis of dermatan sulfate disaccharide.

4-[(Phenylcarboxy)methyl]benzyl N-Benzyl-N-[5-((4-*O*-Benzoyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranosyl)oxy)pentyl]carbamate (172): Linker **83** (92 mg, 0.20 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and was added to a solution of the donor **81** (190 mg, 0.25 mmol) in dry CH_2Cl_2 containing activated 4 Å molecular sieves. The mixture was cooled until -20 °C and catalytic amount of TMSOTf (5 µL, 0.030 mmol, 0.15 eq.) was added. After stirring for 10 min. at -20 ºC, the reaction was allowed to reach room temperature. After 1h, the reaction was quenched by addition of triethylamine and concentrated. The residue was purified by column chromatography (hexane/ EtOAc, 9/1 to 7/3) to afford compound 4-[(phenylcarboxy)methyl]benzyl *N*-Benzyl-N-[5-((4-*O*-Benzoyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-D-galactopyranosyl)oxy)pentyl]carbamate **172** (184 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$, 50 °C) δ 8.08 (d, *J* = 8.1 Hz, 4H, aromatic), 7.57 (m, 2H, aromatic), 7.49 – 7.15 (m, 18H, aromatic), 6.80 (bd, 1H, NH), 5.73 – 5.70 (m, 1H, H-4), 5.43 (dd, *J* = 11.3, 3.1 Hz, 1H, H-3), 5.36 (s, 2H, CH_2-Ph_{Bz}), 5.18 (s, 2H, CH_2-Ph_{Carba}), 4.79 (d, *J* = 8.0 Hz, 1H, H-1), 4.49 (m, 3H, CH_2Ph, CH_2-PhN), 4.42 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.13 – 4.05 (m, 1H, H-2), 3.97 (t, *J* = 6.3 Hz, 1H, H-5), 3.89 (s, 1H, $OCH_{2Linker}$), 3.66 – 3.57 (m, 1H, H-6), 3.49 (s, 1H, $OCH_{2Linker}$), 3.23 (s, 2H, $NCH_{2Linker}$), 2.70 – 2.63 (m, 1H, CH_{2Lev}), 2.60 – 2.37 (m, 3H, CH_{2Lev}), 2.03 (s, 3H, CH_{3Lev}), 1.63 – 1.50 (m, 4H, $CH_{2Linker}$), 1.38 – 1.29 (m, 2H, $CH_{2Linker}$) ppm. ^{13}C NMR (500 MHz, $CDCl_3$, from HSQCed. experiment) δ 133.4 – 127.2, 100.7 (C-1), 73.6 (C_{Bn}), 72.6 (C-5), 70.02 (C-3, $OCH_{2Linker}$), 67.8 (C-4, C-6), 66.9 (CH_2-Ph_{Carba}), 66.4 (CH_2-Ph_{Bz}), 53.8 (C-2), 47.2, 46.2 ($NCH_{2Linker}$), 37.8 (CH_{2Lev}), 29.6 (CH_{3Lev}), 28.9 ($CH_{2Linker}$), 27.9 (CH_{2Lev}), 23.3 ($CH_{2Linker}$) ppm.

4-[(Phenylcarboxy)methyl]benzyl N-Benzyl-N-[5-((4-*O*-Acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranosyl)oxy)pentyl]carbamate (173): The linker **83** (26 mg, 0.057 mmol) and the trichloroacetimidate donor **82** (60 mg, 0.085 mmol) were dissolved in dry CH_2Cl_2 (0.85 mL) in the presence of activated 4 Å molecular sieves. After 20 min. stirring at -20 °C, catalytic amount of TMSOTf (1µL, 8 µmol) was added. The reaction was allowed to reach room temperature and stirred for 90 min. The reaction was quenched by addition of triethylamine and concentrated. The

reaction crude was purified by column chromatography (hexane/ EtOAc, 9/1 to 7/3) to afford compound **173** (50 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$; mixture of rotamers) δ 8.10 – 8.02 (d, *J* = 7.6 Hz, 2H, aromatic), 7.60 – 7.52 (t, *J* = 7.4 Hz, 1H, aromatic), 7.50 – 7.21 (m, 15H, aromatic), 7.15 (d, *J* = 7.6Hz, 1H, aromatic), 7.00 (d, *J* = 8.7 Hz, 0.6 H, NH), 6.77 (d, *J* = 8.7 Hz, 0.4H, NH), 5.44 (d, *J* = 3.2 Hz, 1H, H-4), 5.39 – 5.33 (m, 2H, CH_2-Ph_{Bz}), 5.32 – 5.26 (m, 1H, H-3), 5.19 – 5.14 (m, 2H, CH_2-Ph_{Carba}), 4.73 – 4.63 (m, 1H, H-1), 4.54 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.51 – 4.45 (m, 2H, CH_2-PhN), 4.42 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.11 – 4.00 (m, 1H, H-2), 3.93 – 3.78 (m, 2H, H-5, $OCH_{2Linker}$), 3.62 – 3.33 (m, 3H, H-6, $OCH_{2Linker}$), 3.28 – 3.11 (m, 2H, $NCH_{2Linker}$), 2.78 – 2.71 (m, 1H, CH_2Lev), 2.66 – 2.52 (m, 2H, CH_2Lev), 2.46 – 2.39 (m, 1H, CH_2Lev), 2.14 (s, 3H, CH_3Lev), 2.06 (s, 3H, CH_{3Ac}), 1.60 – 1.42 (m, 4H, $CH_{2Linker}$), 1.36 – 1.28 (m, 2H, $CH_{2Linker}$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$; mixture of rotamers) δ 206.19, 172.17, 170.23, 166.58, 162.17, 137.91, 137.62, 135.89, 133.22, 130.17, 129.83, 128.68, 128.58, 128.53, 128.38, 128.16, 128.05, 127.99, 127.45, 127.28, 100.86, 100.73 (C-1), 92.58 (Cq, TCA), 73.68 (C_{Bn}), 72.32 (C-5), 70.06 (C-3), 69.88 ($OCH_{2Linker}$), 67.63 (C-6), 67.27 (C-4), 66.94 (CH_2-Ph_{Carba}), 66.49 (CH_2-Ph_{Bz}), 53.42 (C-2), 50.64, 50.46 (CH_2-PhN), 47.32, 46.23 ($NCH_{2Linker}$), 37.87 (CH_{2Lev}), 29.81 (CH_{3Lev}), 28.97 ($CH_{2Linker}$), 27.90 (CH_{2Lev}), 27.35, 23.27 ($CH_{2Linker}$), 20.82 (CH_{3PMP}) ppm. HRMS (ESI): *m/z* calcd. for $C_{50}H_{55}Cl_3N_2O_{13}$ $[M+Na]^+$ 1019.2662, found 1019.2644

4-[(Phenylcarboxy)methyl]benzyl N-Benzyl-N-[5-((4-*O*-Benzoyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)oxy)pentyl]carbamate (174):

The compound **172** (175 mg, 0.165 mmol) was dissolved in CH_2Cl_2/ MeOH (9:1, 4.4 mL), hydrazine acetate (30 mg, 0.33 mmol) was added at room temperature and the reaction was stirred for 2 hours. The crude was concentrated and purified by column chromatography (hexane/EtOAc, 6/4) to obtain the acceptor **174** as a white foam (137 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$; mixture of rotamers) δ 8.12 – 8.09 (m, 4H, aromatic), 7.65 – 7.56 (m, 2H, aromatic), 7.49 – 7.39 (m, 7H, aromatic), 7.35 – 7.20 (m, 11H, aromatic), 7.20 (d, 0.5H, NH), 6.97 (d, *J* = 5.6 Hz, 0.5H, NH), 5.68 – 5.66 (m, 1H, H-4), 5.42 – 5.35 (m, 2H, CH_2-Ph_{Bz}), 5.25 – 5.15 (m, 2H, CH_2-Ph_{Carba}), 4.84 (d, *J* = 8.3 Hz, 1H, H-1), 4.56 – 4.49 (m, 3H, CH_2-PhN, CH_2Ph), 4.47 – 4.39 (m, 2H, CH_2Ph, H-3), 3.99 – 3.88 (m, 2H, H-5, $OCH_{2Linker}$), 3.86 – 3.76 (m, 1H, H-2), 3.68 – 3.62 (m, 2H, H-6), 3.58 – 3.44 (m, 1H, $OCH_{2Linker}$), 3.32 – 3.17 (m, 2H, $NCH_{2Linker}$), 3.06 (s, 1H, OH), 1.67 – 1.48 (m, 4H, $CH_{2Linker}$), 1.42 – 1.30 (m, 2H, $CH_{2Linker}$) ppm. ^{13}C NMR (126 MHz,

$CDCl_3$; mixture of rotamers) δ 166.76, 162.72, 162.56, 156.76, 156.27, 137.89, 137.66, 137.08, 136.90, 135.91, 133.59, 133.22, 130.13, 129.82, 129.73, 129.33, 128.94, 128.68, 128.60, 128.52, 128.48, 128.39, 128.31, 128.17, 128.00, 127.90, 127.87, 127.81, 127.78, 127.46, 127.43, 127.26, 100.09 (C-1), 92.58 (Cq, TCA), 73.74 (C_{Bn}), 72.86 (C-5), 70.56 (C-4), 70.04 ($OCH_{2Linker}$), 69.56, 69.42 (C-3), 68.28 (C-6), 66.95 (CH_2-Ph_{Carba}), 66.48 (CH_2-Ph_{Bz}), 57.16 (C-2), 50.62, 50.40 (CH_2Ph-N), 47.29, 46.23 ($NCH_{2Linker}$), 29.33, 29.16, 28.02, 27.43, 23.35 ($CH_{2Linker}$) ppm. HRMS (ESI): *m/z* calcd. for $C_{50}H_{51}Cl_3N_2O_{11}$ $[M+NH_4]^+$ 978.2897, found 978.2964

4-[(Phenylcarboxy)methyl]benzyl N-Benzyl-N-[5-((4-*O*-Acetyl-6-*O*-benzyl-2-deoxy--2-trichloroacetamido-β-D-galactopyranosyl)oxy)pentyl]carbamate (175): Hydrazine acetate (84 mg, 0.094 mmol) was added to a solution of compound **173** (50 mg, 0.047 mmol) in CH_2Cl_2/ MeOH (9/1, 1.4 mL), at room temperature and the reaction was stirred for 2 hours. The crude was concentrated and purified by column chromatography (hexane/EtOAc, 8/2) to obtain the compound **175** (37 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$, 323K) δ 8.10 – 8.06 (m, 2H, aromatic), 7.58 – 7.53 (m, 1H, aromatic), 7.47 – 7.15 (m, 16H, aromatic), 6.99 – 6.75 (bd, 1H, NH), 5.40 – 5.38 (m, 1H, H-4), 5.37 (s, 2H, CH_2-Ph_{Bz}), 5.18 (s, 2H, CH_2-Ph_{Carba}), 4.73 – 4.69 (d, *J* = 8.2 Hz, 1H, H-1), 4.55 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.50 – 4.45 (m, 3H, CH_2-PhN, H-3), 4.32 – 4.26 (m, 1H, $CH_{2Linker}$), 3.90 – 3.78 (m, 2H, $OCH_{2Linker}$, H-5), 3.66 – 3.62 (m, 1H, H-2), 3.61 – 3.53 (m, 2H, H-6), 3.49 – 3.40 (bs, 1H, $OCH_{2Linker}$), 3.30 – 3.15 (bs, 2H, $NCH_{2Linker}$), 2.77 – 2.71 (bs, 1H, OH), 2.08 (s, 3H, CH_{3Ac}), 1.60 – 1.44 (m, 4H, $CH_{2Linker}$), 1.35 – 1.30 (s, 2H, $CH_{2Linker}$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$, 50 °C) δ 171.28, 166.58, 162.74, 138.10, 137.97, 137.18, 136.13, 133.18, 133.14, 130.45, 129.90, 129.86, 128.72, 128.62, 128.56, 128.41, 128.25, 128.01, 127.50, 100.17 (C-1), 92.79 (Cq, TCA), 73.86 (C_{Bn}), 72.85 (C-5), 70.14 (C-4), 70.00 ($OCH_{2Linker}$), 69.61 (C-3), 68.40 (C-6), 67.02 (CH_2-Ph_{Carba}), 66.52 (CH_2-Ph_{Bz}), 57.27 (C-2), 50.74 (CH_2-PhN), 29.84, 29.31, 23.46 ($CH_{2Linker}$), 20.82 (CH_{3Ac}). HRMS (ESI): *m/z* calcd. for $C_{45}H_{49}Cl_3N_2O_{11}$ $[M+Na]^+$ 921.2294, found 921.2294.

4-[(Phenylcarboxy)methyl]benzyl N-Benzyl-N-[5-((3-*O*-(2-*O*-benzoyl-3-*O*-benzyl-4-O-levulinoyl-6-*O*-(*p*-methoxyphenyl)-α/β-L-idopyranosyl)-4-*O*-Benzoyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)oxy)pentyl]carbamate (176):

Method 1: Compound **174** (27 mg, 0.028 mmol) and the trichloroacetimidate donor **30** (25 mg, 0.025 mmol) were dissolved in dry CH_2Cl_2 (0.3 mL) in the presence of powdered 4 Å molecular sieves. The mixture was cooled to -20 °C and catalytic amount of TMSOTf (0.8 µL, 4.2 µmol) was added. The reaction mixture was allowed to reach room temperature and stirred for 90 min. The reaction was quenched by addition of triethylamine, concentrated and purified by preparative TLC (hexane/EtOAc, 8/2) to afford compound **176:** α-anomer (28 mg, 65%) and β-anomer (3 mg, 7%).

Method 2: The acceptor **174** (10 mg, 0.016 mmol) and trichloroacetimidate donor **30** (14 mg, 0.019 mmol) were dissolved in dry CH_2Cl_2 (0.2 mL) in the presence of activated 4 Å molecular sieves and activators ($Yb(OTf)_3$ or $Cu(OTf)_2$, 0.5 eq) were added at 0 °C. The reaction was stirred for 2 hours at room temperature and was quenched by addition of triethylamine. The reaction was analyzed by UPLC-MS and 10% of the β-isomer was also detected.

Method 3: The acceptor **174** (10 mg, 0.010 mmol) and thioglycoside donor **27** (10 mg, 0.015 mmol) were dissolved in dry CH_2Cl_2 (0.2 mL) in the presence of powdered 4 Å molecular sieves. NIS (3 mg, 0.015 mmol) and TMSOTf (0.2 eq.; 30 µL of a solution 0.1M) added at -20 °C. The reaction was stirred for 2 hours at room temperature and was quenched by addition of triethylamine. The reaction was analyzed by UPLC-MS and 10% of the β-isomer was also detected.

α-anomer: ^{1}H NMR (500 MHz, $CDCl_3$; mixture of rotamers) δ 8.12 – 8.01 (m, 4H, aromatic), 7.97 (d, *J* = 7.4 Hz, 2H, aromatic), 7.58 – 7.48 (m, 3H, aromatic), 7.46 – 7.11 (m, 27H, aromatic, NH), 7.07 (dd, *J* = 7.4, 1.9 Hz, 2H, aromatic), 6.93 – 6.89 (m, 2H, aromatic$_{PMP}$), 6.82 – 6.77 (m, 2H, aromatic$_{PMP}$), 5.97 – 5.93 (m, 1H, H-4), 5.38 – 5.32 (m, 2H, CH_2-Ph$_{Bz}$), 5.20 – 5.10 (m, 3H, CH_2-Ph$_{Carba}$, H-1′), 5.06 (ps, 1H, H-4′), 5.04 – 4.99 (m, 2H, H-2′, H-1), 4.92 (t, *J* = 5.5 Hz, 1H, H-5′), 4.73 – 4.67 (m, 1H, H-3), 4.48 (bs, 2H, CH_2-PhN), 4.41 (d, *J* = 12.6 Hz, 1H, CH_2Ph), 4.32 (d, *J* = 12.6 Hz, 1H, CH_2Ph), 4.20 – 4.15 (m, 1H, H-6′), 4.03 (dd, *J* = 9.8, 4.7 Hz, 1H, H-6′), 4.00 – 3.95 (m, 1H, H-5), 3.95 – 3.81 (m, 2H, H-2, OCH$_{2Linker}$), 3.76 (s, 3H, CH$_{3PMP}$), 3.66 (ps, 1H, H-3′), 3.62 – 3.58 (m, 2H, H-6), 3.53 – 3.40 (m, 1H, OCH$_{2Linker}$), 3.29 – 3.13 (m, 2H,

$NCH_{2Linker}$), 2.58 – 2.41 (m, 3H, CH_{2Lev}), 2.34 - 2.26 (m, 1H, CH_{2Lev}), 2.02 (s, 3H, CH_{3Lev}), 1.60 – 1.44 (m, 4H, $CH_{2Linker}$), 1.37 – 1.28 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$; mixture of rotamers) δ 205.98, 171.97,166.56, 165.74, 164.90, 162.22, 162.15, 156.78, 156.28, 154.05, 153.18, 137.96, 137.73, 137.14, 136.94, 135.89, 133.59, 133.22, 130.11, 129.88, 129.85, 129.46, 129.20, 128.69, 128.54, 128.47, 128.42, 128.27, 128.23, 127.90, 127.77, 127.63, 127.45, 127.28, 115.79, 114.69 (C_{PMP}), 100.12 (C-1′, $J_{C1',H1'}$= 170.0 Hz), 99.34 (C-1, $J_{C1,H1}$= 163.0 Hz), 92.40 (Cq, TCA), 73.88 (C-3), 73.78 (C_{Bn}), 73.50 (C-5), 72.47 (C-3′), 71.91 (C_{Bn}), 70.41 (C-4), 70.20 ($OCH_{2Linker}$), 68.95 (C-6), 67.90 (C-6′), 67.76 (C-4′, C-2′), 66.96 (CH_2-Ph_{Carba}), 66.51 (CH_2-Ph_{Bz}), 65.38 (C-5′), 56.93 (C-2), 55.87 (CH_{3PMP}), 50.67, 50.43 (CH_2Ph), 47.33, 46.24 ($NCH_{2Linker}$), 37.92 (CH_{2Lev}), 29.74 (CH_{3Lev}), 29.34, 29.13 ($CH_{2Linker}$), 28.00 (CH_{2Lev}), 27.46 ($CH_{2Linker}$), 23.50, 23.36 ($CH_{2Linker}$) ppm. HRMS (ESI): *m/z* calcd. for $C_{82}H_{83}Cl_3N_2O_{20}$ $[M+Na]^+$ 1543.4497, found 1543.4458.

β-anomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 – 8.04 (d, *J* = 7.7 Hz, 1H, 2H, aromatic), 8.03 – 8.00 (d, *J* = 7.6 Hz, 2H, aromatic), 7.91 – 7.84 (d, *J* = 7.2 Hz, 2H, aromatic), 7.57 – 7.10 (m, 28H, aromatic), 6.88 – 6.78 (m, 4H, $aromatic_{PMP}$), 5.80 – 5.76 (m, 1H), 5.35 – 5.33 (ps, 2H, CH_2-Ph_{Bz}), 5.28 – 5.22 (d, *J* = 1.6 Hz, 1H, H-1′), 5.21 – 5.08 (m, 3H, CH_2-Ph_{Carba}), 5.00 – 4.91 (m, 2H), 4.83 – 4.75 (ps, 1H), 4.74 – 4.67 (d, *J* = 11.3 Hz, 1H), 4.55 – 4.33 (m, 6H, CH_2-PhN), 4.20 – 4.15 (m, 1H), 4.02 – 3.95 (m, 2H), 3.93 – 3.83 (m, 2H), 3.81 – 3.72 (m, 2H, CH_3), 3.67 – 3.56 (m, 2H), 3.50 – 3.38 (m, 1H, $OCH_{2Linker}$), 3.27 – 3.12 (m, 2H, $CH_{2Linker}$), 2.53 – 2.43 (m, 1H, CH_{2Lev}), 2.35 – 2.24 (m, 3H, CH_{2Lev}), 2.07 – 1.98 (s, 1H), 1.96 (s, 3H, CH_{3Lev}), 1.55 – 1.44 (m, 4H, $CH_{2Linker}$), 1.38 – 1.27 (m, 2H, $CH_{2Linker}$). HRMS (ESI): *m/z* calcd. for $C_{82}H_{83}Cl_3N_2O_{20}$ $[M+Na]^+$ 1543.4497, found 1543.4463.

4-[(Phenylcarboxy)methyl]benzyl N-Benzyl-N-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-(*p*-methoxyphenyl)-α-L-idopyranosyl)-(1→3)-4-*O*-Acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)oxy)pentyl]carbamate (177): To a solution of acceptor **175** (150 mg, 0.166 mmol) and idose trichloroacetimidate **30** (173 mg, 0.24 mmol) in dry CH_2Cl_2 (2 mL) with activated 4 Å molecular sieves, TMSOTf (5 µL, 27.6 µmol) was added at -20 °C. After 10 min, the reaction was allowed to reach room temperature and stirred for 90 min. The reaction mixture was quenched by addition of triethylamine and the crude product was purified by column chromatography (hexane/EtOAc, 6/4 to 8/ 2) to obtain compound **177** (206

mg, 85%). $[\alpha]_D^{20}$ = + 1.8° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$; mixture of rotamers) δ 8.08 – 8.06 (m, 2H, aromatic), 8.02 – 8.00 (m, 2H, aromatic), 7.58 – 7.54 (m, 2H, aromatic), 7.45 – 7.22 (m, 22H, aromatic), 7.18 – 7.09 (m, 2H, aromatic, NH), 6.90 – 6.87 (m, 2H, aromatic$_{PMP}$), 6.79 – 6.77 (m, 2H, aromatic$_{PMP}$), 5.67 (d, 1H, H-4), 5.36 – 5.32 (m, 2H, CH_2-Ph$_{Bz}$), 5.21 – 5.06 (m, 5H, H-1′, CH_2-Ph$_{Carba}$, H-2′, H-4′), 4.94 (d, *J* = 7.8 Hz, 1H, H-1), 4.86 – 4.83 (m, 1H, H-5′), 4.76 – 4.69 (m, 2H, CH_2Ph), 4.63 – 4.55 (m, 1H, H-3), 4.53 – 4.43 (m, 4H, CH_2-PhN, CH_2Ph), 4.19 – 4.13 (m, 1H, H-6′), 4.03 – 3.98 (m, 1H, H-6′), 3.91 – 3.83 (m, 2H, H-5, OCH$_{2Linker}$), 3.80 – 3.78 (m, 1H, H-3′), 3.76 – 3.64 (m, 4H, CH$_{3PMP}$, H-2) , 3.56 – 3.35 (m, 3H, H-6, OCH$_{2Linker}$), 3.26 – 3.11 (m , 2H, CH$_{2Linker}$), 2.56 – 2.43 (m, 3H, CH$_{2Lev}$), 2.37 – 2.28 (m, 1H, CH$_{2Lev}$), 2.03 (s, 3H, CH$_{3Lev}$), 1.74 (s, 3H, CH$_{3Ac}$), 1.58 – 1.42 (m, 4H, CH$_{2Linker}$), 1.35 – 1.26 (m, 2H, CH$_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$; mixture of rotamers) δ 205.97, 172.07, 170.17, 166.54, 164.98, 162.22, 162.13, 156.74, 156.25, 154.05, 153.14, 137.93, 137.88, 137.82, 137.10, 136.92, 135.89, 133.64 (Cq), 133.20, 130.19, 129.90, 129.83, 129.43, 128.67, 128.60, 128.53, 128.51, 128.46, 128.40, 128.30, 128.19, 127.95, 127.89, 127.83, 127.45, 127.25, 115.79 (C$_{PMP}$), 114.66 (C$_{PMP}$), 100.10 (C-1′), 99.19 (C-1), 92.39 (Cq, TCA), 73.73 (C$_{Bn}$), 73.65, 73.56 (C-3), 73.39 (C-3′), 73.12 (C-5), 72.75 (C$_{Bn}$), 70.18 (OCH$_{2Linker}$), 69.65 (C-4), 68.72 (C-6), 67.92 (C-4′, C-6′), 67.77 (C-2′), 66.94 (CH$_2$-Ph$_{Carba}$), 66.49 (CH$_2$-Ph$_{Bz}$), 65.26 (C-5′), 56.67 (C-2), 55.83 (CH$_{3PMP}$), 50.65, 50.42 (CH$_2$-PhN), 47.34, 46.24 (NCH$_{2Linker}$), 37.90 (CH$_{2Lev}$), 29.73 (CH$_{3Lev}$), 29.29, 29.00, 28.00 (CH$_{2Lev}$), 27.40, 23.53 (CH$_{2Linker}$), 23.31 (CH$_{2Linker}$), 20.40 (CH$_{3Ac}$) ppm. HRMS (ESI): *m/z* calcd. for $C_{77}H_{81}Cl_3N_2O_{20}$ $[M+Na]^+$ 1481.4340, found 1481.4324.

4-[(Phenylcarboxy)methyl]benzyl N-Benzyl-N-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyl)-(1→3)-4-*O*-Acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)oxy)pentyl]carbamate (178): The compound **177** (97 mg, 0.066 mmol) was dissolved in acetonitrile/water (4/ 1, 2.4 mL) and CAN (160 mg, 0.291 mmol) was added at 0 °C and was stirred for 10 min. The reaction was quenched by adding solid $NaHCO_3$ and was washed with saturated $NaHCO_3$ aq solution, water and brine. The crude produces was purified by column chromatography (hexane/EtOAc, 7/3 to 2/8) to obtain the title compound as a white solid (89 mg, 82%).$[\alpha]_D^{20}$ = + 7.3° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, mixture of rotamers) δ 8.09 – 8.05 (m, 2H, aromatic), 8.05 – 7.99 (m, 2H, aromatic),

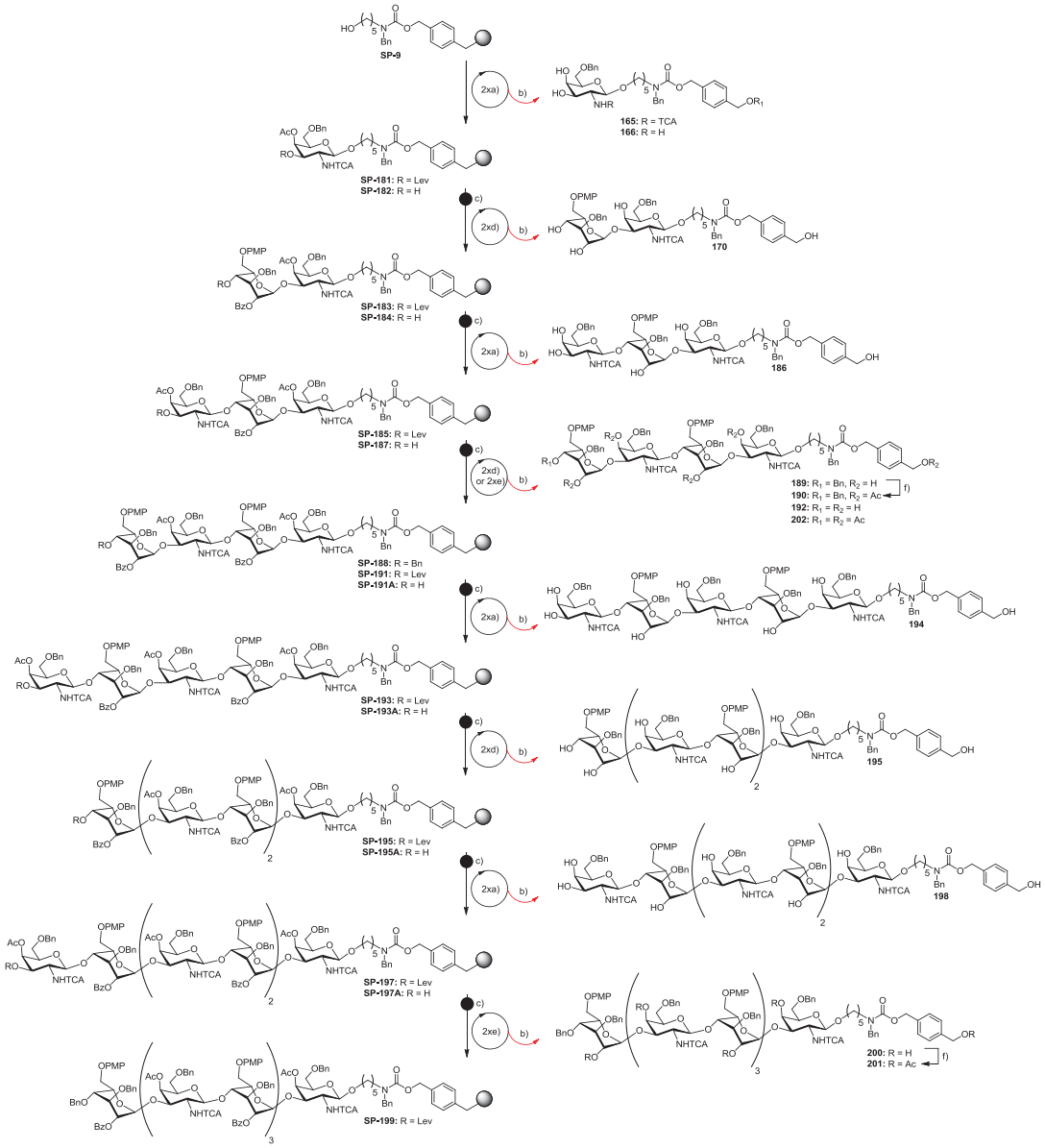
7.61 – 7.53 (m, 2H, aromatic), 7.49 – 7.19 (m, 23H, aromatic, NH), 7.18 – 7.09 (d, 1H, aromatic), 6.96 – 6.86 (d, *J* = 7.1 Hz, 0.4H, NH), 5.60 – 5.55 (d, *J* = 3.2 Hz, 1H, H-4), 5.40 – 5.31 (d, *J* = 12.1 Hz, 2H, CH_2-Ph_{Bz}), 5.23 – 5.05 (m, 4H, CH_2-Ph_{Carba}, H-1′, H-2′), 5.01 – 4.97 (s, 1H, H-4′), 4.96 – 4.91 (d, *J* = 8.2 Hz, 1H, H-1), 4.77 – 4.70 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.69 – 4.63 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.62 – 4.53 (t, *J* = 13.3 Hz, 1H, H-3), 4.53 – 4.42 (m, 4H, CH_2-PhN, CH_2Ph), 4.39 – 4.32 (t, *J* = 7.0 Hz, 1H, H-5′), 3.94 – 3.81 (m, 2H, $OCH_{2Linker}$, H-5), 3.80 – 3.78 (m, 1H, H-3′), 3.77 – 3.69 (m, 2H, H-6′), 3.68 – 3.58 (m, 1H, H-2), 3.55 – 3.50 (dd, *J* = 10.0, 6.1 Hz, H-6), 3.50 – 3.35 (m, 2H, H-6, $OCH_{2Linker}$), 3.26 – 3.11 (m, 2H, $NCH_{2Linker}$), 2.68 – 2.45 (m, 3H, CH_{2Lev}), 2.39 – 2.26 (m, 1H, CH_{2Lev}), 2.06 (s, 3H, CH_{3Lev}), 1.76 (s, 3H, CH_{3Ac}), 2.13 – 2.02 (s, 1H), 1.61 – 1.41 (m, 4H, $CH_{2Linker}$), 1.34 – 1.28 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$; 25°C, mixture of rotamers) δ 206.19, 172.39, 171.27, 166.55, 165.04, 162.37, 156.75, 156.27, 137.91, 137.85, 137.78, 137.09, 136.87, 135.89, 133.67, 133.21, 130.18, 129.90, 129.83, 129.39, 128.68, 128.61, 128.53, 128.45, 128.39, 128.17, 128.07, 128.00, 127.93, 127.91, 127.45, 127.24, 100.99 (C-1′), 99.15 (C-1), 92.34 (cq, TCA), 74.99 (C-3), 73.77 (C_{Bn}), 73.27, 73.15 (C-3′), 72.80 (C-5), 72.64 (C_{Bn}), 70.28 ($OCH_{2Linker}$), 70.20 (C-4), 68.53 (C-6), 67.82 (C-4), 67.66 (C-2′), 66.93 (CH_2-Ph_{Carba}), 66.76 (C-5′), 66.48 (CH_2-Ph_{Bz}), 62.01 (C-6′), 56.57 (C-2), 50.64, 50.43 (CH_2-PhN), 47.35, 46.21 ($NCH_{2Linker}$), 37.94 (CH_{2Lev}), 29.74 (CH_{3Lev}), 29.26, 28.90 ($CH_{2Linker}$), 27.99 (CH_{2Lev}), 27.35, 23.60, 23.27 ($CH_{2Linker}$), 20.60 (CH_{3Ac}) ppm. LRMS (ESI): *m/z* calcd. for $C_{70}H_{75}Cl_3N_2O_{19}$ $[M+NH_4]^+$ 1370.4, found 1370.2.

4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((3-*O*-benzyl-α-L-idopyranosiduronic acid)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (179): The compound **178** (107 mg, 0.079 mol) was dissolved in a mixture acetonitrile/ water (1/1, 1.6 mL), TEMPO (3 mg, 0.019 mmol) and BAIB (63 mg, 0.195 mmol) were added at 0 °C and the mixture was stirred overnight at room temperature. The reaction mixture was partitioned between EtOAc and 1M $Na_2S_2O_4$ aq solution, and the organic phase was washed with brine. The crude product was purified by column chromatography (hexane/EtOAc, 6/4 to 4/6 containing 1% of acetic acid). ^{1}H NMR (500 MHz, $CDCl_3$; mixture of rotamers) δ 8.09 – 8.08 (m, 2H, aromatic), 8.02 – 7.97 (m, 2H, aromatic), 7.59 – 7.52 (m, 2H, aromatic), 7.50 – 7.23 (m, 24H, aromatic, NH), 7.17 – 7.15 (m, 1H, aromatic), 7.13 – 07 (bs, 0.4H, NH), 6.35 – 5.85 (bs, 1H, OH), 5.53 – 5.50 (d, *J* = 3.4Hz, 1H, H-4), 5.40 – 5.33 (m, 3H, CH_2-

Ph_{Bz}, H-5′), 5.31 – 5.27 (d, *J* = 9.3Hz, 1H, H-1′), 5.24 – 5.09 (m, 4H, CH_2-Ph_{carba}, H-2′, H-4′), 4.91 – 4.82 (m, 1H, H-1), 4.81 – 4.70 (m, 2H, CH_2Ph), 4.63 – 4.55 (m, 1H, H-3), 4.53 – 4.43 (m, 4H, CH_2-PhN, CH_2Ph), 3.91 – 3.73 (m, 4H, H-2, $OCH_{2Linker}$, H-3′, H-5), 3.57 – 3.34 (m, 3H, H-6), 3.31 – 3.11 (m, 2H, $NCH_{2Linker}$), 2.58 – 2.47 (m, 2H, CH_{2Lev}), 2.44 – 2.35 (m, 1H, CH_{2Lev}), 2.34 – 2.19 (m, 1H. CH_{2Lev}), 2.06 – 2.00 (m, 3H, CH_{3Lev}), 1.73 – 1.64 (m, 3H, CH_{3Ac}), 1.61 – 1.43 (m, 4H, $CH_{2Linker}$), 1.37 – 1.27 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$; mixture of rotamers) δ 206.8, 176.0, 171.6, 170.7, 170.5, 166.6, 164.8, 162.4, 162.2, 156.4, 137.8, 137.6, 136.8, 135.9, 133.7, 133.2, 130.2, 129.9, 129.8, 129.2, 128.7, 128.6, 128.5, 128.5, 128.5, 128.4, 128.2, 128.0, 128.0, 127.9, 127.5, 127.3, 100.7 (C-1′), 99.5 (C-1), 92.5 (Cq, TCA), 73.7 (C_{Bn}), 72.8 (C_{Bn}), 72.2 (C-3), 72.1 (C-5′), 70.1 ($OCH_{2Linker}$), 69.3 (C-4), 68.5 (C-6), 67.9, 67.8 (C-2′), 67.0 (CH_2-Ph_{carba}), 66.5 (CH_2-Ph_{Bz}), 66.2 (C-4′), 56.2 (C-2), 50.6, 50.5 (CH_2-PhN), 47.4, 46.2 ($NCH_{2Linker}$), 37.8 (CH_{2Lev}), 29.6 (CH_{3Lev}), 29.2, 28.9 ($CH_{2Linker}$), 28.0 (CH_{2Lev}), 27.3, 23.4 ($CH_{2Linker}$), 20.3 (CH_{3Ac}) ppm. The compound was dissolved in methanol (0.5 mL) and sodium methoxide (1 mL) was added at 0°C and was stirred overnight. The reaction crude was quenched with Amberlite® IR-120(H) and was purified by Sephadex® LH-20 (CH_2Cl_2/MeOH, 2/1) to obtain compound (58 mg, 70% over 2steps). ^1H NMR (500 MHz, CD_3OD; mixture of rotamers) δ 7.46 – 7.40 (d, *J* = 7.5 Hz, 2H, aromatic), 7.39 – 7.19 (m, 16H, aromatic), 7.19 – 7.12 (d, *J* = 7.2 Hz, 1H, aromatic), 5.20 – 5.00 (m, 3H, CH_2-Ph_{Linker}, H-1′), 4.94 – 4.82 (m, 1H, H-5′, under H_2O signal), 4.75 – 4.67 (d, *J* = 11.3 Hz, 1H, CH_2Ph), 4.65 – 4.49 (m, 6H, H-1, CH_2Ph, CH_2-Ph_{carba}), 4.50 – 4.44 (s, 2H, CH_2-PhN), 4.25 – 4.17 (t, *J* = 9.5 Hz, 1H, H-2), 4.14 – 4.05 (s, 1H, H-4′), 4.04 – 3.96 (m, 1H, H-4), 3.93 – 3.75 (m, 3H, H-2′, H-3, $OCH_{2Linker}$), 3.74 – 3.64 (m, 4H, H-3′, H-6, H-5), 3.50 – 3.37 (m, 1H, $OCH_{2Linker}$), 3.26 – 3.12 (m, 2H, $NCH_{2Linker}$), 1.57 – 1.41 (m, 4H, $CH_{2Linker}$), 1.36 – 1.21 (m, 2H, $CH_{2Linker}$) ppm. ^{13}C NMR (126 MHz, CD_3OD; mixture of rotamers) δ 164.28, 158.39, 157.89, 142.68, 139.57, 139.09, 137.01, 129.58, 129.46, 129.42, 129.38, 129.06, 128.86, 128.76, 128.69, 128.65, 128.33, 128.10, 104.93 (C-1′), 102.38 (C-1), 94.36 (Cq, TCA), 81.54 (C-3′), 77.63 (C-3), 75.00 (C-5), 74.36 (C_{Bn}), 72.92 (C_{Bn}), 70.64 (C-6), 70.51 ($OCH_{2Linker}$), 70.04 (C-5′, C-4′), 69.69 (C-4), 68.19 (CH_2-Ph_{Linker}), 67.38 (C-2′), 64.88 (CH_2-Ph_{carba}), 54.79 (C-2), 51.53, 51.33 (CH_2-PhN), 48.37, 47.56 ($NCH_{2Linker}$), 30.30, 28.94, 28.43, 24.32 ($CH_{2Linker}$) ppm. HRMS (ESI): *m/z* calcd. for $C_{49}H_{57}Cl_3N_2O_{15}$ $[M+NH_4]^+$ 1041.2717, found 1041.2715.

4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((3-*O*-benzyl-2,4-di-*O*-sodium sulfonato-α-L-idopyranosyluronate)-(1→3)-6-*O*-benzyl-2-deoxy-4-*O*-sodium sulfonato-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (180): The compound **179** (59 mg, 0.058 mmol) and the sulfur trioxide pyridine complex (5eq per OH group, 185 mg, 1.16 mmol) were dissolved in dry pyridine (3.5 mL) and heated at 60 °C for 15 min using microwave irradiation. Additional amount of sulfur trioxide pyridine complex (46 mg, 0.29 mmol) was added and stirred for 30 min. at the same conditions. The reaction vessel was cooled and triethylamine (500 µL), MeOH (1mL) and CH_2Cl_2 (1mL) were added. The solution was layered on the top of a Sephadex® LH-20 chromatography column wich was eluted with CH_2Cl_2/MeOH (1/1) to obtain the product as triethylamonium salt. The residue was converted into the sodium salt **180** by elution from a column of Dowex 50WX2-Na^+ with MeOH (61 mg, 73%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.55 – 7.08 (m, 19H, aromatic), 5.37 (s, 1H, H-1´), 5.30 (s, 1H, H-5´), 5.15 (m, 2H, CH_2-Ph), 5.04 – 4.98 (m, 2H, CH_2-Ph_{Carba}), 4.96 – 4.93 (m, 1H, H-4), 4.77 – 4.70 (m, 2H, H-4´, CH_2Ph), 4.63 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.59 (s, 1H, H-3´), 4.56 (s, 2H, CH_2Ph), 4.49 (s, 2H, CH_2-PhN), 4.44 – 4.38 (m, 2H, H-1, H-2´), 4.18 (t, *J* = 9.4 Hz, 1H, H-2), 4.00 (dd, *J* = 11.1, 2.7 Hz, 1H, H-3), 3.91 (d, *J* = 5.5 Hz, 1H, H-6), 3.85 – 3.74 (m, 2H, H-5, $OCH_{2Linker}$), 3.51 – 3.39 (m, 1H, $OCH_{2Linker}$), 3.27 – 3.19 (m, 2H, $NCH_{2Linker}$), 3.17 (q, Et_3N), 1.58 – 1.43 (m, 4H, $CH_{2Linker}$), 1.27 (t, Et_3N), 1.36 – 1.21 (m, 2H, CH_2linker).^{13}C NMR (126 MHz, CD_3OD, selected data from HSQC experiment) δ 128.4 – 127.5 (Caromatic), 102.5 (C-1´), 102.0 (C-1), 80.9 (C-3), 75.5 (C-4), 74.7 (C-2´), 74.3 (C-5), 73.0 (C_{Bn}), 72.0 (C-4´), 71.4 (C_{Bn}), 70.7 (C-6), 70.3 (C-3´), 69.6 ($OCH_{2Linker}$), 69.0 (CH_2-Ph_{Carba}), 67.5 (C-5´), 53.0 (C-2), 50.2 (CH_2-PhN), 46.4 ($NCH_{2Linker}$), 29.1, 27.5 ($CH_{2Linker}$), 23.2 ($CH_{2Linker}$) ppm.

5.9.3 Solid-Phase synthesis of DS tetrasaccharide and octasaccharide precursors.



Scheme 5.2. Assembly of a DS tetrasaccharide precursor **190** and octasaccharide precursor **201**. a) **82**, 15% TMSOTf, -20 °C to rt; b) NaOMe (cat), MeOH, 40 °C, MW; c) hydrazine acetate, CH_2Cl_2: MeOH (4:1); d) **30**, 20% TMSOTf, -20 °C to rt; e) **41**, 20% TMSOTf, -20 °C to rt; f) Ac_2O, pyridine, 0 °C to rt.

Resin-Bound 4-(hydroxymethyl)benzyl N-benzyl-N-[5-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-181): The reaction was performed according to general procedure G employing one cycle on resin **SP-9** (480 mg, 0.22 mmol/g, 0.106 mmol) with trichloroacetimidate donor **82** (1 x 5 eq., 369 mg, 0.53 mmol) and TMSOTf (3 µL, 0.016 mmol). The conversion was determined after analytical NaOMe cleavage. Conversion: 97%; UPLC-MS (ESI): 67% as compound **165** (*m/z*[M+H]$^+$ Calcd for $C_{36}H_{43}Cl_3N_2O_9H$ 753.20, found 752.91) and 30% as compound **166** (*m/z* [M+H]$^+$ Calcd for $C_{34}H_{44}N_2O_8H$ 609.30, found 609.25). The partial loss of trichloroacetamide could be avoided when the cleavage reaction was carried out at 40°C instead of 55°C.

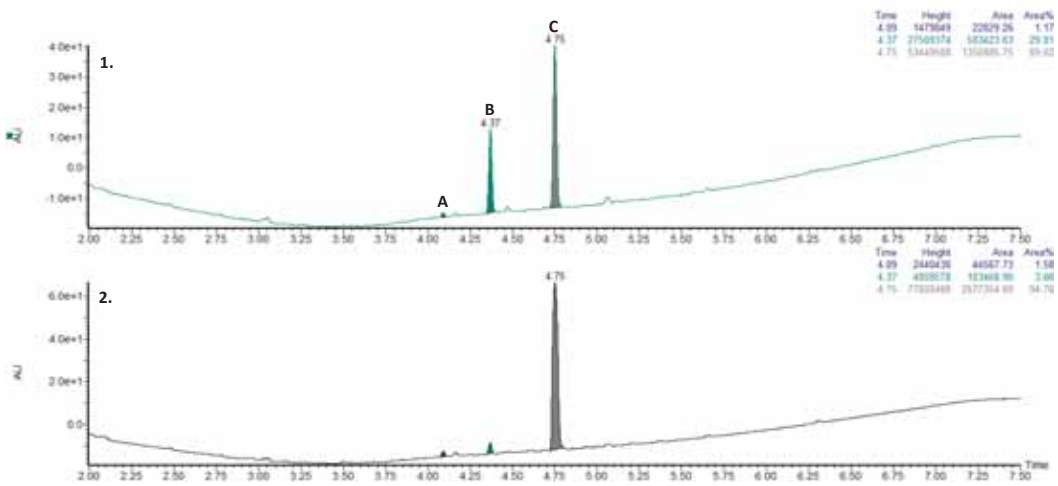


Figure 5.15. UPLC-MS chromatograms of analytical cleavage conversion of linker **SP-9** (retention time (t_r) at 4.09 min., peak **A** (*m/z* calcd for $C_{21}H_{27}NO_4$[M+H]$^+$ 358.19 found 358.24)) to monosaccharide **SP-181** (t_r at 4.37 min., peak **C** (**165**, *m/z* calcd for $C_{36}H_{43}Cl_3N_2O_9$ [M+NH$_4$]$^+$ 770.20 found 770.06); peak **B** (**166**, t_r at 4.75 min. *m/z* calcd for $C_{34}H_{44}N_2O_8$[M+H]$^+$ 609.30 found 609.25)). Trace 1: analytical cleavage at 55 °C; trace 2: analytical cleavage at 40 °C.

SP-182

The resin **SP-181** was transformed to resin-bound 4-(hydroxymethyl)benzyl N-benzyl-N-[5-(4-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyloxy)pentyl]carbamate **SP-2** using general procedure F.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-O-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-183): Disaccharide formation was performed according to general procedure G using 2 cycles on resin **SP-182** (0.390 g, 0.087 mmol) with trichloroacetimidate donor **30** (5 equiv, 0.315 g, 0.44 mmol) and TMSOTf (3 µL, 0.017 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) n.a (not analyzed); (cycle 2) 99% as compound **170** (*m/z* $[M+NH_4]^+$ calcd for $C_{56}H_{65}Cl_3N_2O_{15}$ NH_4 1128.34, found 1127.93.

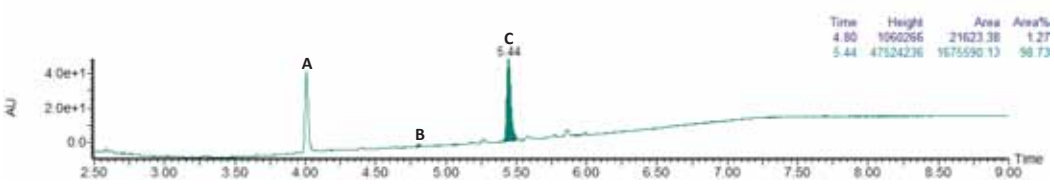


Figure 5.16. UPLC-MS chromatograms of analytical cleavage conversion of monosaccharide **SP-181** (t_r at 4.80 min. (peak **B**: compound **149**, *m/z* calcd for $C_{36}H_{43}Cl_3N_2O_9$ $[M+NH_4]^+$ 770.20 found 770.06)) to disaccharide **SP-183** (t_r at 5.44 min., peak **C** (**170**, *m/z* calcd for $C_{56}H_{65}Cl_3N_2O_{15}$ $[M+NH_4]^+$ 1128.34 found 1128.27. Peak **A** (no mass detectable).

SP-184

The resin **SP-183** was transformed to resin-bound resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyloxy)pentyl]carbamate **SP-184** using general procedure F.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyloxy)pentyl]carbamate (SP-185): Trisaccharide formation was performed according to general procedure G using 2 cycles on resin **SP-184** (378 mg, 0.083 mmol) with trichloroacetimidate donor **82** (5 equiv, 0.290 g, 0.415 mmol) and TMSOTf (3 µL). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) n.a (not

analyzed); (cycle 2) 97%, as compound **186** (*m/z* $[M+NH_4]^+$ calcd for $C_{71}H_{81}Cl_6N_3O_{20}NH_4$ 1523.35, found 1523.31).

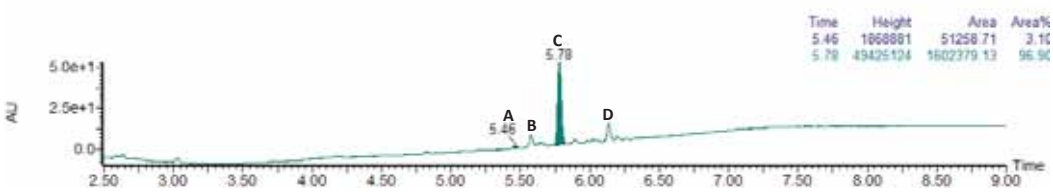


Figure 5.17. UPLC-MS chromatograms of analytical cleavage conversion of disaccharide **SP-184** (t_r at 5.46 min. (peak **A**, as **170**, *m/z* calcd for $C_{56}H_{65}Cl_3N_2O_{15}$ $[M+NH_4]^+$ 1128.34 found 1128.31)) to trisaccharide **SP-185** (t_r at 5.78 min., peak **C** (as **186**, *m/z* calcd for $C_{71}H_{81}Cl_6N_3O_{20}$ $[M+NH_4]^+$ 1523.35 found 1523.31)). Peak **B** (as **186-TCA,** *m/z* calcd for $C_{69}H_{82}Cl_3N_3O_{19}$ $[M+H]^+$ 1362.42 found 1362.41), peak **D** (as **186+Bn**, *m/z* calcd for $C_{78}H_{87}Cl_6N_3O_{20}$ $[M+NH_4]^+$ 1613.44 found 1613.41).

SP-187

The resin **SP-185** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate **SP-187** using general procedure F.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-O-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate (SP-188): Linker **SP-9** (500 mg, 0.22 mmol/g, 0.11 mmol) was glycosylated in 1 cycle according to general procedure A with trichloroacetimidate donor **82** (5 equiv, 384 mg 0.55 mmol) and TMSOTf (3 μL, 0.022 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) 98%, 94% as compound **165** (*m/z* for $C_{36}H_{43}Cl_3N_2O_9$ $[M+NH_4]^+$ 770.20, found 770.20 and 4% as compound **166** (*m/z* for $C_{34}H_{44}N_2O_8$ $[M+H]^+$ 609.30, found 609.25; (cycle 2) n.a (not analyzed). After capping and delevulination following procedure B, disaccharide formation **SP-183** was performed according to general procedure G using 2 cycles on resin **SP-182** (490 mg,

0.11 mmol) with trichloroacetimidate donor **2** (3 equiv, 521 mg, 0.72 mmol) and TMSOTf (2 µL, 0.011 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) n.a (not analyzed); (cycle 2) 99% as compound **170** (*m/z* $[M+NH_4]^+$ calcd for $C_{56}H_{65}Cl_3N_2O_{15}NH_4$ 1128.34, found 1127.93. After capping and delevulination, trisaccharide formation **SP-185** was performed according to general procedure G using 2 cycles on resin **SP-184** (480 mg, 0.11 mmol) with trichloroacetimidate donor **82** (3 equiv, 230 mg, 0.33 mmol) and TMSOTf (3 µL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) n.a (not analyzed); (cycle 2) 95%, 74% as compound **186** (*m/z* for $[M+NH_4]^+$ calcd $C_{71}H_{81}Cl_6N_3O_{20}NH_4$ 1523.35, found 1523.31) and 21% as **186-TCA** (*m/z* $[M+H]^+$ calcd for $C_{69}H_{82}Cl_3N_3O_{19}H$ 1362.42, found 1362.35). After capping and delevulination, procedure H was applied consecutively to synthesized the tetrasaccharide **SP-188** on resin **SP-187** (400 mg, 0.088 mmol) using trichloroacetimidate donor **41** (3 equiv, 186 mg, 0.26 mmol) and TMSOTf (25 µL of 0.1M solution in CH_2Cl_2). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) n.a (not analyzed); (cycle 2) 97%, 71% as compound **189+Bz** with one remaining Bz group (*m/z* $[M+2NH_4]^{2+}$ calcd for $C_{105}H_{113}Cl_6N_3O_{27}(NH_4)_2$ 1046.78, found 1046.78), 19% as **189** (*m/z* $[M+NH_4]^+$ calcd for $C_{98}H_{109}Cl_6N_3O_{26}$ NH_4 1971.54, found 1971.47) and 6% as **189+Bz+Bn** (*m/z* $[M+NH_4]^+$ calcd for $C_{98}H_{109}Cl_6N_3O_{26}$ NH_4 1971.54, found 1971.47).

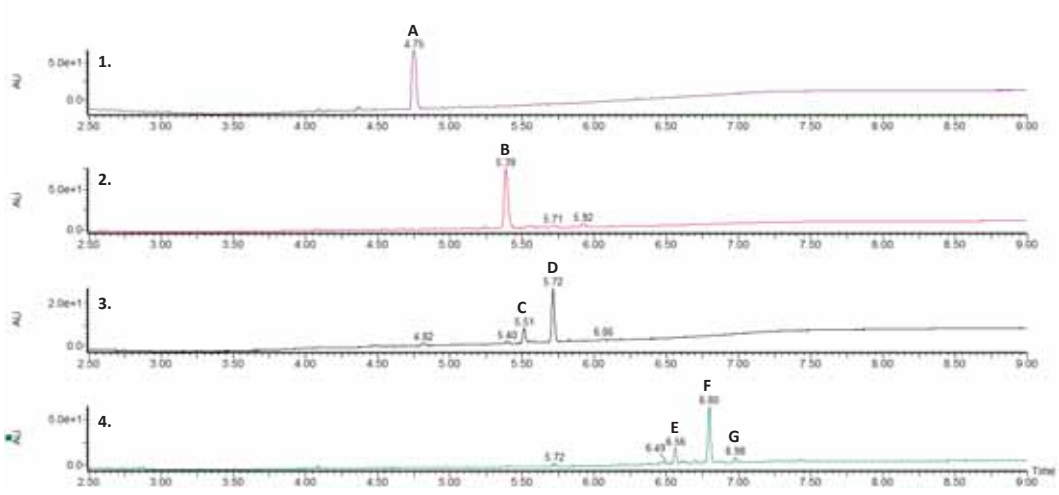


Figure 5.18 UPLC-MS analysis of tetrasaccharide **SP-188** synthesis. **(1)** UPLC-MS chromatogram from analytical cleavage of monosaccharide **SP-181** (t_r at 4.75 min., peak **A** (cleaved as **165**, *m/z* calcd for $C_{36}H_{43}Cl_3N_2O_9$ $[M+NH_4]^+$ 770.20 found 770.06). **(2)** UPLC-MS

chromatogram from analytical cleavage of disaccharide **SP-183** (t_r at 5.39 min., peak **B** (cleaved as **170**, *m/z* calcd for $C_{56}H_{65}Cl_3N_2O_{15}$ $[M+NH_4]^+$ 1128.34 found 1128.27). **(3)** UPLC-MS chromatogram from analytical cleavage conversion of trisaccharide **SP-185** (t_r at 5.72 min., peak **D** (cleaved as **186,** *m/z* calcd for $C_{71}H_{81}Cl_6N_3O_{20}$ $[M+NH_4]^+$ 1523.35 found 1523.24); t_r at 5.51 min., peak **C** (cleaved as **186-TCA**, *m/z* calcd for $C_{69}H_{82}Cl_3N_3O_{19}$ $[M+H]^+$ 1362.42 found 1362.35). **(4)** UPLC-MS chromatogram of analytical cleavage conversion of trisaccharide **SP-187** (cleaved as **186**, t_r at 5.72 min.) to tetrasaccharide **SP-188** (t_r at 6.56 min., peak **E** (cleaved as **189**, *m/z* calcd for $C_{98}H_{109}Cl_6N_3O_{26}$ $[M+NH_4]^+$ 1971.54 found 1971.47); t_r at 6.80 min., peak **F** (cleaved as **189+Bz**, *m/z* calcd for $C_{105}H_{113}Cl_6N_3O_{27}$ $[M+NH_4]^{2+}$ 1046.78 found 1046.78) and t_r at 6.98 min., peak **G** (cleaved as **189+Bz+Bn**, *m/z* calcd for $C_{112}H_{119}Cl_6N_3O_{27}$ $[M+2NH_4]^{2+}$ 1091.80 found 1091.81).

4-(Acetoxymethyl)benzyl N-benzyl-N-[5-((2-*O*-acetyl-3,4-di-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (190): The resin **SP-188** (580 mg of resin) was swollen in dry CH_2Cl_2 (5 mL) and quantitative cleavage was performed according to general procedure C using 8 cycles of NaOMe/MeOH (500µL). The crude was treated with additional amount of 0.25 M NaOMe solution until UPLC-MS showed the deprotection of all acyl groups **(189)**. Acetylation reaction was performed overnight at room temperature using acetic anhydride (0.25 mL) and catalytic amount of DMAP in pyridine (0.5 mL). The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with 1M HCl, saturated $CuSO_4$ aq solution, water and brine. The organic layer was dried over anhydrous MgSO4, filtered and concentrated.The crude product was purified by column chromatography (hexane/acetone; 8/2 to 1/1) and preparative HPLC (C-18 (250x21.20 mm, 5 µm); flow rate 10 $mL \cdot min^{-1}$; eluents: 20mM NH_4CO_3 in water/MeCN; gradient: initial 10% water/90% MeCN; 30 min: 1% water/99% MeCN to obtain compound **190** (71 mg, resin after cleavage 340 mg; 45% overall yield; 92% for each step). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.14 (m, 34H, aromatic), 7.10 (d, *J* = 7.5 Hz, 1H, NH), 6.92 (d, *J* = 7.4 Hz, 1H, NH), 6.89 (d, *J* = 9.0 Hz, 2H, aromatic$_{PMP}$), 6.84 – 6.70 (m, 6H, aromatic$_{PMP}$), 5.56 (dd, *J* = 16.1, 3.3 Hz, 2H, H-4$_{GalNAc}$), 5.21 – 5.14 (m, 2H, CH_2Ph_{carba}), 5.11 – 5.05 (m, 2H, CH_2Ph_{Ac}), 4.98 – 4.92 (m, 2H, 2xH-1$_{Ido}$), 4.92 – 4.89 (m, 1H, H-2$_{Ido}$), 4.86 –

4.80 (m, 3H, 2xH-1_{GalN}, H-2_{Ido}), 4.68 – 4.62 (m, 2H, H-5_{Ido}, CH_2Ph), 4.59 – 4.53 (m, 3H, H-5_{Ido}, CH_2Ph), 4.56 – 4.45 (m, 7H, CH_2Ph, CH_2PhN), 4.45 – 4.39 (m, 2H, CH_2Ph, H-3_{GalNAc}), 4.39 – 4.32 (m, 3H, CH_2Ph, H-3_{GalNAc}), 4.21 (dd, *J* = 10.2, 7.6 Hz, 1H, H-6_{Ido}), 4.18 – 4.12 (m, 2H, H-6_{Ido}, H-3_{Idocap}), 3.94 (dd, *J* = 10.2, 4.7 Hz, 1H, H-6_{Ido}), 3.92 – 3.80 (m, 2H, H-6_{Ido}, $OCH_{2Linker}$), 3.79 – 3.71 (m, 9H, H-5_{GalNAc}, 2xH-2_{GalNAc}, 2xCH_{3PMP}), 3.69 (t, *J* = 3.4 Hz, 1H, H-3_{Ido}), 3.64 (t, *J* = 6.0 Hz, 1H, H-5_{GalNAc}), 3.61 – 3.58 (m, 1H, H-$4_{Idocapp}$), 3.57 – 3.53 (m, 1H, H-4_{Ido}), 3.52 – 3.35 (m, 3H, H-6_{GalNAc}, $OCH_{2Linker}$), 3.29 (dd, *J* = 9.5, 5.7 Hz, 1H, H-6_{GalNAc}), 3.26 – 3.09 (m, 3H, H-6_{GalNAc}, $NCH_{2Linker}$), 2.09 (s, 3H, CH_{3Ac}), 1.99 – 1.98 (2s, 6H, CH_{3Ac}), 1.75 (s, 3H, CH_{3Ac}), 1.70 (s, 3H, CH_{3Ac}), 1.58 – 1.43 (m, 4H, $CH_{2Linker}$), 1.35 – 1.26 (m, 2H, $CH_{2Linker}$) ppm. $\delta^{13}C$ (126 MHz, $CDCl_3$): 171.0, 170.1, 169.9, 169.7, 169.6, 162.0, 161.8, 154.2, 154.1, 153.2, 153.0, 138.3, 138.0, 138.0, 137.7, 137.6, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.0, 128.0, 127.9, 127.9, 127.8, 127.5, 127.3, 116.5, 115.8, 114.7, 101.1 (C-1_{GalNAc}), 100.2 (C-1_{Ido}), 100.1 (C-1_{Ido}), 99.6 (C-1_{GalNAc}), 92.7 (Cq, TCA), 92.3 (Cq, TCA), 76.3 (C-$4_{Idocapp}$), 73.8 (C-3_{GalNAc}), 73.7 (C_{Bn}), 73.6 (C_{Bn}), 73.3 (C-4_{Ido}), 73.3 (C-3_{GalNAc}), 72.9 (C-5_{GalNAc}), 72.6 (C-5_{GalNAc}), 72.6 (C_{Bn}), 72.3 (C-3_{Ido}), 70.0 ($OCH_{2Linker}$), 69.7, 69.7 (C-4_{GalNAc}), 68.9 (C-6_{GalNAc}),, 68.6 (C-6_{GalNAc}), 68.5 (C-2_{Ido}), 68.3 (C-6_{Ido}), 68.0 (C-2_{Ido}), 67.9 (C-6_{Ido}), 67.2 (C-5_{Ido}), 66.9 (CH_2Ph_{carba}), 66.1 (CH_2Ph_{Ac}), 66.0 (C-5_{Ido}), 56.5 (C-2_{GalNAc}), 56.4 (C-2_{GalNAc}), 55.9 (CH_{3PMP}), 50.7, 50.5 (CH_2PhN), 47.4, 46.3 ($NCH_{2Linker}$), 29.2, 29.1, 29.1, 28.0, 27.4 ($CH_{2Linker}$), 23.4, 22.8 ($CH_{2Linker}$), 21.2, 21.1, 21.1, 20.4, 20.3 (CH_{3Ac}) ppm. HRMS (ESI) *m/z*: $[M+NH_4]^+$ Calcd for $C_{108}H_{119}Cl_6N_3O_{31}NH_4$ 2181.6297; Found 2182.6685.

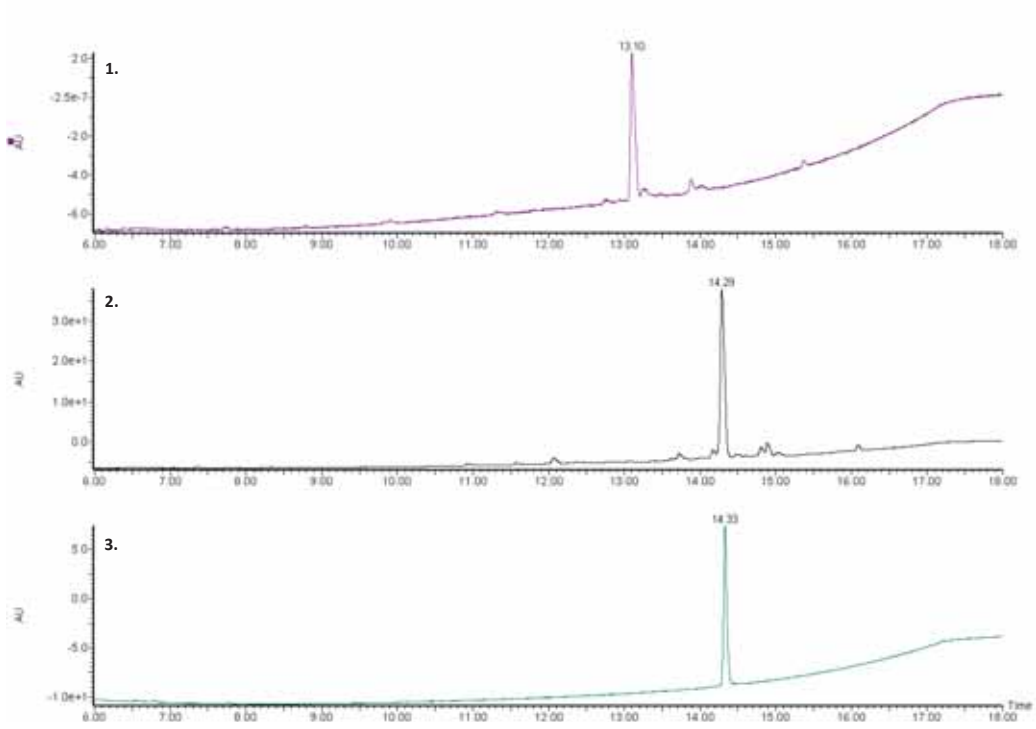


Figure 5.19. UPLC-MS data for the synthesis of tetrasaccharide **190**. (1) chromatogram of the crude **189** from preparative cleavage, (2) chromatogram after acetylation reaction **190**, (3) chromatogram after preparative HPLC purification of **190**.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-O-levulinoyl-6-*O*- *p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate (SP-191): Tetrasaccharide formation was performed according to general procedure G using 2 cycles on resin **SP-187** (372 mg, 0.082 mmol) with trichloroacetimidate donor **30** (5 equiv, 296 mg, 0.41 mmol) and TMSOTf (3 μL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) 86%; (cycle 2) 98% as compound **192** (*m/z* $[M+NH_4]^+$ calcd for $C_{91}H_{103}Cl_6N_3O_{26}$ NH_4 1881.49 found 1881.46).

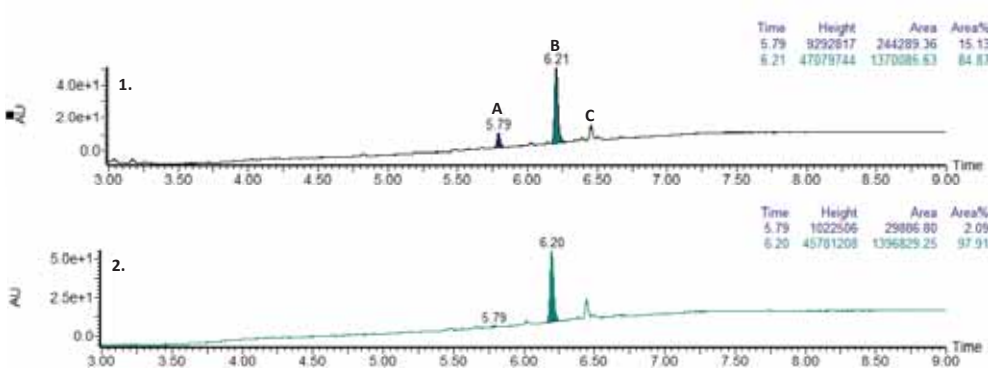


Figure 5.20. UPLC-MS chromatogram of analytical cleavage conversion of trisaccharide **SP-187** (cleaved as **186**, t_r at 5.79 min., peak **A** (*m/z* calcd for $C_{71}H_{81}Cl_6N_3O_{20}$ $[M+NH_4]^+$ 1523.35 found 1523.33)) to tetrasaccharide **SP-191** (cleaved as **192**; t_r at 6.20 min., peak **B** (*m/z* calcd for $C_{91}H_{103}Cl_6N_3O_{26}$ $[M+NH_4]^+$ 1881.49 found 1881.46)). Peak **C** (as **192+Bn**, t_r at 6.55 min. for *m/z* $C_{98}H_{109}Cl_6N_3O_{26}$ $[M+NH_4]^+$ 1971.54 found 1971.51).

SP-191A

The resin **SP-191** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate **SP-191A** using general procedure F.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-levulinoyl-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-193): Pentasaccharide formation was performed according to general procedure G using 2 cycles on resin **SP-191A** (370 mg, 0.081 mmol) with trichloroacetimidate donor **82** (5 equiv, 283 mg, 0.41 mmol) and TMSOTf (3 μL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) n.a; (cycle 2) full conversion as compound **S-16** (*m/z* $[M+2NH_4]^{2+}$ calcd for $C_{106}H_{119}Cl_9N_4O_3(NH4)_2$ 1147.25 found 1147.23).

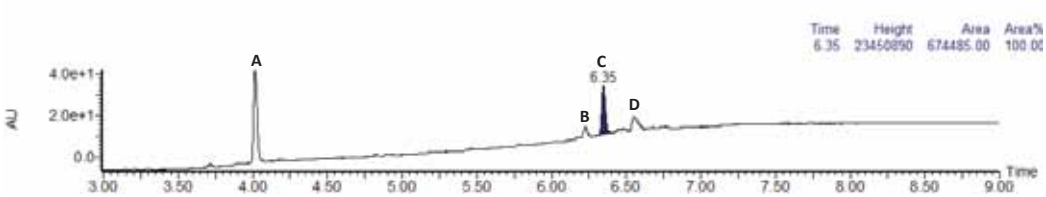


Figure 5.21. UPLC-MS chromatogram of analytical cleavage of pentasaccharide **SP-193** synthesis (t_r at 6.35 min., peak C (as **194**, t_r at 6.35 min *m/z* calcd for $C_{106}H_{119}Cl_9N_4O_{31}$ $[M+2NH_4]^{2+}$ 1147.25 found 1147.23)); peak B (cleaved as **194-TCA**, at 6.25 min *m/z* calcd for $C_{104}H_{120}Cl_6N_4O_{30}$ $[M+H+NH_4]^{2+}$ 1066.80 found 1066.78)); peak D (as **194+Bn**, t_r at 6.55 min. for *m/z* $C_{113}H_{125}Cl_9N_4O_{31}$ $[M+2NH_4]^{2+}$ 1192.27 found 1192.25). Unidentified: peak **A** (no mass detectable).

The resin **SP-193** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate **SP-193A** using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-O-levulinoyl-6-*O*- *p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido- β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-195): Hexasaccharide formation was performed according to general procedure G using 2 cycles on resin **SP-194A** (367 mg, 0.081 mmol) with trichloroacetimidate donor **30** (5 equiv, 290 mg, 0.40 mmol) and TMSOTf (3 µL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) n.a; (cycle 2) 90% as compound **196** *m/z* $[M+2NH_4]^{2+}$ calcd for $C_{126}H_{141}Cl_9N_4O_{37}(NH_4)_2$ 1326.32 found 1326.26).

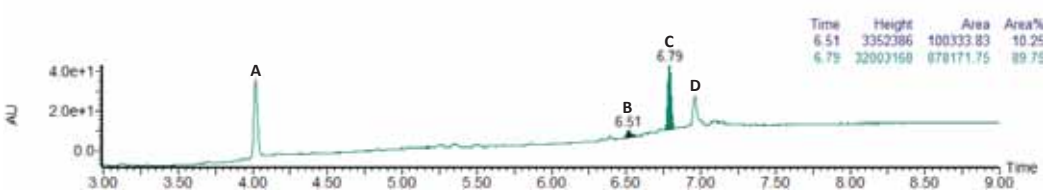


Figure 5.22. UPLC-MS chromatogram of analytical cleavage data of hexasaccharide **SP-195**. Conversion of pentasaccharide **SP-193A** (t_r at 6.51 min., peak **B** (as **194**, m/z calcd for $C_{106}H_{119}Cl_9N_4O_{31}$ $[M+2NH_4]^{2+}$ 1147.25 found 1147.20) to the hexasaccharide **SP-195** (as **196,** t_r at 6.79 min., peak **C** (*m/z* calcd for $C_{126}H_{141}Cl_9N_4O_{37}$ $[M+2NH_4]^{2+}$ 1326.32 found 1326.26)); peak **D** (as **196+Bn**, t_r at 6.96 min. *m/z* calcd for $C_{133}H_{147}Cl_9N_4O_{37}$ $[M+2NH_4]^{2+}$ 1371.34 found 1371.28). Unidentified: peak **A** (no mass detectable).

SP-195A

The resin **SP-195** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate **SP-195A** using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-levulinoyl 2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-197): Heptasaccharide formation was performed according to general procedure G using 2 cycles on resin **SP-195A** (364 mg, 0.080 mmol) with trichloroacetimidate donor **82** (5 equiv, 290 mg, 0.40 mmol) and TMSOTf (3 μL, 0.016 mmol). The conversion was determined by analytical NaOMe

cleavage. UPLC-MS (ESI): (cycle 1) 94%; (cycle 2) full conversion as compound **198** (*m/z*[M+2NH$_4$]$^{2+}$ calcd for $C_{141}H_{157}Cl_{12}N_5O_{42}(NH_4)_2$ 1523.82 found 1523.65).

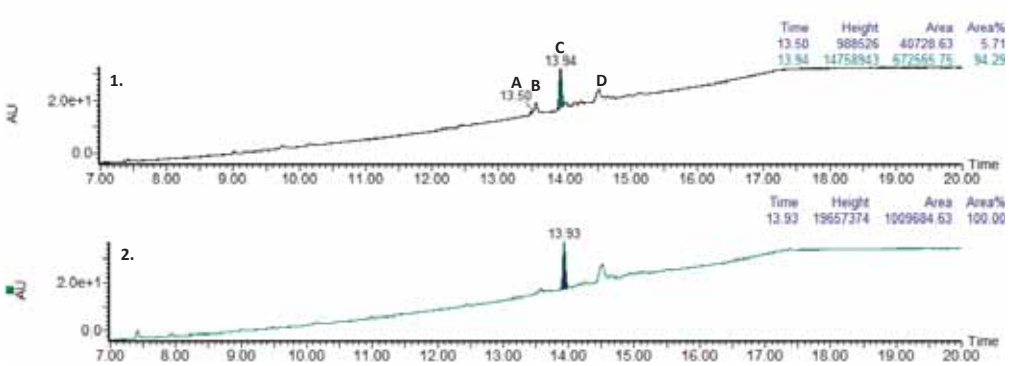


Figure 5.23. UPLC-MS chromatogram of analytical cleavage data conversion of hexasaccharide **SP-195A** (t_r at 13.50 min., peak **A** (as **196**, m/z calcd for $C_{126}H_{141}Cl_9N_4O_{37}$ [M+2NH$_4$]$^{2+}$ 1326.32 found 1326.26) to the heptasaccharide **SP-197** (as **S-198**, t_r at 13.93 min., peak **C** (*m/z* calcd for $C_{141}H_{157}Cl_{12}N_5O_{42}$ [M+2NH$_4$]$^{2+}$ 1523.82 found 1523.65)); peak **B** (as **198-TCA**, t_r at 5.18 min. *m/z* calcd for $C_{139}H_{158}Cl_9N_5O_{41}$ [M+NH$_4$+H]$^{2+}$ 1443.40 found 1443.19), peak **D** (as **198+Bn**, t_r at 14.51 min. (*m/z* calcd for $C_{148}H_{163}Cl_{12}N_5O_{42}$ [M+2NH$_4$]$^{2+}$ 1568.85, found 1568.66). (1) cycle 1, (2) cycle 2.

SP-197A

The resin **SP-197** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate **197A** using general procedure H.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-

benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-199): Octasaccharide formation was performed according to general procedure G using 2 cycles on resin **SP-197A** (358 mg, 0.080 mmol) with trichloroacetimidate donor **41** (5 equiv, 281 mg, 0.40 mmol) and TMSOTf (2.8 μL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. UPLC-MS (ESI): (cycle 1) n.a; (cycle 2) 97% as compound **200** (*m/z:* $[M+2NH_4]^{2+}$ calcd for $C_{141}H_{157}Cl_{12}N_5O_{42}$ NH_4 1523.82 found 1523.65).

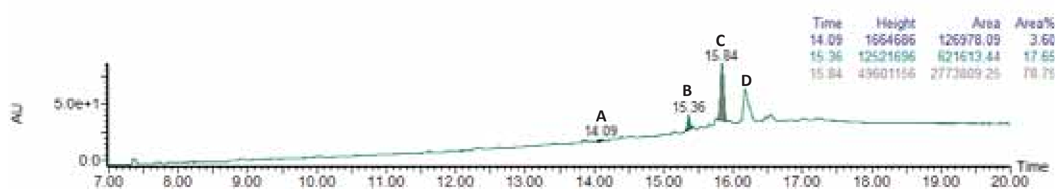


Figure 5.24. UPLC-MS analysis of analytical cleavage data conversion of heptasaccharide **SP-197A** (**198**, t_r at 14.09 min., peak **A** (*m/z* calcd for $C_{141}H_{157}Cl_{12}N_5O_{42}$ $[M+2NH_4]^{2+}$ 1523.82 found 1523.65) to the octasaccharide **SP-199** (as **200**, t_r at 15.36 min., peak **B** (*m/z* calcd for $C_{168}H_{185}Cl_{12}N_5O_{48}$ $[M+2NH_4]^{2+}$ 1747.90 found 1747.98); peak **C** (as **200+Bz**, t_r at 15.84 min. *m/z* calcd for $C_{175}H_{189}Cl_{12}N_5O_{49}$ $[M+2NH_4]^{2+}$ 1443.40 found 1443.19), peak **D** (as **200+Bz+Bn**, t_r at 16.18 min. (*m/z* calcd for $C_{182}H_{195}Cl_{12}N_5O_{49}$ $[M+2NH_4]^{2+}$ 1844.96, found 1845.04).

4-(Acetoxymethyl)benzyl N-benzyl-N-[5-((2-*O*-acetyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate (201): The resin **SP-199** (570 mg of resin, resin after cleavage 340 mg; 0.075 mmol) was swollen in dry CH_2Cl_2 (5mL) and quantitative cleavage was performed according to general procedure C using 8 cycles of NaOMe/MeOH (500μL). The crude was treated with additional

amount of 0.25 M NaOMe solution until UPLC-MS showed the deprotection of all acyl groups affording intermediate **200** (see Figure 5.25). Acetylation was performed overnight at room temperature with acetic anhydride (0.25 mL) and a catalytic amount of DMAP in pyridine (0.5 mL). The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with 1M HCl, saturated $CuSO_4$ aq solution, water and brine. The organic layer was dried over MgSO4 and the crude product was purified by column chromatography (hexane/EtOAc; 7/3 to 1/1) and preparative HPLC (column: C-18 (21.2x250 mm 5 µm); flow rate 10 mL·min^{-1}; eluents: 20mM NH_4CO_3 in water/MeCN; gradient: initial 10% water/90% MeCN; 30 min: 1% water/99% MeCN to obtain compound **201** (27 mg, 9.4% overall yield) and **201+Bn** (16 mg, 5.6%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.13 (m, 59H, aromatic), 7.06 (bs, 1H, NH), 6.94 – 6.86 (m, 8H, aromatic$_{PMP}$, NH), 6.85 – 6.72 (m, 10H, aromatic$_{PMP}$), 5.59 – 5.52 (m, 4H, 4xH-4$_{GalNAc}$), 5.19 – 5.04 (m, 4H, CH_2-Ph$_{Carba}$, CH_2-Ph$_{Ac}$), 4.98 – 4.91 (m, 4H, 4xH-1$_{Ido}$), 4.91 – 4.88 (s, 1H, H-2$_{Ido}$), 4.84 – 4.76 (m, 7H, 4xH-1$_{GalNAc}$, 3x H-2$_{Ido}$), 4.67 – 4.60 (m, 4H, 2xH-5$_{Ido}$, CH_2Ph), 4.58 – 4.25 (m, 24 H, CH_2PhN, 8xCH_2Ph, 4xH-3$_{GalNAc}$, 2xH-5$_{Ido}$, CH_2Ph), 4.23 – 4.11 (m, 7H, 3xH-3$_{Ido}$, 2xH-6$_{Ido}$), 3.96 – 3.84 (m, 5H, 2xH-6$_{Ido}$, OCH$_{2Linker}$), 3.83 – 3.71 (m, 17H, 4CH$_{3PMP}$, 4xH-2$_{GalN}$, H-5$_{GalNAc}$), 3.69 (t, *J* = 3.4 Hz, 1H, H-3$_{Ido}$), 3.65 – 3.55 (m, 6H, 3xH-5$_{GalNAc}$, 3xH-4$_{Ido}$), 3.55 – 3.51 (bs, 1H, H-4$_{Idocapp}$), 3.51 – 3.33 (m, 3H, H-6$_{GalNAc}$, OCH$_{2Linker}$), 3.32 – 3.08 (m, 8H, CH_2N_{Linker}, 3xH-6$_{GalN}$), 2.12 (1s, 3H, CH$_{3Ac}$), 2.01 – 1.95 (s, 12H, CH$_{3Ac}$), 1.74 (s, 3H, CH$_{3Ac}$), 1.69 (s, 3H, CH$_{3Ac}$), 1.59 – 1.58 (2s, 6H, CH$_{3Ac}$), 1.55 – 1.44 (m, 4H, CH$_{2Linker}$), 1.35 – 1.28 (m, 2H, CH$_{2Linker}$) ppm; δ^{13}C (126 MHz, $CDCl_3$): 173.0, 170.1, 169.8, 169.7, 169.6, 169.5, 162.1, 162.0, 156.7, 156.2, 154.0, 153.9, 153.0, 152.9, 138.2, 138.2, 137.9, 137.6, 137.6, 137.4, 136.9, 135.7, 131.0, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.4, 127.2, 116.2 (C$_{PMP}$), 115.5 (C$_{PMP}$), 114.6 (C$_{PMP}$), 114.5 (C$_{PMP}$), 101.6 (C-1$_{GalN}$, J_{CH} = 162.0 Hz), 101.4 (C-1$_{GalN}$, J_{CH} = 162.0 Hz) , 99.9 (4xC-1$_{Ido}$, J_{CH} = 172.0 Hz, C-1$_{GalNAc,}$ J_{CH} = 160.0 Hz), 92.8, 92.5 (2x), 92.5 (Cq, TCA), 76.5 (C-4$_{Ido}$), 73.7 (C$_{Bn}$, C-3$_{GalNAc}$), 73.6 (C-3$_{GalNAc}$), 73.3 (C-4$_{Ido}$), 73.1 (C-5$_{GalNAc}$, C-4$_{Ido}$), 72.8 (C-2$_{GalNAc}$), 72.5 (C$_{Bn}$), 72.3, 72.2 (C-3$_{Ido}$), 69.9 (OCH$_{2Linker}$), 69.6, 69.4 (C-4$_{GalNAc}$), 68.8, 68.5, 68.4, 68.3, 68.2 (C-6$_{GalNAc}$, C-2$_{Ido}$), 67.9 (C-6$_{Ido}$), 67.7 (C-2$_{Ido}$), 66.9 (CH_2Ph$_{Carba}$, C-5$_{Ido}$), 66.1 (CH_2Ph$_{Ac}$, C-2$_{Ido}$), 56.0, 55.9, 55.8 (CH$_{3PMP}$), 50.6, 50.4 (CH_2PhN), 47.3, 46.3 (NCH$_{2Linker}$), 29.4, 29.4, 29.2, 29.1, 28.0, 27.4, 23.3, 22.8, 21.1, 21.0, 20.4, 20.3, 20.2 ppm; HRMS (ESI) *m/z:* [M+2Na]$^{2+}$ calcd for $C_{186}H_{203}Cl_{12}N_5O_{57}Na_2$ 1941.9593, found 1941.9615.

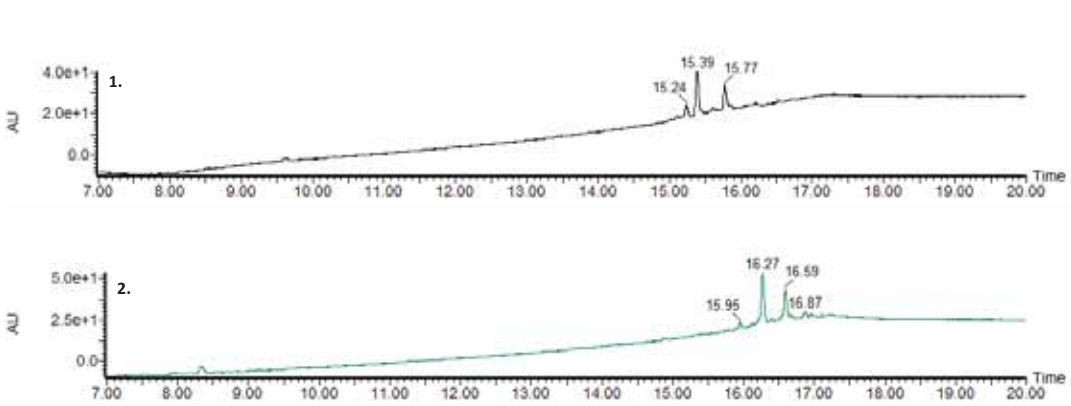


Figure 5.25. UPLC-MS data for the synthesis of octasaccharide **201**. **1)** chromatogram after quantitative cleavage (unidentified peak, t_r = 15.24 min, *m/z* 1808.47; as **200** (t_r = 15.39 min, *m/z* calcd for $C_{168}H_{185}Cl_{12}N_5O_{48}$ $[M+2NH_4]^{2+}$ 1747.95 found 1747.97); as **200+Bn** (t_r = 15.77 min, *m/z* calcd for $C_{175}H_{191}Cl_{12}N_5O_{48}$ $[M+2NH_4]^{2+}$ 1792.98 found 1792.95). **2)** chromatogram after acetylation (unidentified peak, t_r = 15.95 min, *m/z* 1885.64); as **201** (t_r = 16.27 min, *m/z* calcd for $C_{186}H_{203}Cl_{12}N_5O_{57}$ $[M+2NH_4]^{2+}$ 1937.01 found 1936.97); as **201+Bn** (t_r = 16.59 min, *m/z* calcd for $C_{193}H_{209}Cl_{12}N_5O_{57}$ $[M+2NH_4]^{2+}$ 1982.02 found 1981.97).

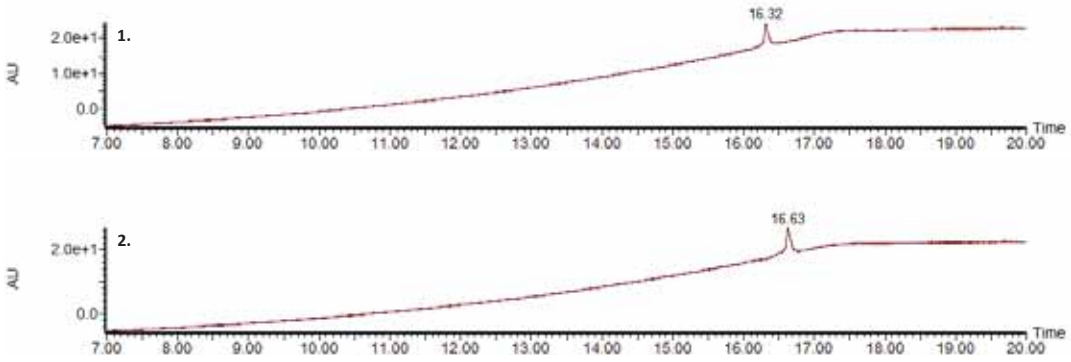


Figure 5.26. UPLC-MS data for the synthesis of octasaccharide **201**. **1)** Chromatogram after preparative HPLC column of pure compound **201**. **2)** Chromatogram after preparative HPLC column of compound **201+Bn**.

5.9.4 Deprotection schemes for DS tetra and octasaccharides.

4-(Acetoxymethyl)benzyl N-benzyl-N-[5-((2,4-di-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl)-(1→3)-(2-acetamido-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl)-(1→3)-2-acetamido-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-β-D-galactopyranosyloxy)pentyl]carbamate (206): The compound **202** (46 mg, 0.022 mmol) was dissolved in dry toluene (0.5 mL) and tributyltinhydride (63 mg, 58 µL) and AIBN (72 µL of a solution 0.06 M) were added at room temperature. The reaction was stirred in the microwave for 4h at 80 °C, additional amount of tributyltinhydride (27 µL) and AIBN (60 µL of a solution 0.06 M) were added and stirred for 2h more. Finally, additional amount of tributyltinhydride (63 mg, 58 µL) and AIBN (72 µL of a solution 0.06 M) were added at room temperature and stirred for 2h more. The reaction crude was washed with hexane (5 x 10mL) to obtain 38 mg of compound 4-(acetoxymethyl)benzyl N-benzyl-N-[5-((2,4-di-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(2-acetamido-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-2-acetamido-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-β-D-galactopyranosyloxy)pentyl]carbamate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.15 (m, 29H, aromatic), 6.89 – 6.83 (m, 4H, aromatic), 6.82 – 6.75 (m, 4H, aromatic), 6.15 (d, *J* = 6.7 Hz, NH), 6.02 (d, *J* = 7.3 Hz, NH), 5.65 (d, *J* = 7.3 Hz, NH), 5.51 (dd, *J* = 12.4, 3.0 Hz, 2H, H-4_{GalNAc}), 5.18 – 5.05 (m, 5H, CH_2-$Ph_{Bz,}$ CH_2-Ph_{carba}, H-1_{GalNAc}), 5.01 (bs, 1H, H-4_{Ido2}), 4.99 – 4.89 (m, 2H, H-1_{GalNAc}, H-1_{Ido}), 4.85 – 4.81 (m, 2H, H-2_{Ido}, H-2_{Ido}), 4.79 (bs, 1H, H-1_{GalNac}), 4.74 – 4.71 (m, 1H, H-5_{Ido2}), 4.67 – 4.44 (m, 12H, CH_2Ph, CH_2-PhN, 1HCH_2Ph, H-3_{GalNac}, H-5_{Ido}), 4.38 – 4.35 (d, *J* = 10.3 Hz, 1H, CH_2Ph), 4.17 – 4.08 (m, 3H, H-6_{Ido}, H-4_{Ido}), 3.99 – 3.94 (m, 2H, H-6_{GalNac}), 3.91 – 3.85 (m, 1H, $OCH_{2Linker}$), 3.84 – 3.80 (t, *J* = 6.1Hz, 1H, H-5_{GalNac}), 3.77 – 3.66 (m, 9H, 2xH-3Ido, H-5_{GalNac}, 2xCH_{3PMP}), 3.53 – 3.46 (m, 2H, H-6_{GalNac}), 3.45 – 3.13 (m, 7H, H-2_{GalNac}, $OCH_{2Linker}$, H-6_{GalNac}, $NCH_{2Linker}$), 2.09 (s, 3H, CH_{3Ac}), 2.06 – 1.98 (3s, 9H, CH_{3Ac}), 2.50 –2.43 (bs, 3H, CH_3NH_{Ac}), 1.76 (s, 3H, CH_{3Ac}, 1.73 – 1.68 (bs, 3H, CH_{3NHAc}), 1.61 (s, 3H, CH_{3Ac}), 1.57 –1.45 (m, 4H, $CH_{2Linker}$), 1.38 – 1.27 (m, 2H, $CH_{2Linker}$) ppm. This compound (0.015g, 78µmol) was dissolved in acetonitrile/water (4/1, 1 mL) and CAN (5eq x OH, 0.043 g) was added at 0 °C and was stirred for 30 minutes. The crude was diluted with EtOAc and was washed with water and brine. The organic phase was dried

over $MgSO_4$ and concentrated. The crude was purified by preparative HPLC (gradient: initial - 5min: 60% water, 40% acetonitrile; 5 – 45 min. (30% to 70% of ACN); 45 min – 55 min. (70% to 99% of acetonitrile) to obtain **206** (6 mg, 54%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.15 (m, 29H, aromatic), 6.09 (d, *J* = 7.4 Hz, NH, 0.45H), 5.93 (d, *J* = 6.8 Hz, NH, 0.73H), 5.46 – 5.43 (dd, *J* = 12.1, 3.4 Hz, 2H, H-4_{GalNAc}), 5.17 – 5.08 (m, 5H, CH_2-$Ph_{Bz,}$ CH_2-Ph_{carba}, H-1_{GalNAc}), 4.98 – 4.85 (m, 4H, H-1 $_{GalNAc}$, H-1_{Ido1}, H-2_{Ido2}, H-4_{Ido2}), 4.84 – 4.78 (m, 2H, H-2_{Ido1}, H-1_{Ido2}), 4.65 – 4.40 (m, 11H, 2xH-3_{GalNAc}, CH_2-PhN, 3xCH_2Ph, CH_2Ph), 4.37 – 4.32 (d, *J* = 11.5Hz, 1H, CH_2Ph), 4.29 – 4.25 (m, 1H, H-5_{Ido2}), 4.18 – 4.13 (m, 1H, H-5_{Ido1}), 4.06 – 4.02 (bs, 1H, H-3_{Ido1}), 3.92 – 3.55 (m, 9H, 2xH-5_{GalNAc}, H-3_{Ido2}, H-4_{Ido1}, 2xH-6_{Ido1}, $OCH_{2Linker}$), 3.52 – 3.40 (m, 3H, $OCH_{2Linker}$, H-6_{GalNac}), 3.38 – 3.14 (m, 6H, H-6 $_{GalNac}$, 2xH-2_{GalNac}, $NCH_{2Linker}$), 2.79 – 2.62 (bs, 1H, OH), 2.58 – 2.45 (bs, 1H, OH), 2.09 – 2.08 (s, 6H, CH_3), 2.05 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.91 – 1.85 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.58 – 1.46 (m, 4H, $CH_{2Linker}$), 1.33 – 1.28 (m, 2H, $CH_{2Linker}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.86, 171.32, 170.95, 170.64, 169.96, 169.70, 156.29, 155.81, 138.34, 137.94, 137.75, 136.92, 135.82, 128.71, 128.59, 128.52, 128.49, 128.37, 128.14, 128.07, 128.03, 127.96, 127.93, 127.90, 127.83, 127.49, 127.28, 101.41 (C-1_{GalNac}, J_{CH} = 164 Hz), 100.86 (C-1_{Ido}, J_{CH} = 171 Hz), 100.36 (C-1_{Ido}, J_{CH} = 171 Hz), 99.7 (C-1_{GalNac}, J_{CH} = 165 Hz), 76.64 (C-4_{Ido}), 75.76, 75.31(C-3_{GalNAc}), 73.70 (C_{Bn}), 73.29 (C-4_{Ido}), 72.67, 72.56 (C-5_{GalNac}, C-3_{Ido}), 72.42 (C-3_{Ido}), 72.26 (C_{Bn}), 70.43, 70.31 (C-4_{GalNac}), 70.11 ($OCH_{2Linker}$), 68.76, 68.60 (C-6_{GalNac}), 67.81, 67.72 (C-2_{Ido}), 67.52 (C-4_{Ido2}), 67.30 (C-5_{Ido}), 66.94 (CH_2-Ph_{carba}), 66.61 (C-5_{Ido}), 66.10 (CH_2-Ph_{Bz}), 62.17, 62.07 (C-6_{Ido}), 56.13 (C-2_{GalNac}), 50.46 (CH_2-PhN), 47.51 ($NCH_{2Linker}$), 29.84, 29.23, 27.97, 27.43 ($CH_{2Linker}$), 23.66 (CH_3), 23.57(CH_3), 23.25 ($CH_{2Linker}$), 21.30, 21.15, 21.08, 21.04, 20.74, 20.42 (CH_3) ppm.

4-(Acetoxymethyl)benzyl N-benzyl-N-[5-((2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-idopyranosyl)-(1→3)-(2-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-acetamido-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-

galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate (210): The compound **201** (28 mg, 0.0062 mmol) was dissolved in toluene (0.5 mL), tributyltin hydride (0.094 mmol, 25 µL) and catalytic amount of AIBN (42 µL of a 30 mM stock solution in toluene) were added. The reaction was heated in the microwave at 80 °C for 2h adding more amount of tributyltin hydride until no intermediates were observed by UPLC-MS. The reaction mixture was concentrated and was stirred with hexane for 1h. The product was filtered and dried in the vacuum pump (22 mg, 90%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.15 (m, 54H, aromatic), 6.92 – 6.84 (m, 6H, aromatic$_{PMP}$, 2xNH) 6.88 – 6.76 (m, 10H, aromatic$_{PMP}$), 6.04 (d, *J* = 7.1Hz, NH), 5.74 – 5.58 (m, 3H, NH), 5.52 – 5.46 (m, 4H, 4xH-4$_{GalNAc}$), 5.18 – 5.08 (m, CH_2-Ph$_{Carba}$, CH_2-Ph$_{Ac}$), 5.03 – 4.89 (m, 5H, 4H-1$_{GalNAc}$, H-1$_{Ido}$), 4.88 – 4.87 (m, 1H, H-2$_{Idocapp}$), 4.84 – 4.74 (m, 6H, 3xH-2$_{Ido}$, 3xH-1$_{Ido}$), 4.61 – 4.33 (m, 28H, CH_2PhN, 9xCH_2Bn, 4xH-5$_{Ido}$, 4xH-3$_{GalNAc}$), 4.15 – 4.09 (m, 7H, 3xH-3$_{Ido}$, 4xH-6$_{Ido}$), 3.99 – 3.85 (m, 5H, 4xH-6$_{Ido}$, OCH$_{2Linker}$), 3.84 – 3.80 (m, 1H, H-5$_{GalNac}$), 3.76 – 3.68 (m, 20H, CH_{3PMP}, 3xH-4$_{Ido}$, 3xH-5$_{GalNAc}$, H-3$_{Idocapp}$), 3.62 – 3.59 (m, 1H, H-4$_{Idocapp}$), 3.50 – 3.48 (m, 2H, H-6$_{GalNAc}$), 3.46 – 3.14 (m, 13H, 3xH-6$_{GalNac}$, 4xH-2$_{GalNAc}$, CH_2N_{Linker}, OCH$_{2Linker}$), 2.09 (s, 3H, CH_3), 2.04 – 2.02 (m, 12H, 4xCH_3), 1.88 – 1.81 (m, 6H, 2xCH_3), 1.76 (s, 3H, CH_3), 1.69 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.65 – 1.63 (m, 9H, 3xCH_3), 1.58 - 1.50 (m, 4H, $CH_{2Linker}$), 1.35 – 1.28 (m, 2H, $CH_{2Linker}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.89, 170.82, 170.20, 169.97, 169.95, 169.77, 154.04, 153.92, 153.17, 153.07, 138.43, 138.39, 137.97, 137.93, 137.87, 137.78, 135.78, 128.69, 128.52, 128.45, 128.34, 128.29, 128.13, 128.08, 127.98, 127.94, 127.87, 127.82, 127.75, 127.46, 127.26 ($C_{aromatic}$), 115.75, 115.69, 114.73, 114.64 ($C_{aromaticPMP}$), 101.43 (C-1$_{GalNAc}$), 100.13 (C-1$_{Ido}$), 99.84 (C-1$_{Ido}$), 75.44 (C-4$_{Ido}$), 74.42, 74.36 (C-3$_{GalNAc}$), 73.88, 73.61 (C_{Bn}), 73.05, 72.97 (C-5$_{GalNAc}$, C-3$_{Ido}$, C-4$_{Ido}$), 72.60 72.54 (C-5$_{GalNAc}$), 72.45, 72.39 (C_{Bn}), 70.08, 70.01, 69.91, 69.85, 69.80 (C-4$_{GalNAc}$, OCH$_{2Linker}$), 69.02 (C-6$_{GalNAc}$), 68.82 (C-6$_{GalNAc}$, C-2$_{Ido}$), 68.21, 68.13, 68.10 (C-2$_{Ido}$), 67.63, 67.29, 67.21 (C-6$_{Ido}$), 66.92 (CH_2-Ph$_{Carba}$), 66.75 (C-5$_{Ido}$), 66.10 (CH_2Ph$_{Ac}$), 65.70, 65.66 (C-5$_{Ido}$), 55.84 (CH_{3PMP}), 55.72 (4xH-2$_{GalNAc}$), 50.59, 50.42 (CH_2PhN), 47.48, 45.70 (NCH$_{2Linker}$), 29.82, 29.25, 27.97, 26.97 ($CH_{2Linker}$), 23.52 (CH_3), 23.38 (CH_3), 23.29 (CH_3, $CH_{2Linker}$), 21.31 (CH_3), 21.26 (CH_3), 21.20 (CH_3), 21.13 (CH_3), 20.55 (CH_3), 20.39 (CH_3), 20.30 (CH_3) ppm. MS (ESI): *m/z* calcd. for $C_{186}H_{215}N_5O_{57}$ $[M+2NH_4]^{2+}$ 1733.2, found: 1732.9. This compound (22 mg, 0.006 mmol) was

dissolved in acetonitrile/ water (4/1, 1.25 mL) and cerium ammonium nitrate (70 mg, 0.128 mmol) was added at 0 °C. The reaction was stirred for 90 min and it was diluted with EtOAc and washed with water, saturated $NaHCO_3$ aq solution and brine. The reaction crude was dried over anhydrous $MgSO_4$ and concentrated. The crude was purified by Sephadex® LH-20 CH_2Cl_2/MeOH (1/1) and preparative HPLC (column: C-18 (diameter, 21.2x250 mm 5 μm); flow rate 10 mL·min^{-1}; eluents: 20mM NH_4CO_3 in water/MeCN; gradient: initial 10% water/90% MeCN; 30 min: 1% water/99% MeCN to obtain compound **210** (6 mg, 32%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.15 (m, 54H, aromatic), 6.08 – 6.06 (d, 0.6H, NH), 5.90 – 5.85 (m, 3H, NH), 5.66 – 5.65 (d, 0.4H, NH), 5.48 – 5.37 (m, 4H, 4xH-4$_{GalNAc}$), 5.20 – 5.03 (m, 7H, CH_2-Ph$_{Carba}$, CH_2-Ph$_{Ac}$, 3xH-1$_{GalNAc}$), 4.98 – 4.75 (m, 9H, H-1$_{GalNAc}$, 4xH-1$_{Ido}$, 4x H-2$_{Ido}$), 4.65 – 4.32 (m, 24H, 4xH-3$_{GalNAc}$, 9xCH_2Ph, CH_2PhN), 4.17 – 4.10 (m, 4H, 4xH-5$_{Ido}$), 4.07 – 4.00 (m, 3H, 3xH-3$_{Ido}$), 3.92 – 3.80 (m, 3H, H-6$_{Ido}$, OCH$_{2Linker}$,H-5$_{GalNAc}$), 3.80 – 3.64 (m, 11H, 1xH-3$_{Ido}$, 3xH-5$_{GalNAc}$, 3x H-6$_{Ido,}$ H-6$_{Ido}$), 3.62 – 3.57 (m, 3H, 3xH-4$_{Ido}$), 3.57 – 3.42 (m, 5H, H-4$_{Ido}$, H-6$_{GalNAc}$, OCH$_{2Linker}$), 3.13 (m, 11H, 4xH-2$_{GalNAc}$, H-6$_{GalNAc}$, OCH$_{2Linker}$, NCH$_{2Linker}$), 2.77 – 2.45 (bs, 4H, OH), 2.09 (s, 3H, CH_3), 2.05 – 2.01 (m, 12H, CH_3), 1.98 (s, 3H, CH_3), 1.96 – 1.92 (2s, 6H, CH_3), 1.91 – 1.78 (m, 6H, CH_3), 1.75 (s, 3H, CH_3), 1.67 – 1.63 (2s, 6H, CH_3Me), 1.58 – 1.42 (m, 4H, CH$_{2Linker}$), 1.36 – 1.28 (m, 2H, CH$_{2Linker}$) ppm; δ^{13}C(126 MHz, $CDCl_3$; selected from HSQCed.): 171.72, 171.55, 170.84, 169.97, 138.29, 137.92, 137.77, 137.67, 128.70 – 127.27 (C$_{aromatic}$), 101.47, 101.42, 101.36 (C-1$_{GalNAc}$), 100.80 (C-1$_{Ido}$), 100.39 (C-1 $_{GalNAc}$), 76.27 (C-4$_{Ido}$), 75.32, 75.22 (C-3$_{GalNAc}$), 74.07 (C-4$_{Ido}$), 73.69 (C$_{Bn}$), 73.40 (C-3$_{Ido}$), 72.90, 72.67, 72.64 (C$_{Bn}$), 72.52 (C-3$_{Ido}$), 72.49 (C-5$_{GalNAc}$), 72.37 (C-5$_{GalNAc}$), 72.28 (C$_{Bn}$), 70.40 (C-4$_{GalNAc}$), 70.24 (C-4$_{GalNAc}$), 70.11 (C-4$_{GalNAc}$, OCH$_{2Linker}$), 69.0 (C-2$_{Ido}$), 68.73, 68.67, 68.60 (C-6$_{GalNAc}$), 68.4 (C-5$_{Ido}$), 67.9, 67.8 (C-2$_{Ido}$), 67.34 (C-5$_{Ido}$), 67.29 (C-5$_{Ido}$), 66.94 (CH_2-Ph$_{Carba}$), 66.10 (CH_2Ph$_{Ac}$), 62.61, 62.14, 62.07, 62.03 (C-6$_{Ido}$), 56.11, 55.98 (C-2$_{GalNAc}$), 50.45 (NCH$_{2Linker}$), 47.49, 46.10 (NCH$_{2Linker}$), 29.49, 29.30, 27.41, 27.24 (CH$_{2Linker}$), 23.61, 23.55 (CH_3), 23.15, 22.82 (CH$_{2Linker}$), 21.25, 21.20, 21.14, 20.73, 20.55, 20.49 (CH_3) ppm. LRMS (ESI): *m/z* calcd. for $C_{158}H_{191}N_5O_{53}$ $[M+2NH_4]^{2+}$ 1521.12, found: 1521.10.

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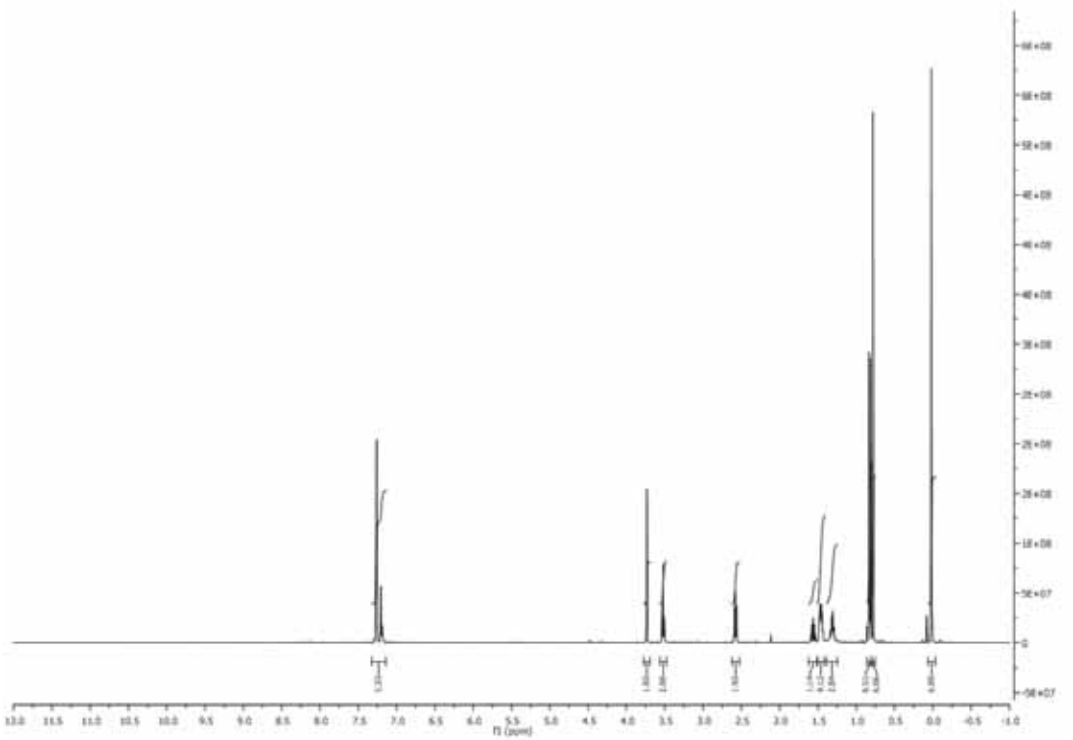
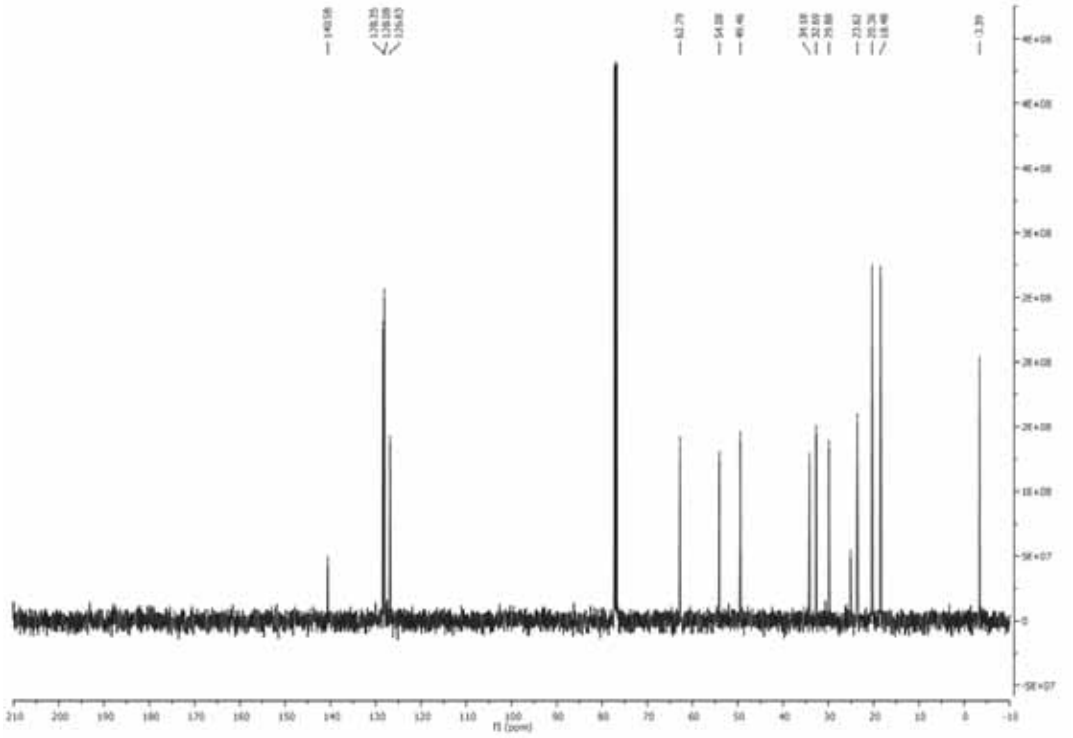
6. APPENDIX

Selected NMR Spectra

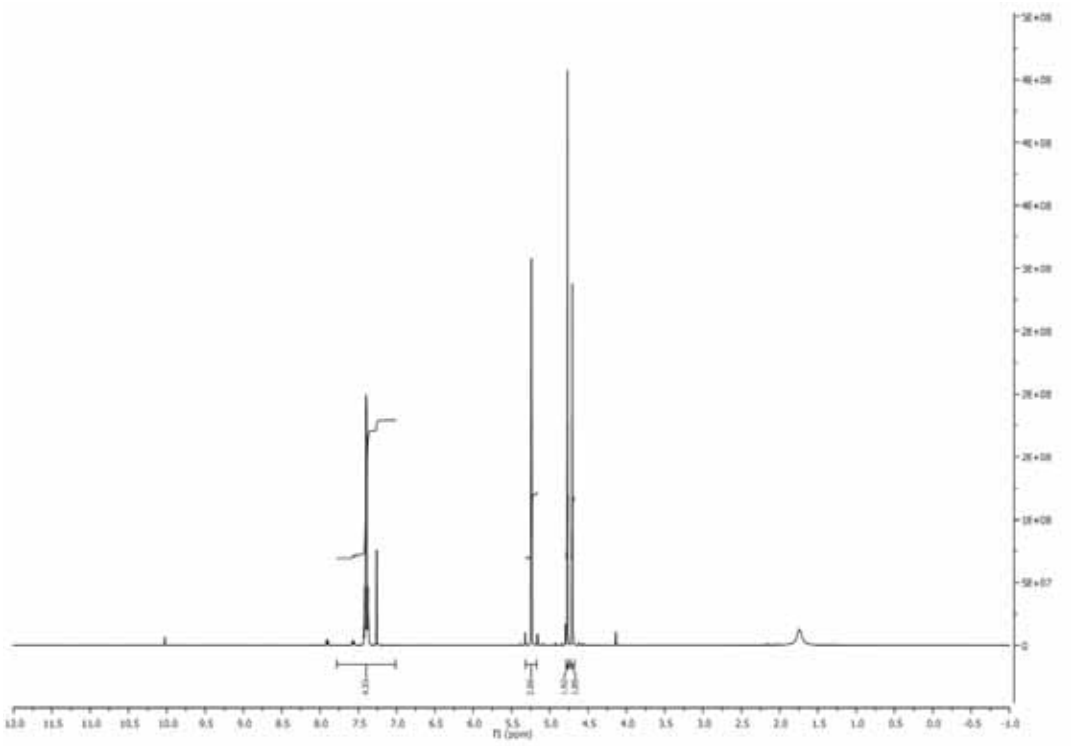
4.5 Linker Synthesis and glycosylation trials on solid support

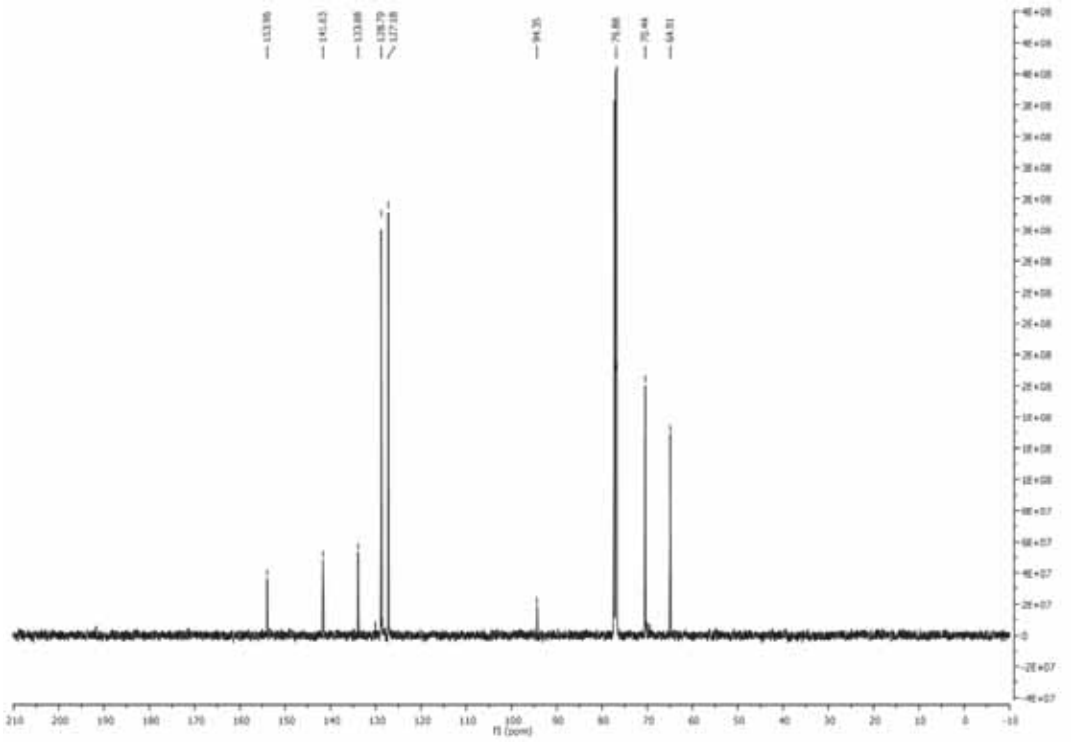
N-Benzyl-5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentan-1-amine (**2**):



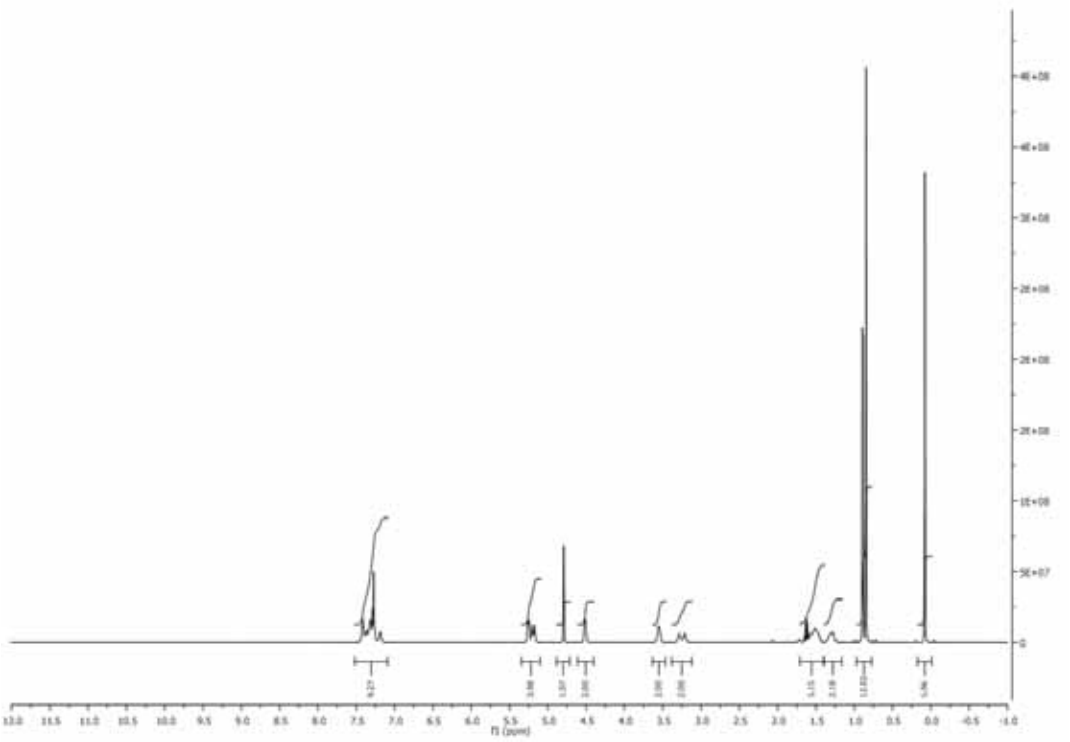


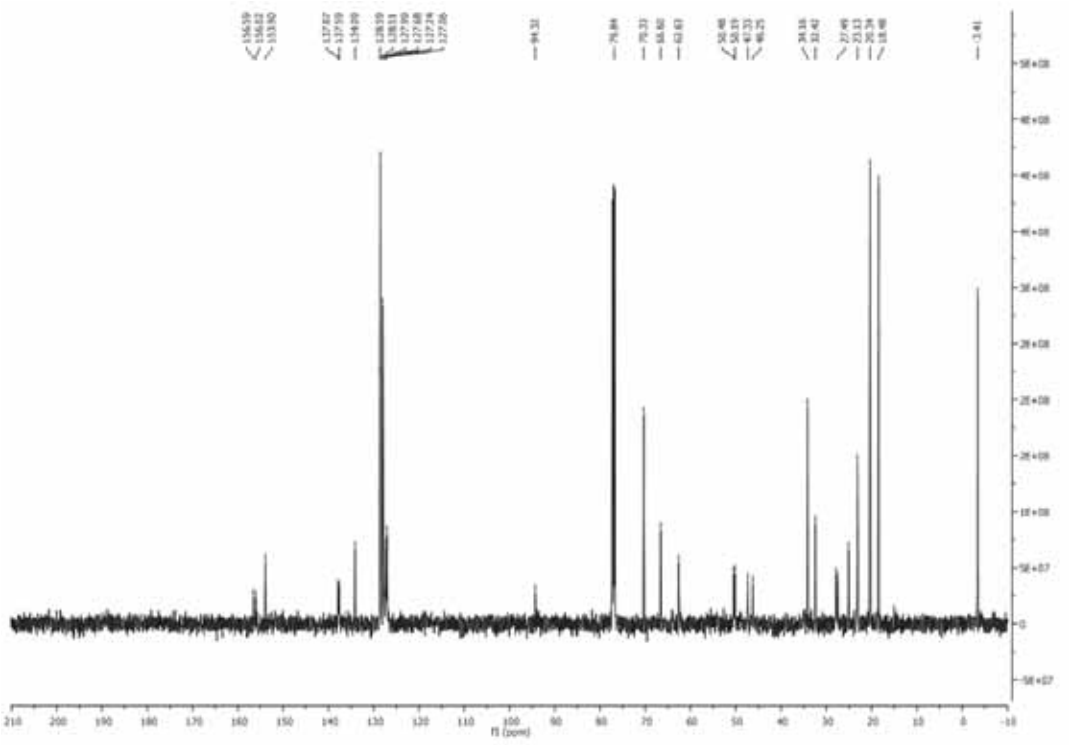
4-(Hydroxymethyl)benzyl 2,2,2-trichloroethyl carbonate (**4**):



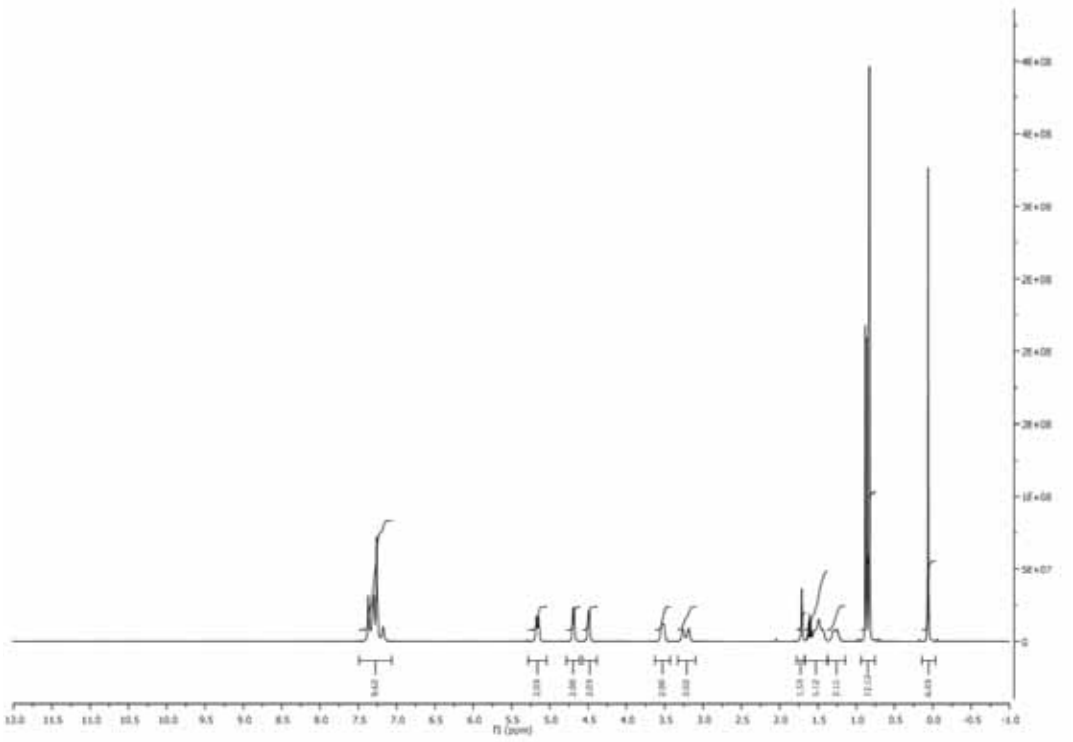


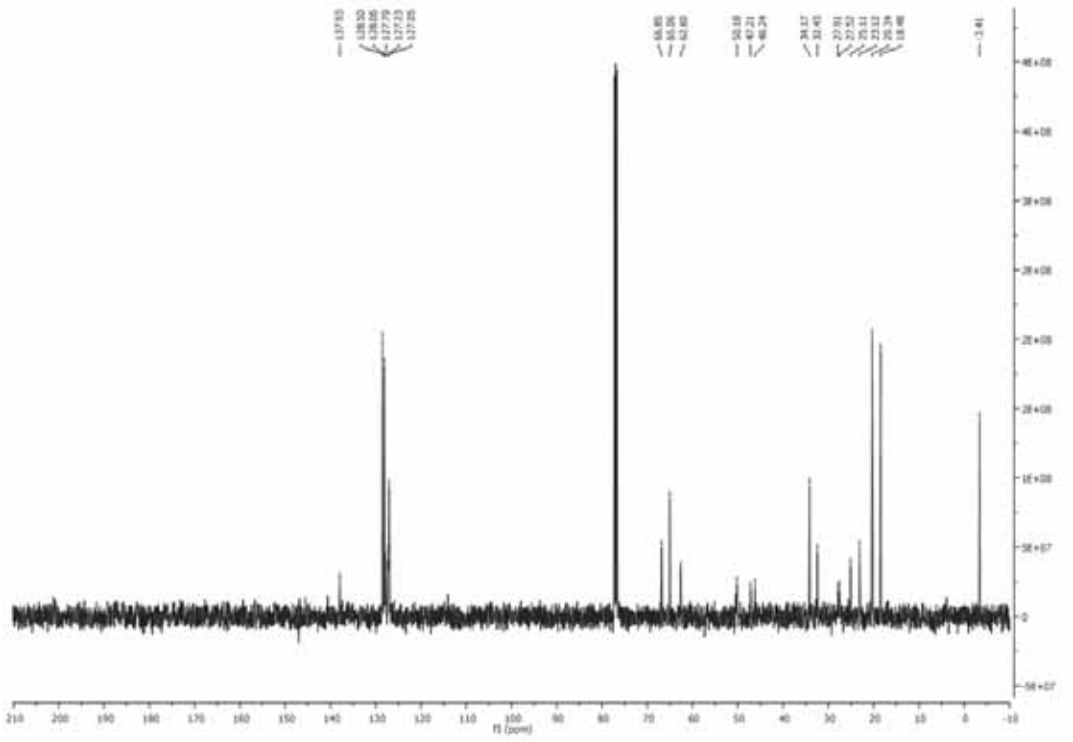
4-(((2,2,2-Trichloroethoxy)carbonyloxy)methyl)benzyl *N*-benzyl *N*-(5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl) carbamate (**6**):



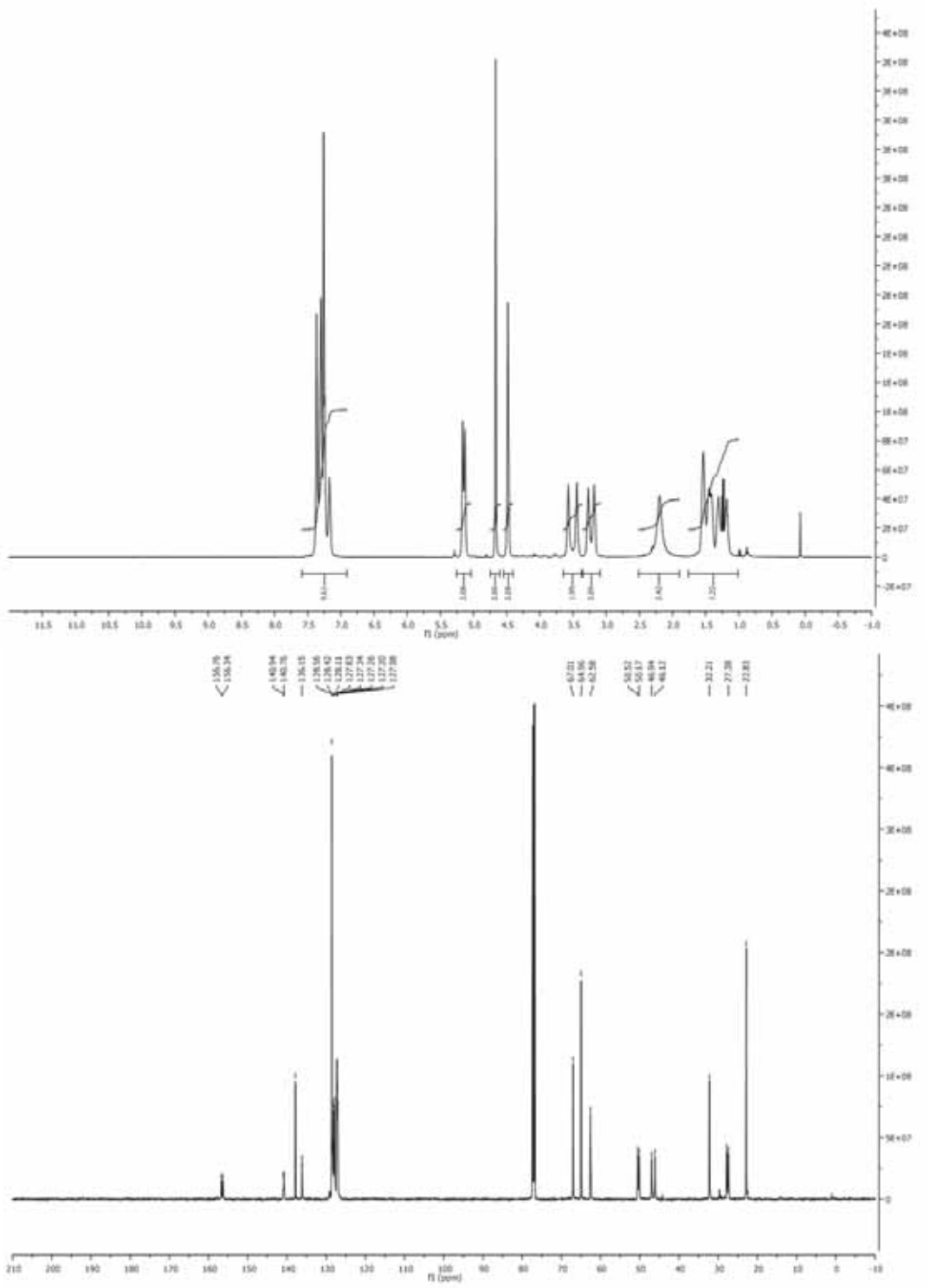


4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl) carbamate (**7**):

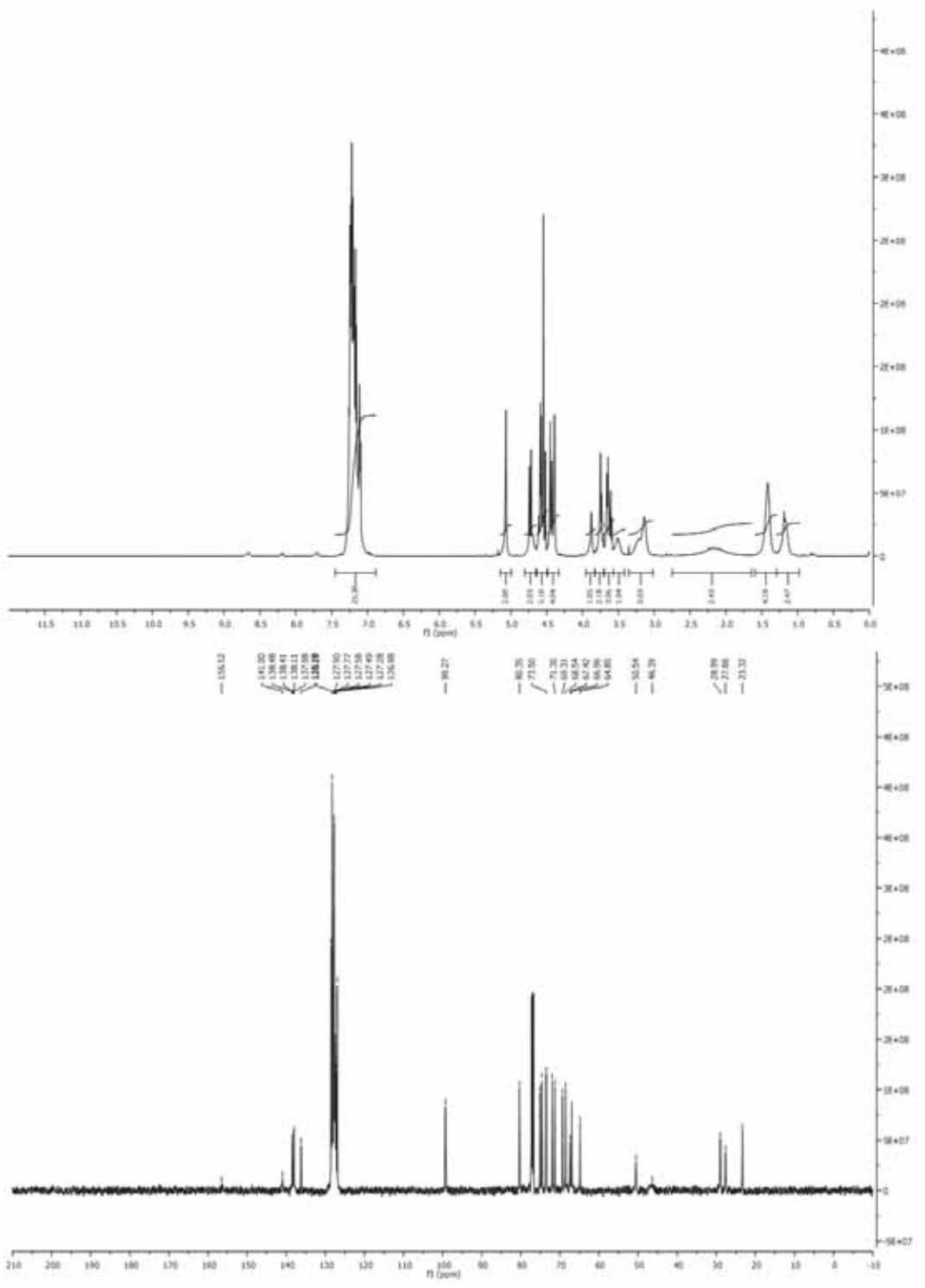




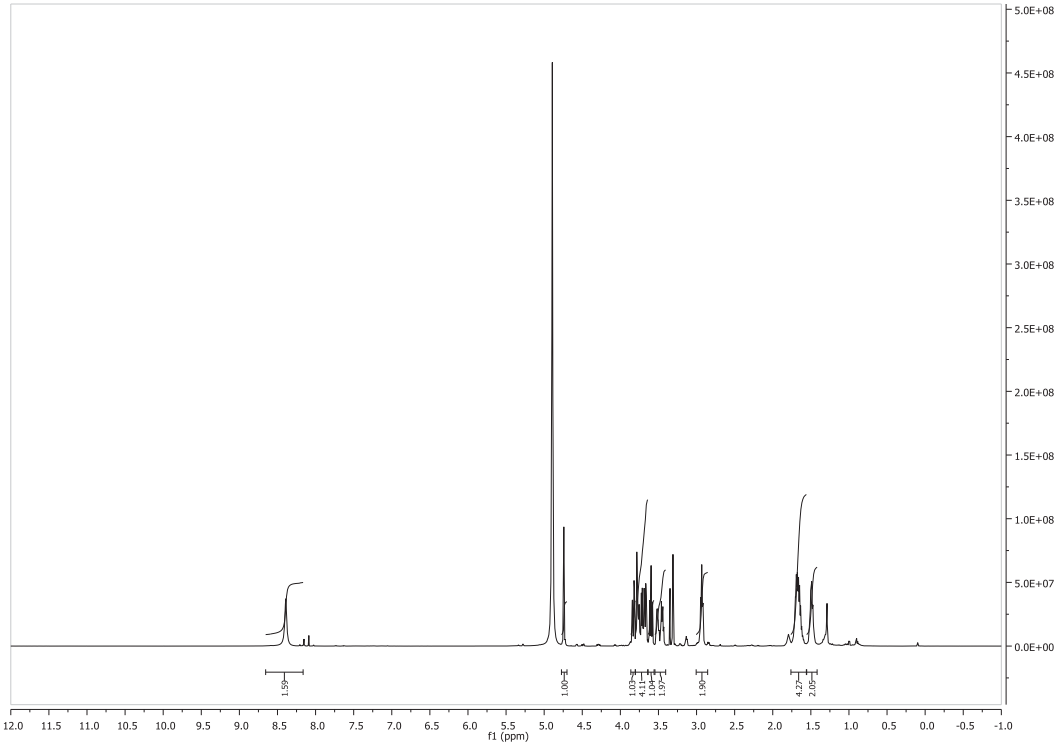
4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-hydroxypentyl) carbamate (10):

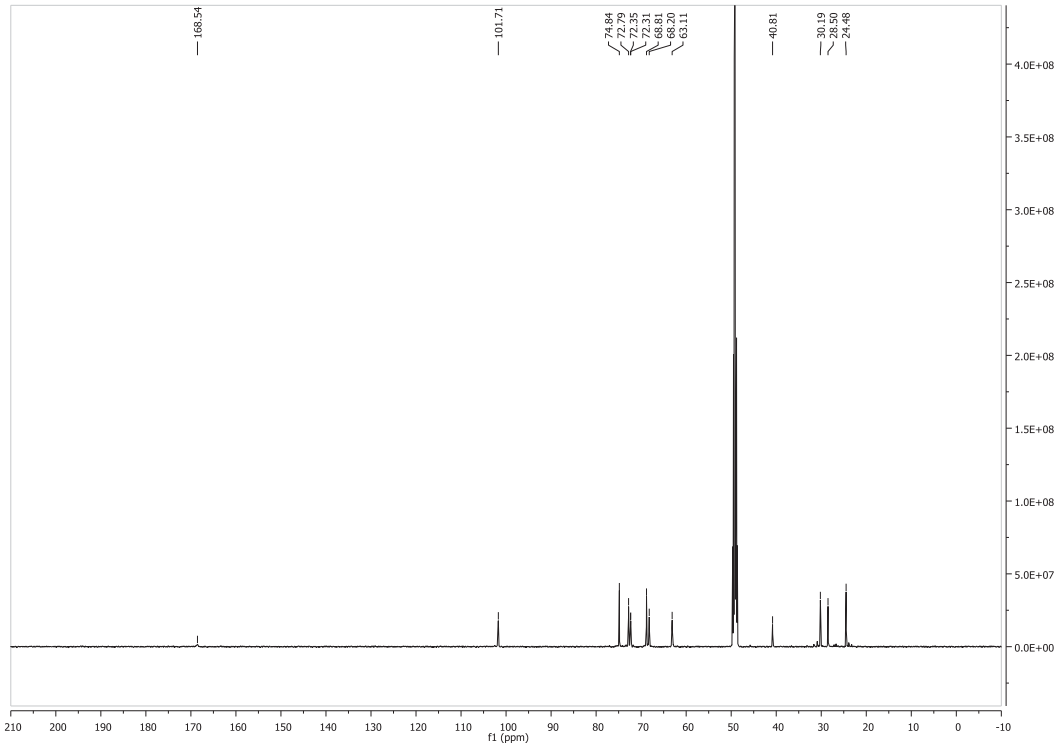


4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyloxy)pentyl) carbamate (**13**):



5-Aminopentyl α-D-mannopyranoside (14):

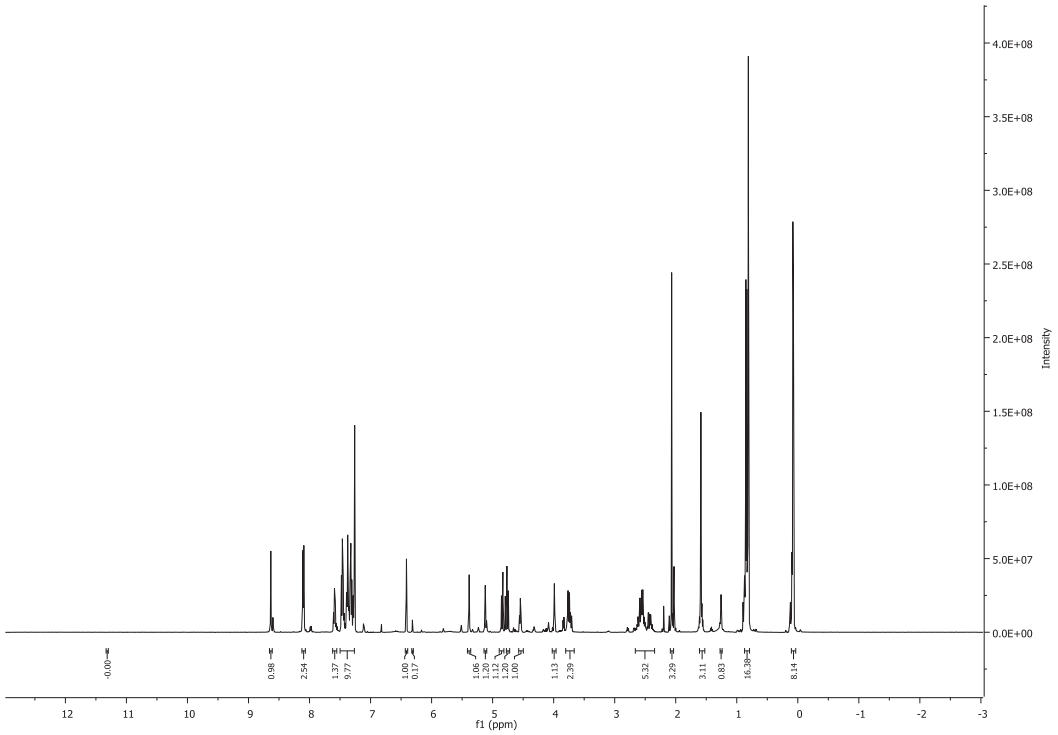




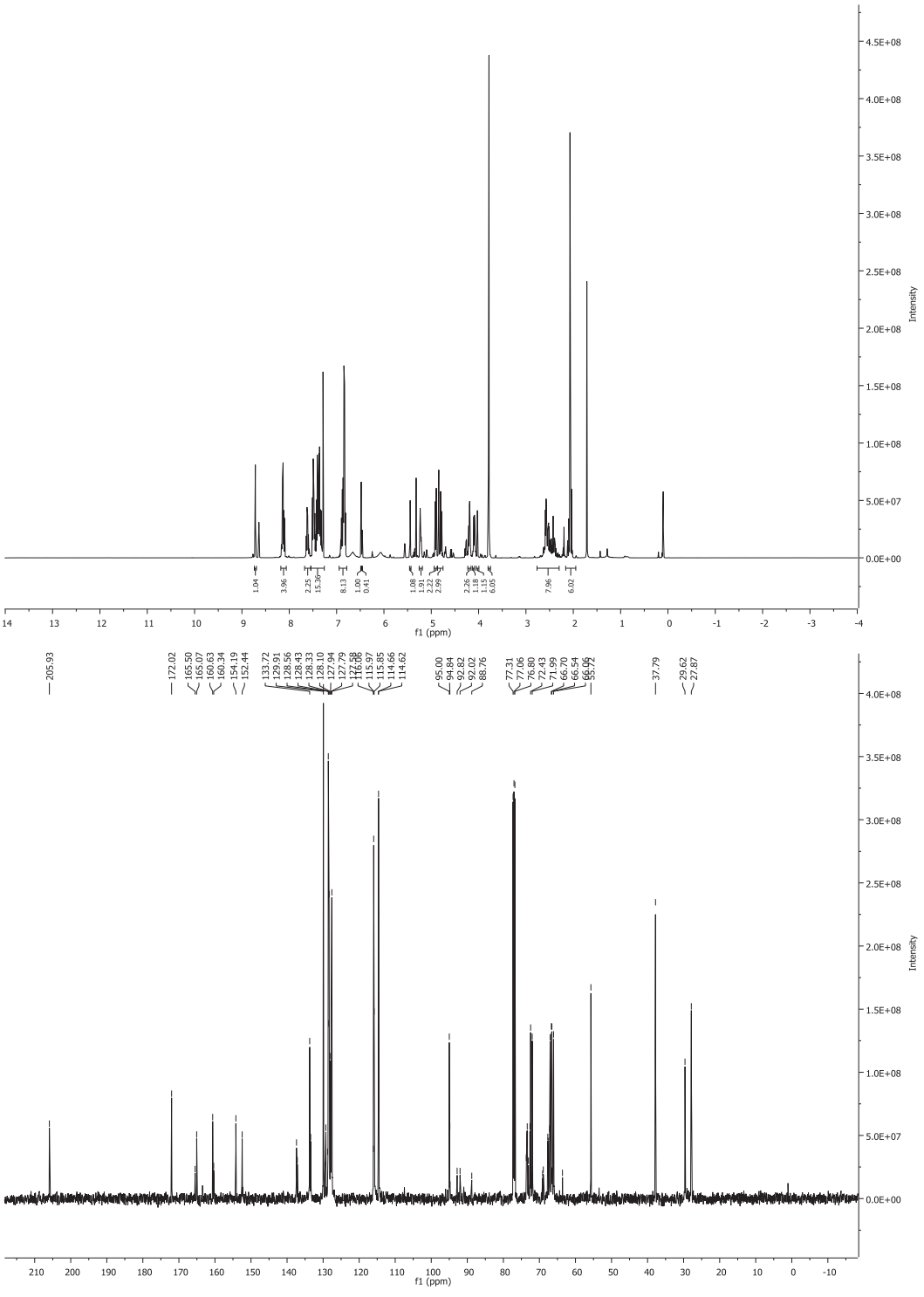
4.6 Building blocks synthesis

4.6.1 Synthesis of Idose Building Block

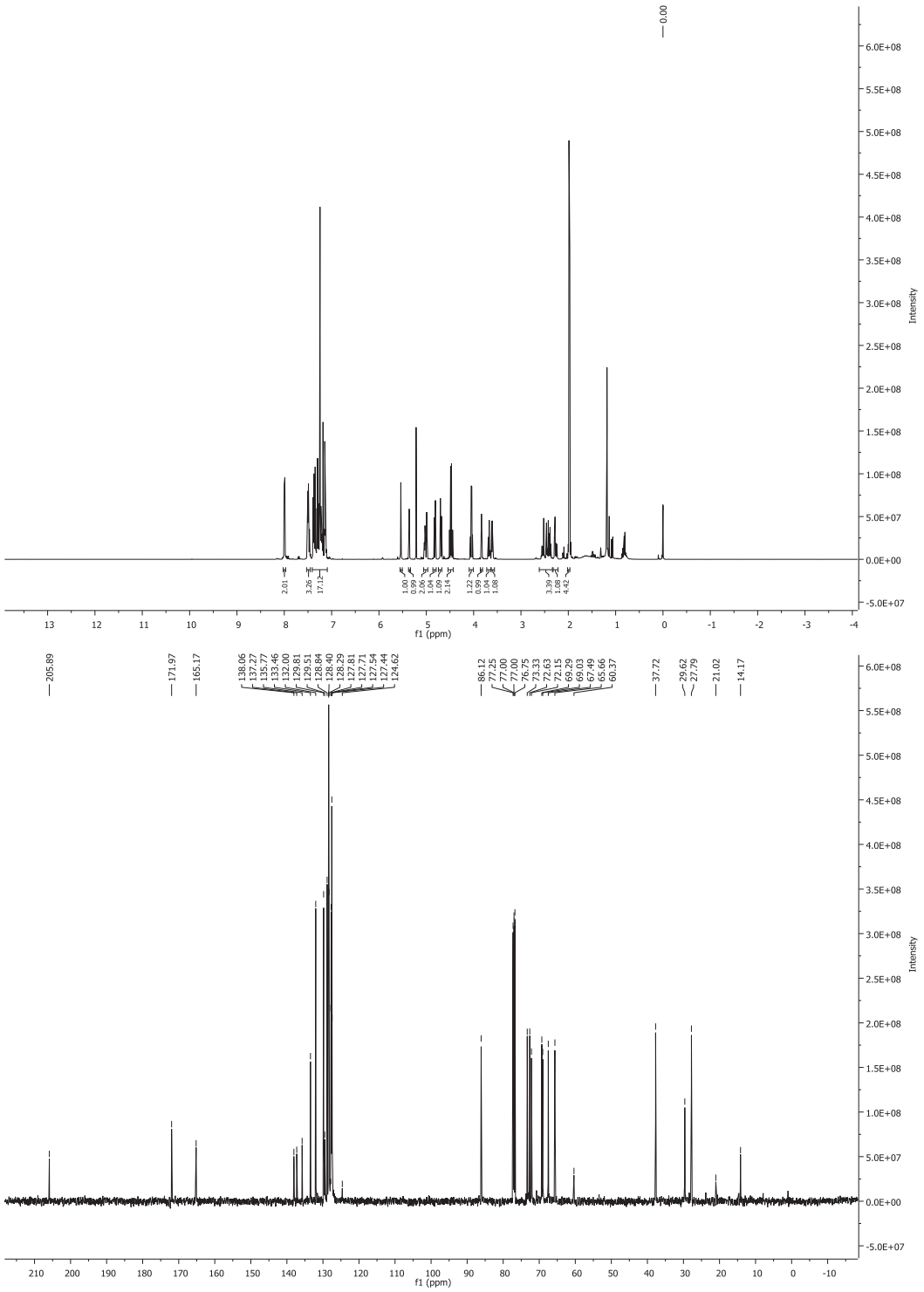
2-*O*-Benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-4-*O*-levulinoyl-α/β-L-idopyranosyl trichloroacetimidate (**29**):



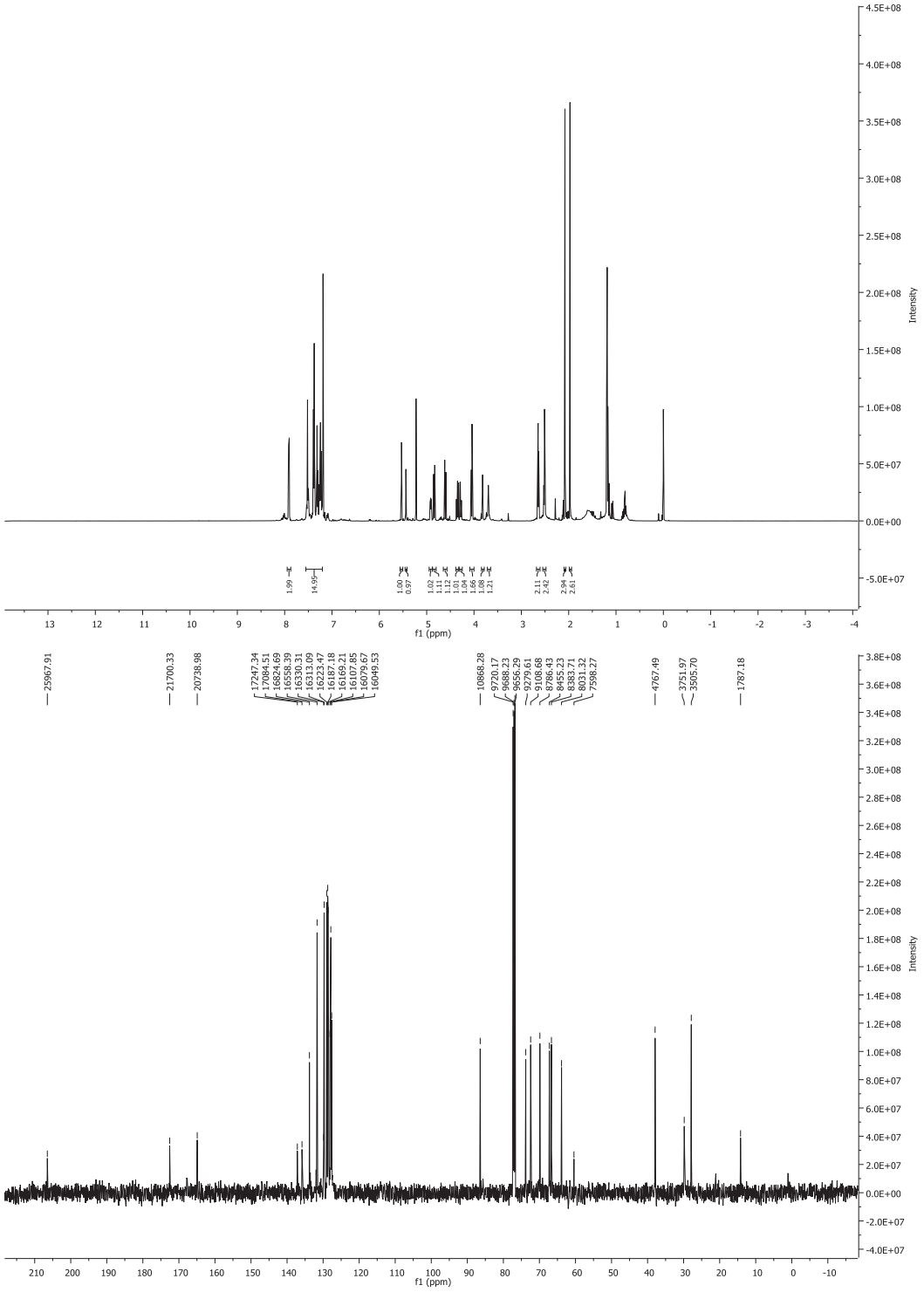
2-*O*-Benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α/β-L-idopyranosyl trichloroacetimidate **(30)**:



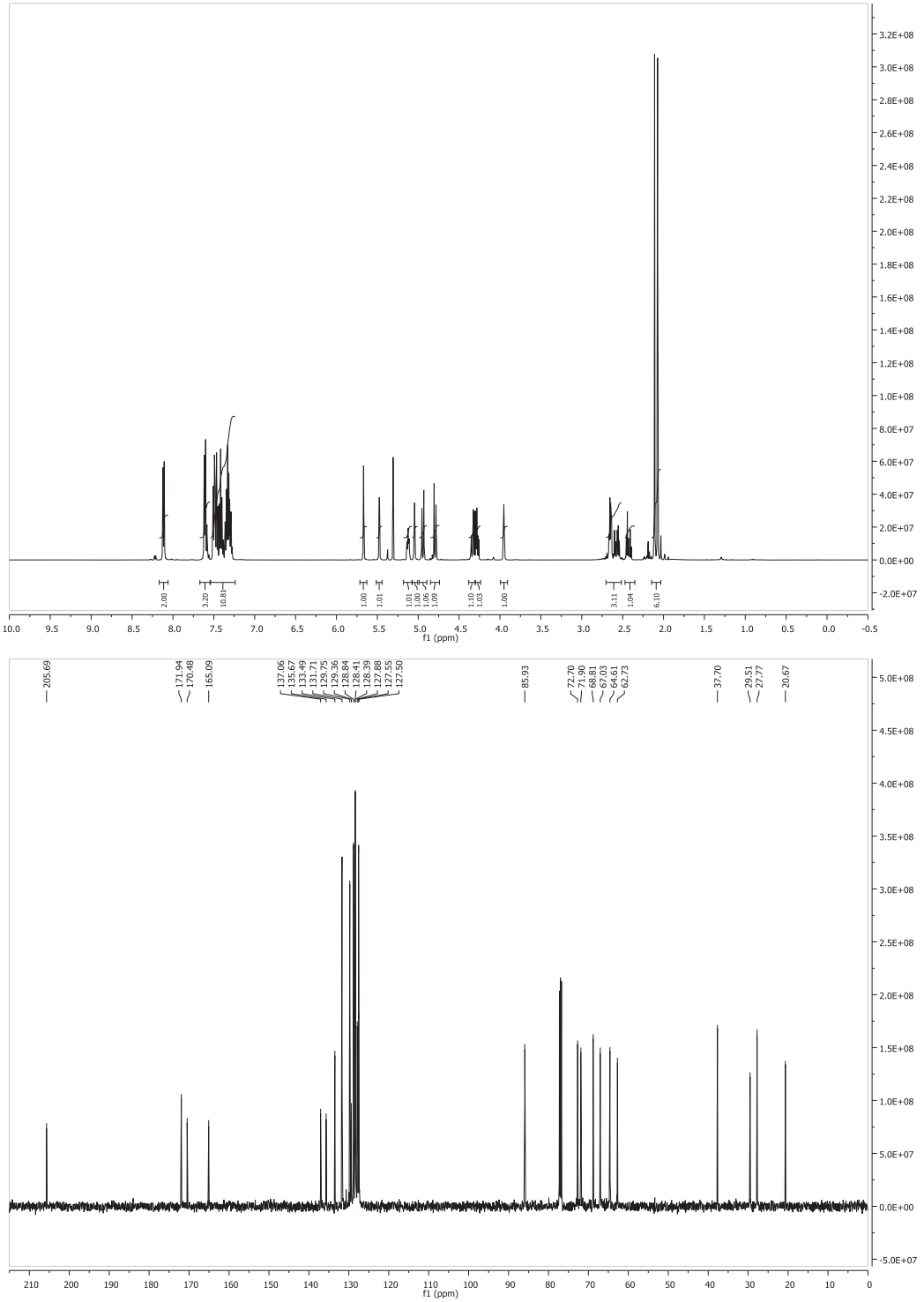
Phenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-levulinoyl-1-thio-α-L-idopyranoside (**35**):



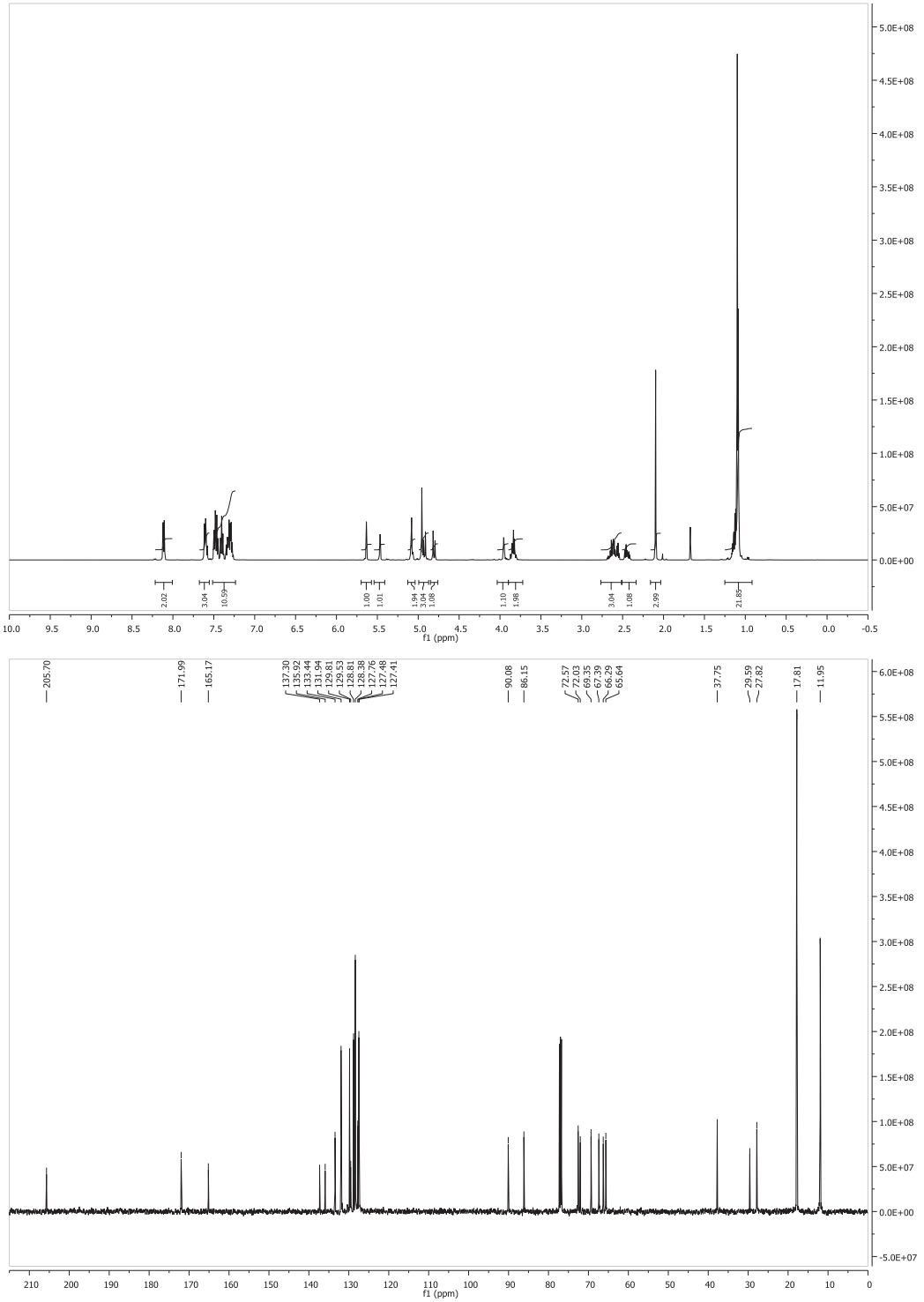
Phenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*para*-methoxybenzyl-1-thio-α-L-idopyranoside (**36**):



Phenyl 6-*O*-acetyl-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-1-thio-α-L-idopyranoside (**37**):

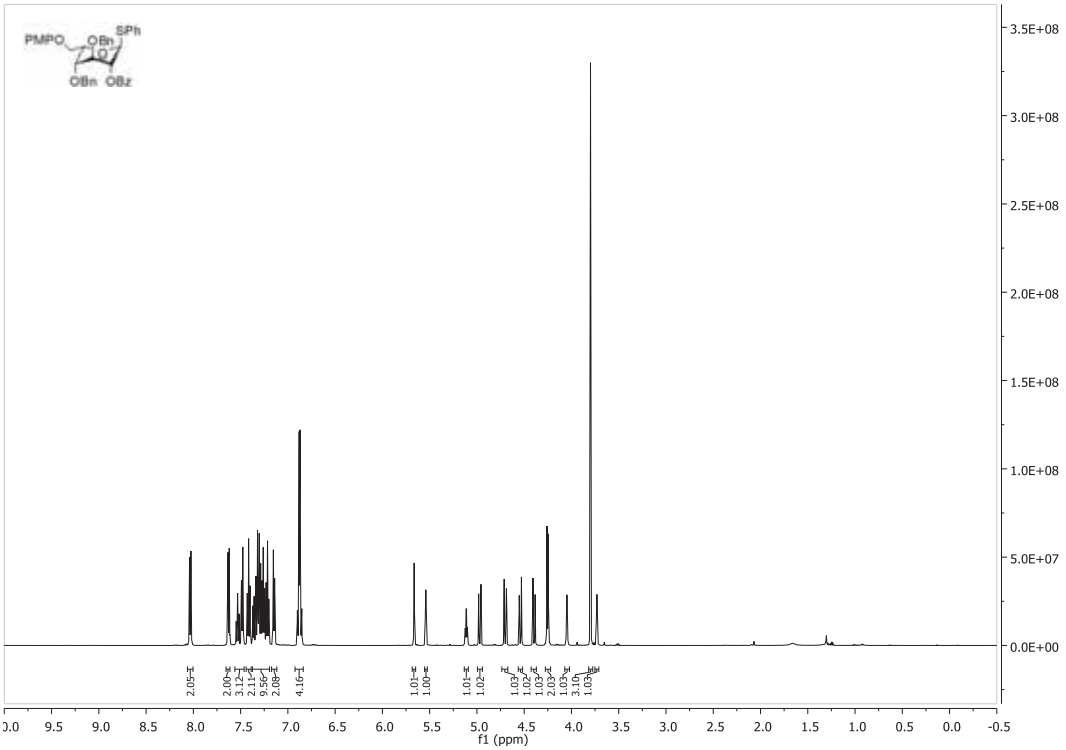


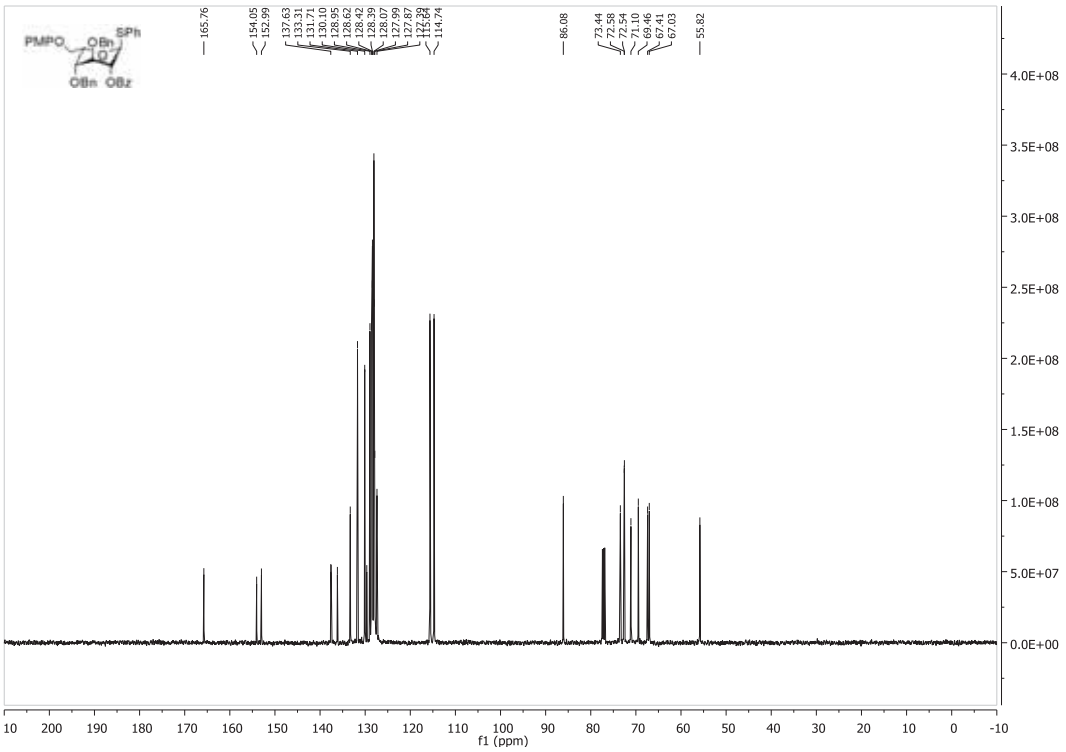
Phenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-1-thio-6-*O*-tri-*iso*-propylsiloxymethyl-α-L-idopyranoside (**38**):



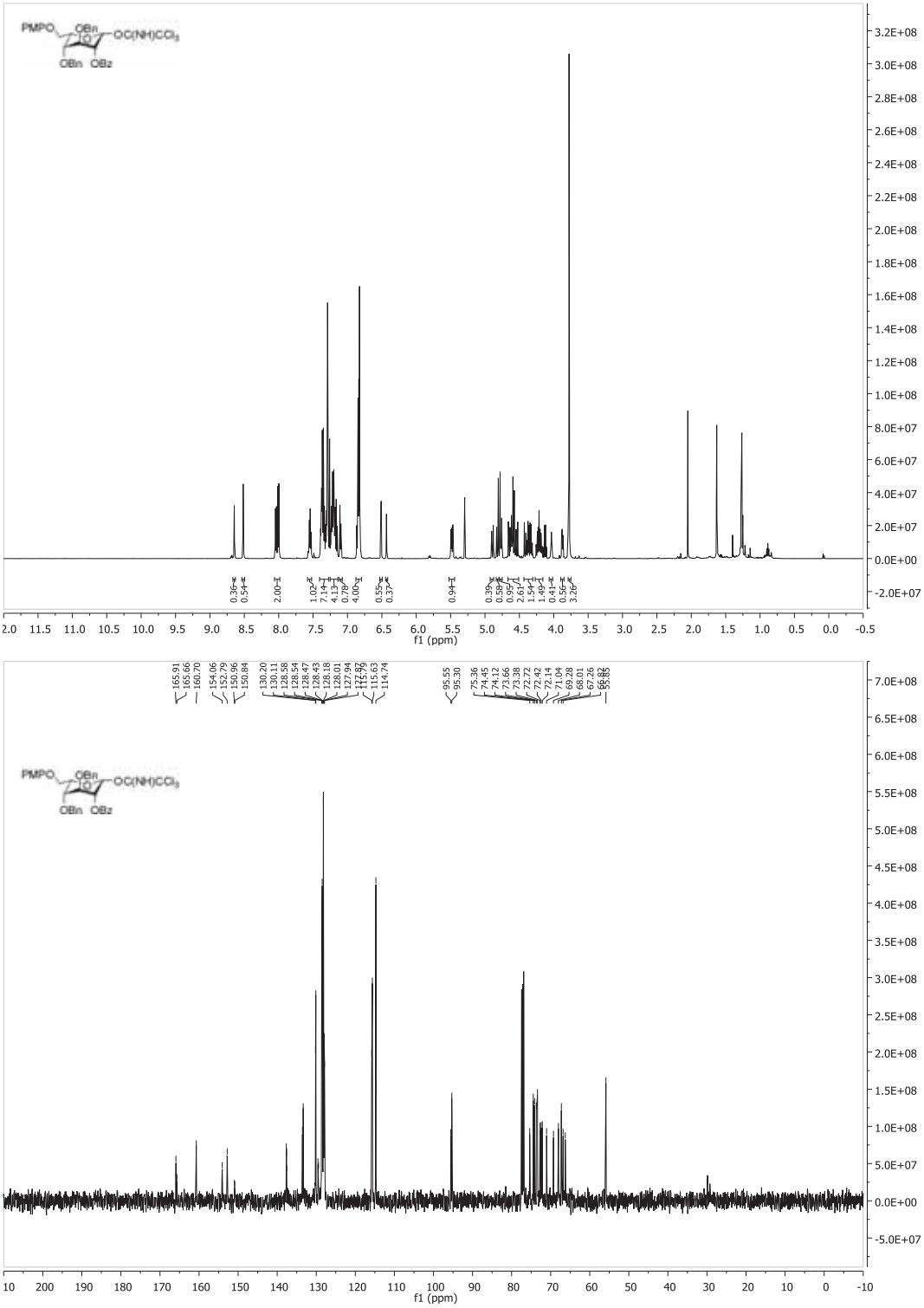
4.6.1.1 Synthesis of the Ido non-reducing end building block

Phenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-1-thio-α-L-idopyranoside (**39**):



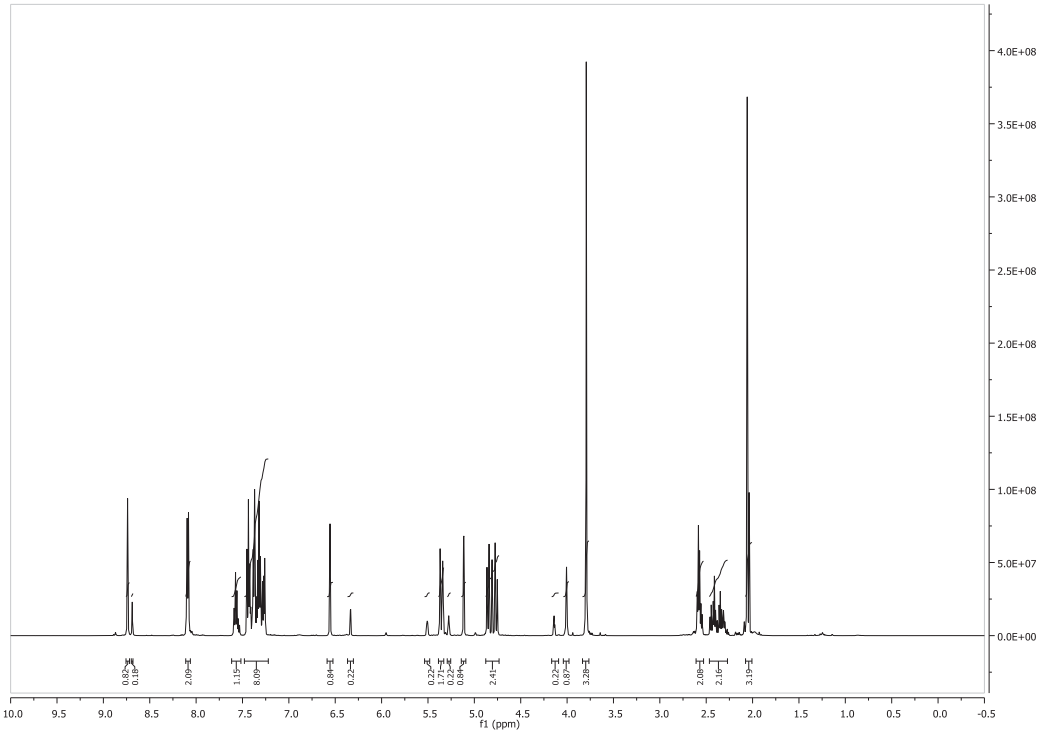


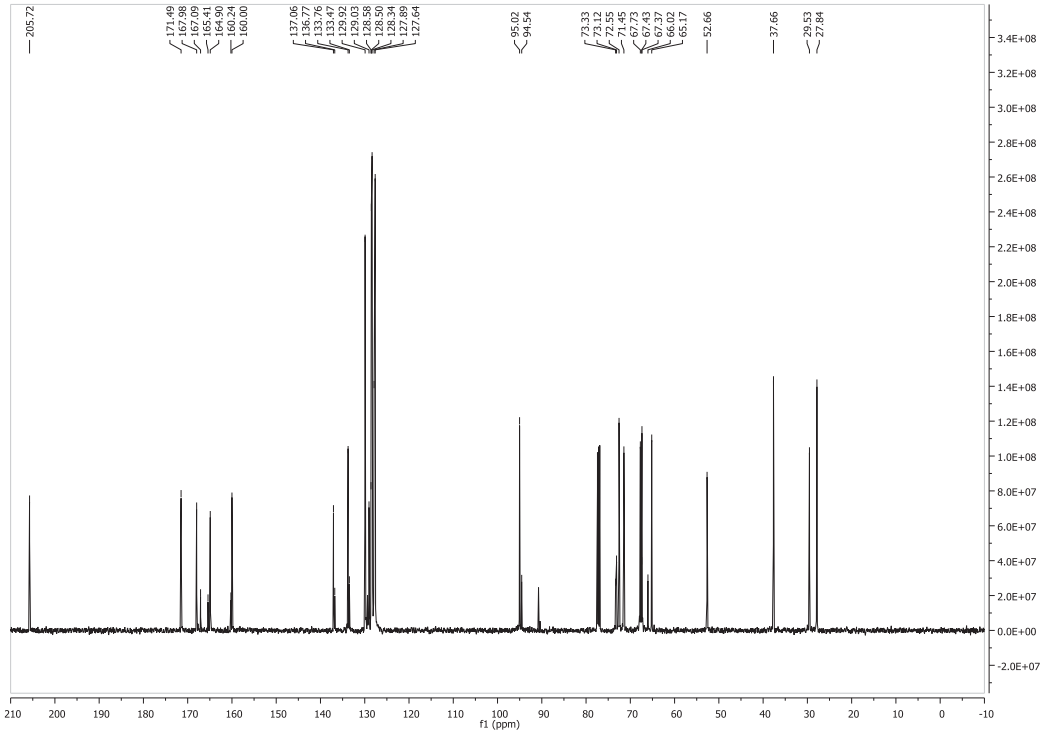
2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α/β-L-idopyranosyl trichloroacetimidate (**41**):



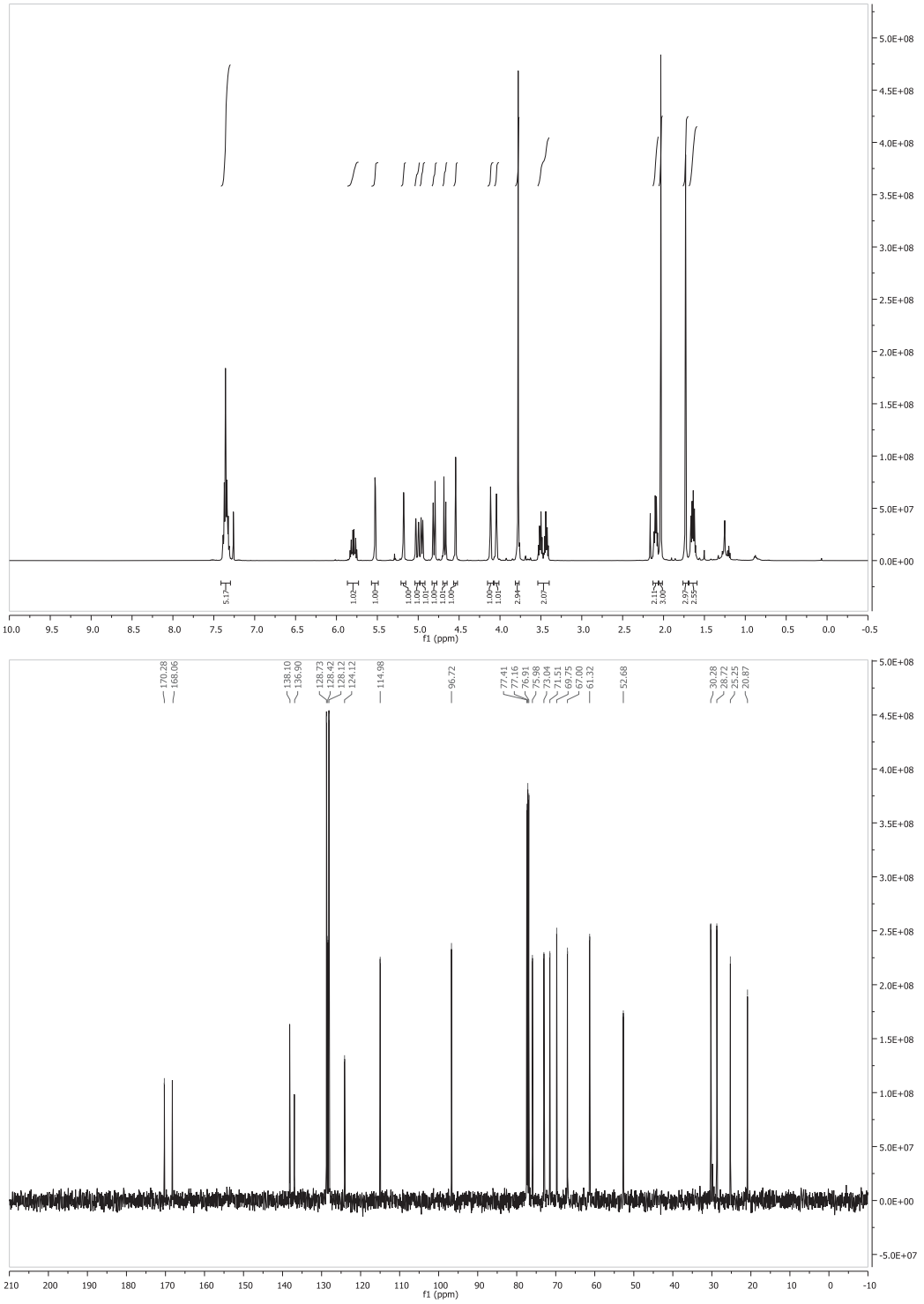
4.6.2 Synthesis of L-iduronic acid (IdoA) building blocks

Methyl (2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α/β-L-idopyranosyluronate) trichloroacetimidate (**44**):

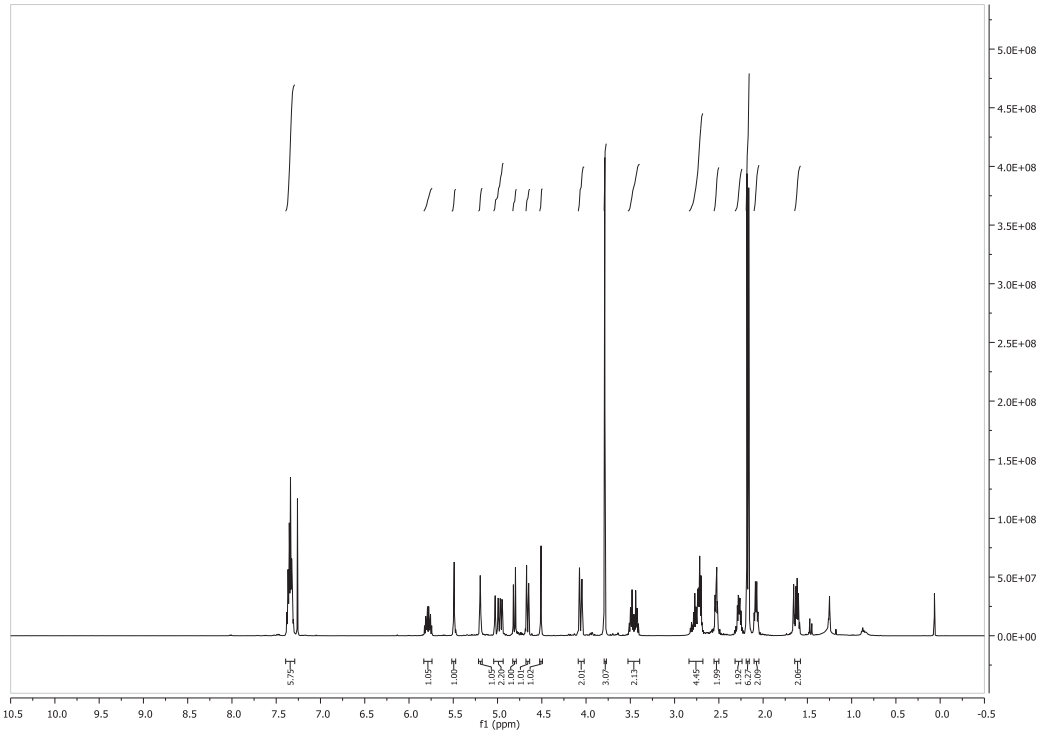


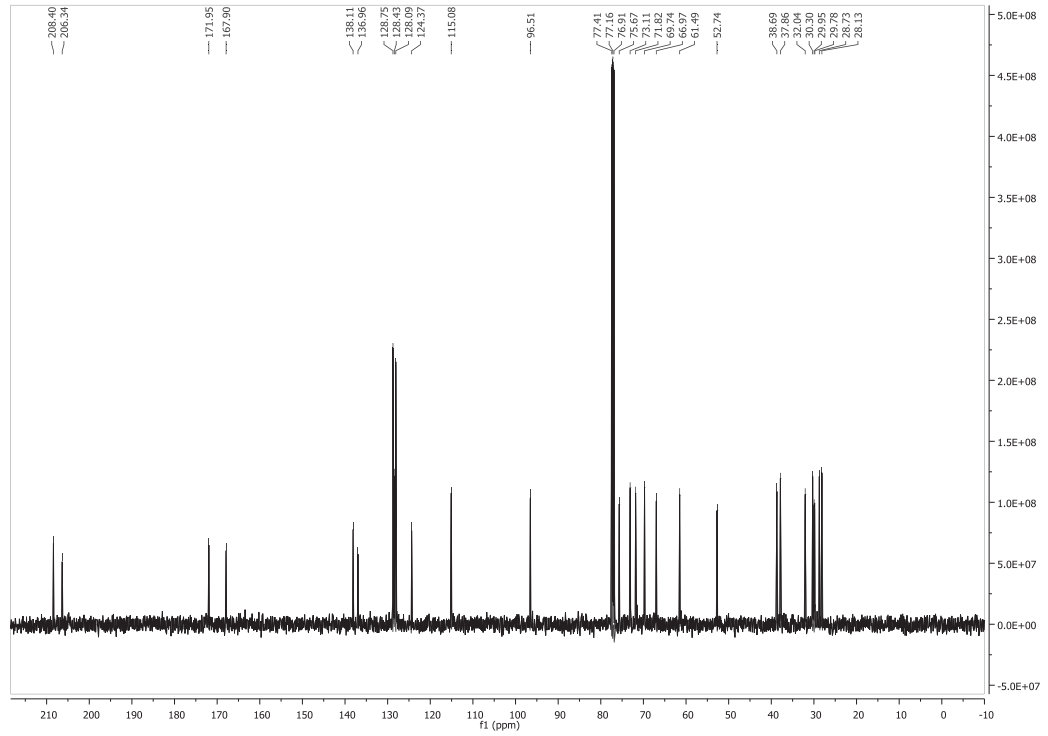


Methyl (4-*O*-acetyl-3-*O*-benzyl-[1,2-*O*-(1-pent-4-enyloxyethylidene)]-β-L-idopyranuronate (**52**):

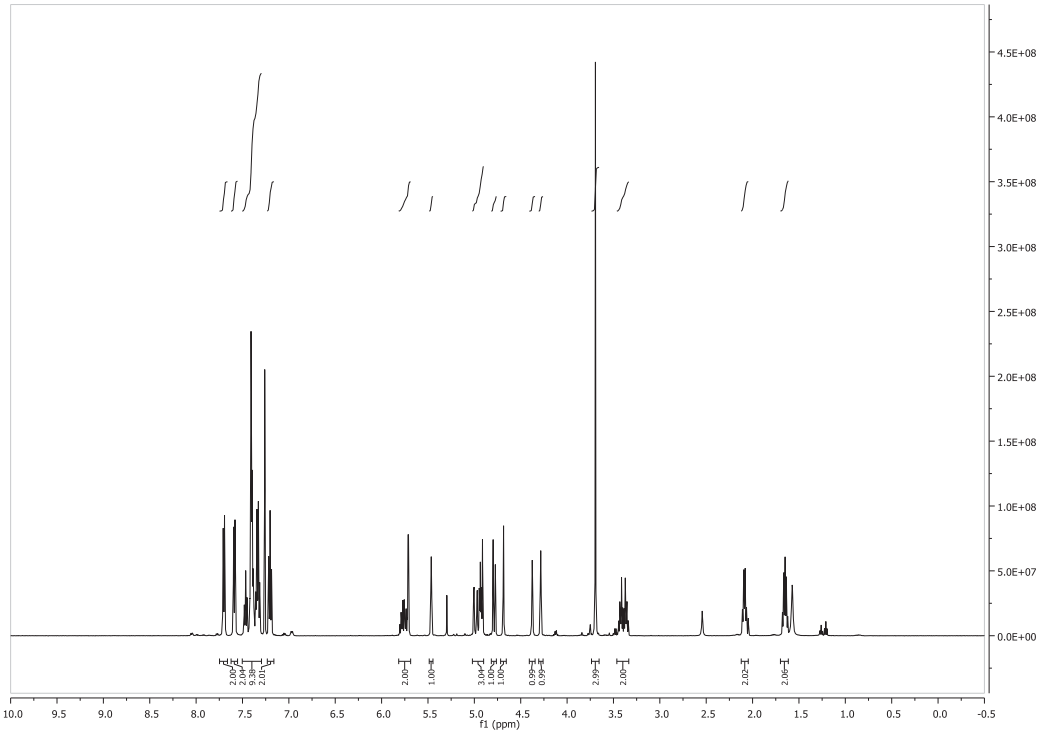


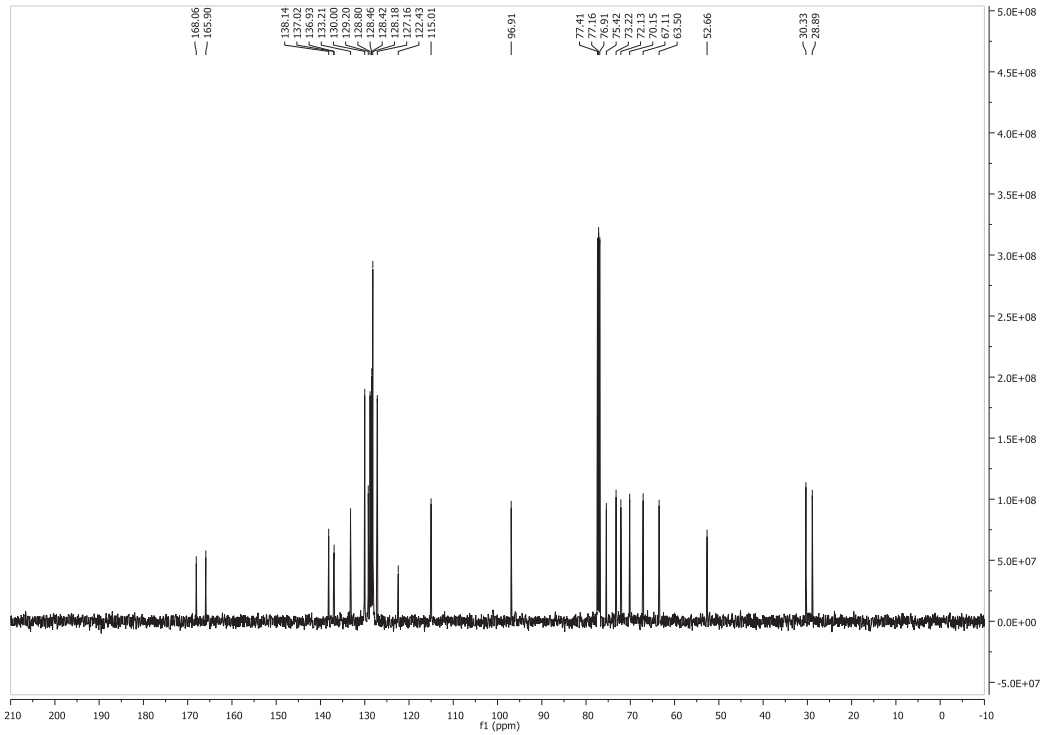
Methyl (3-*O*-benzyl-4-*O*-levulinoyl-[1,2-*O*-(1-pent-4-enyloxylevulinylidene)]-β-L-idopyranuronate (**53**):



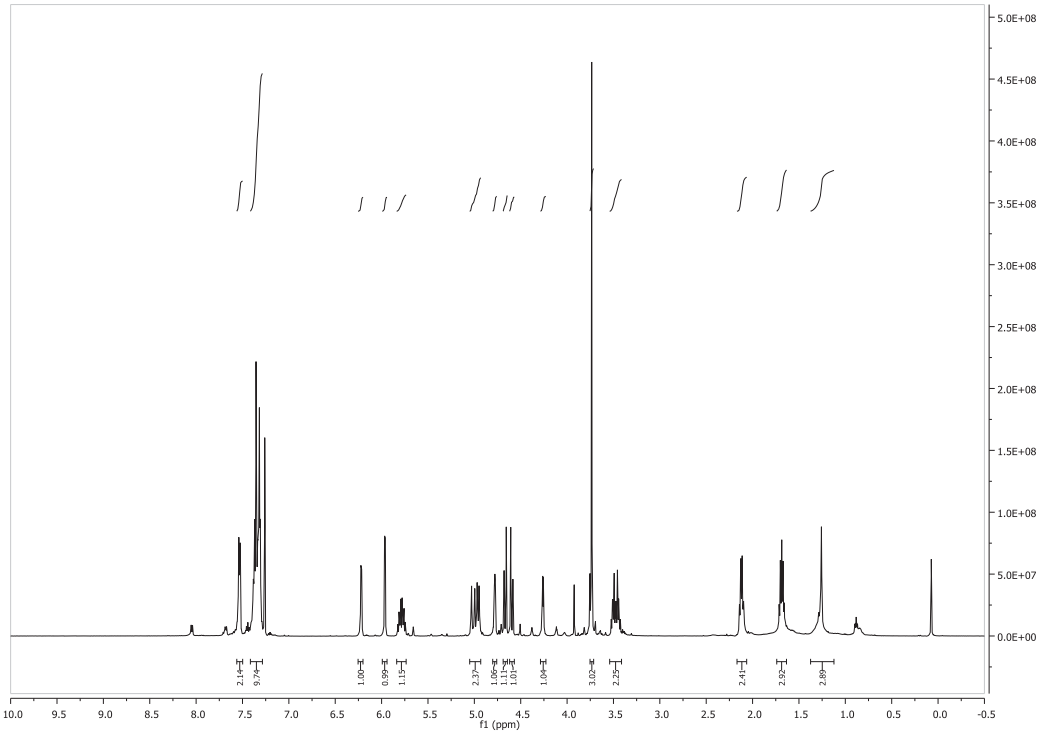


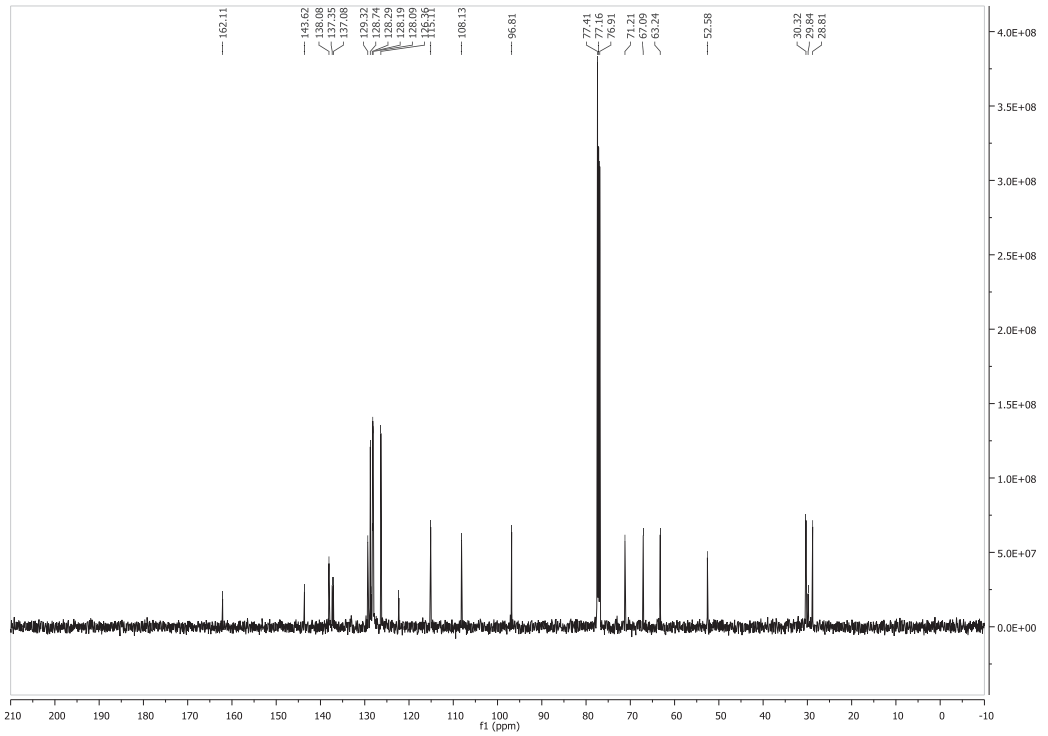
Methyl (4-*O*-benzoyl-3-*O*-benzyl-[1,2-*O*-(1-pent-4-enyloxybenzylidene)]-β-L-idopyranuronate (**54**):



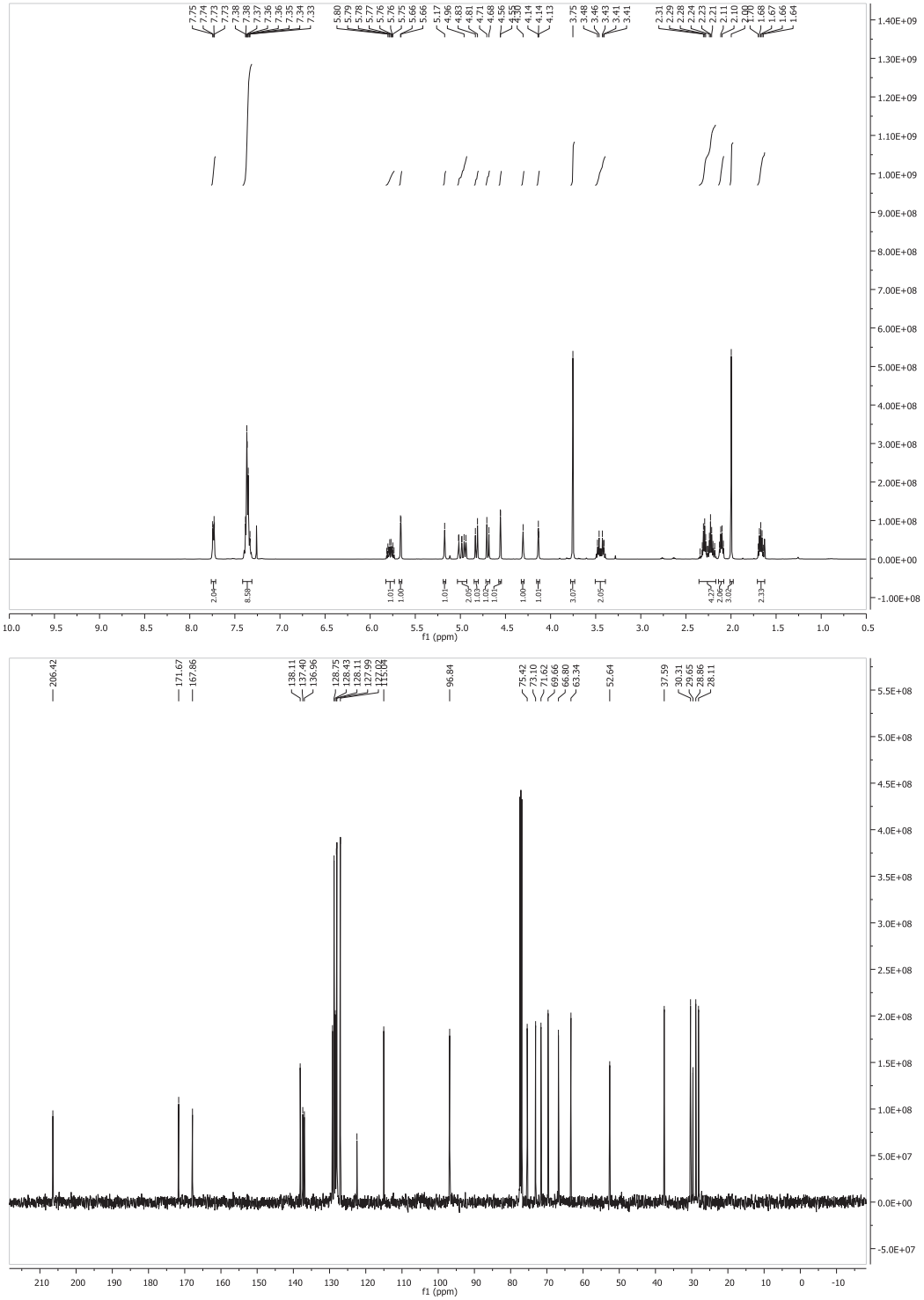


Methyl (3-*O*-benzyl-[1,2-*O*-(1-pent-4-enyloxybenzylidene)]-β-L-*threo*-hex-4-enopyranuronate (**55**):



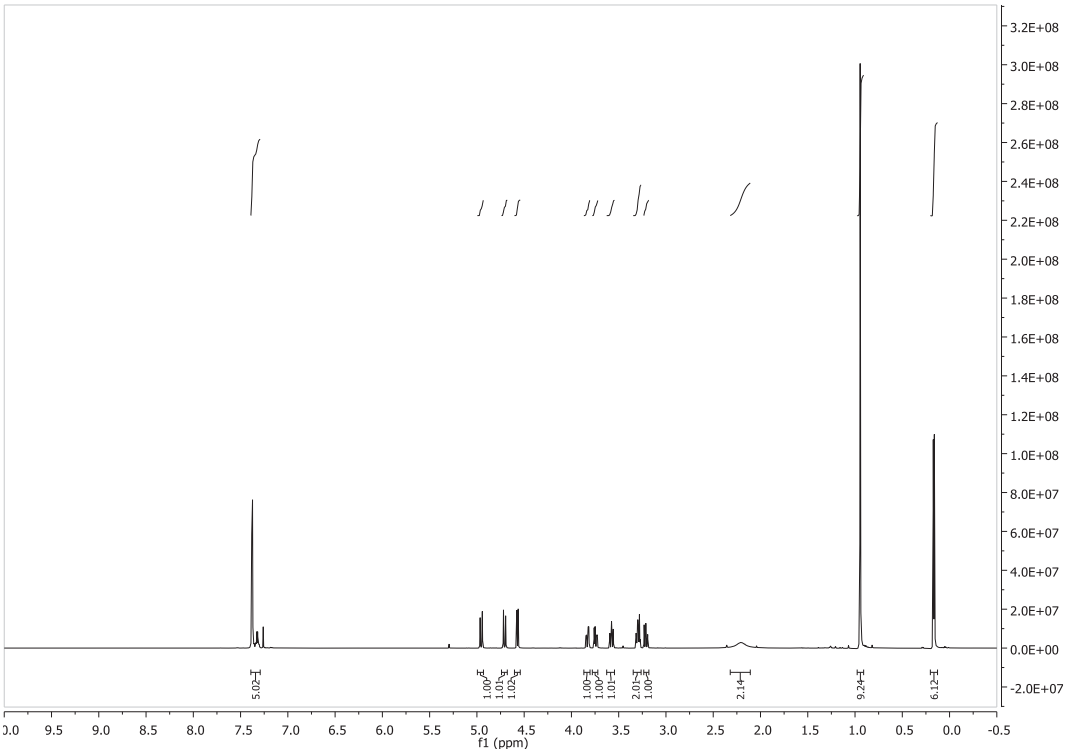


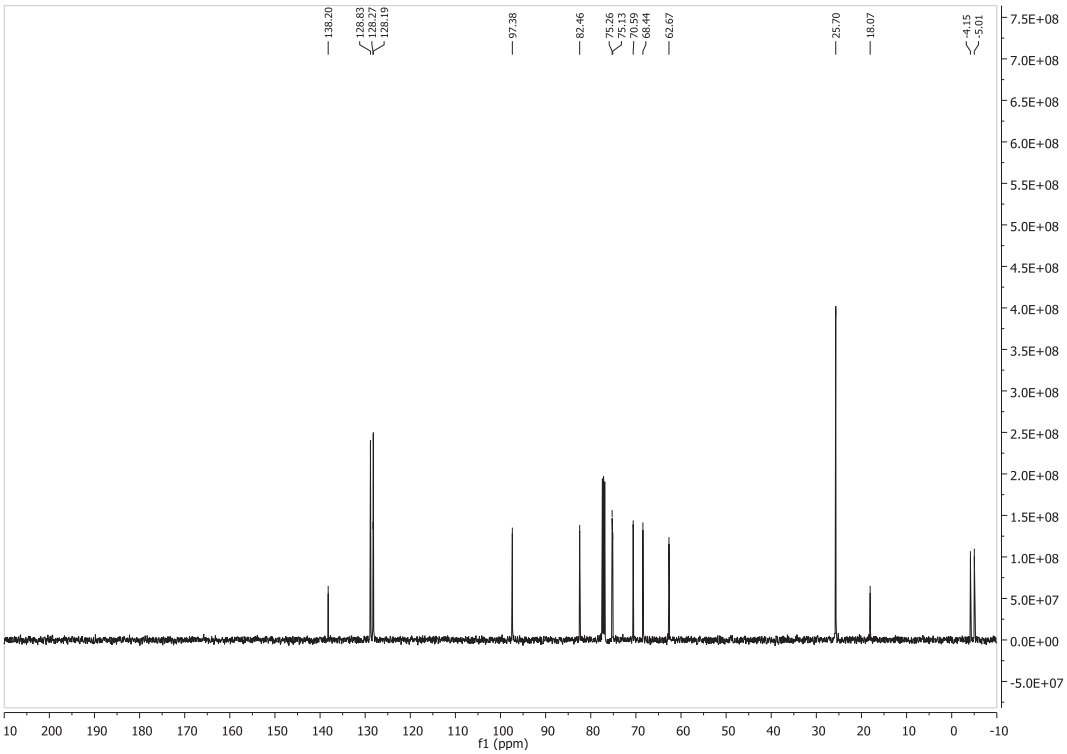
Methyl (3-*O*-benzyl-4-*O*-levulinoyl-[1,2-*O*-(1-pent-4-enyloxybenzylidene)]-β-L-idopyranuronate (**57**):



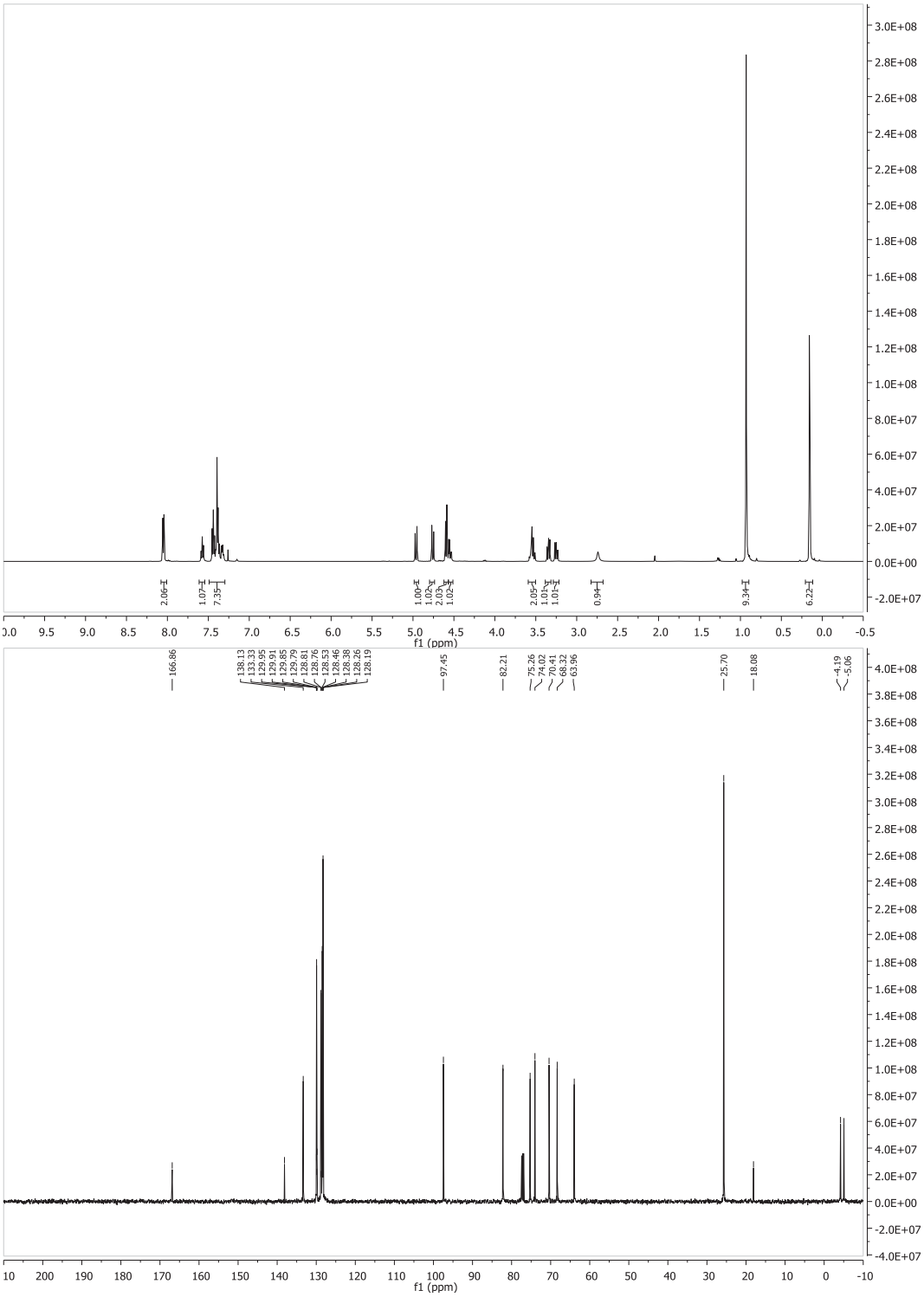
4.6.3 Synthesis of 2-azido-D-glucopyranose (Glc) building blocks

2-Azido-3-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranose (**62**):

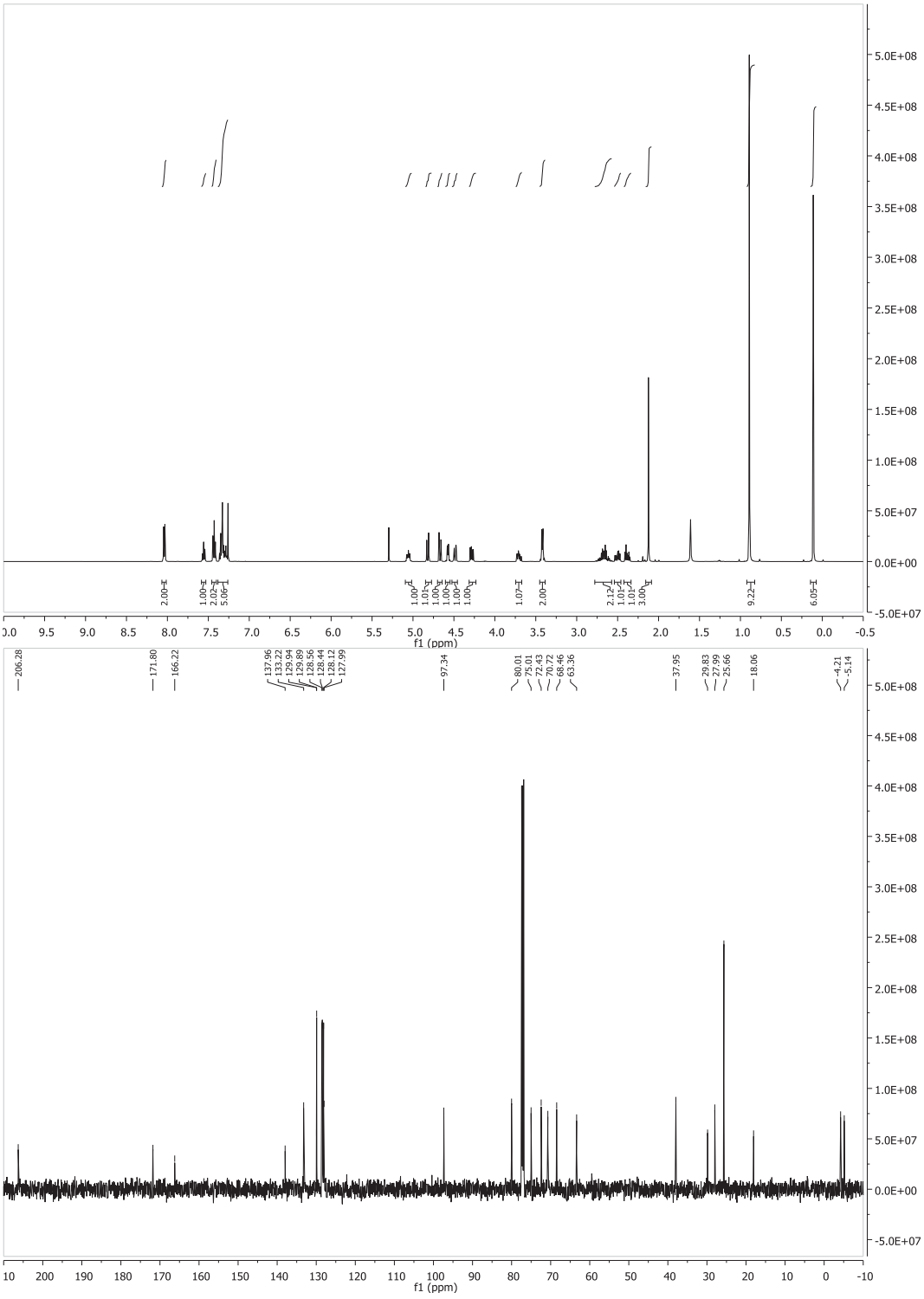




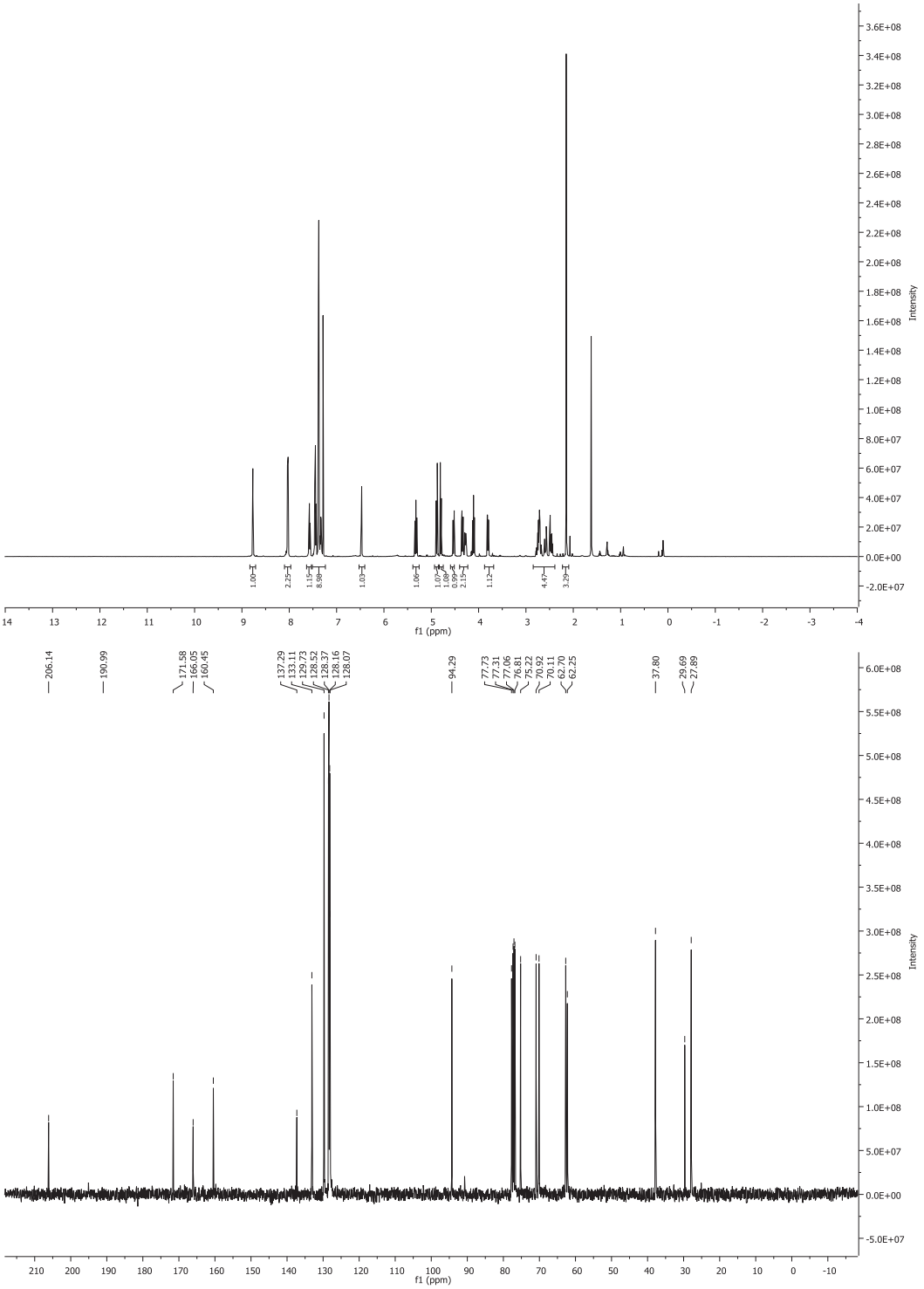
2-Azido-6-*O*-benzoyl-3-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy-β-D-glucopyranose (**63**):



2-Azido-6-*O*-benzoyl-3-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy-4-*O*-levulinoyl-β-D-glucopyranose (**64**):

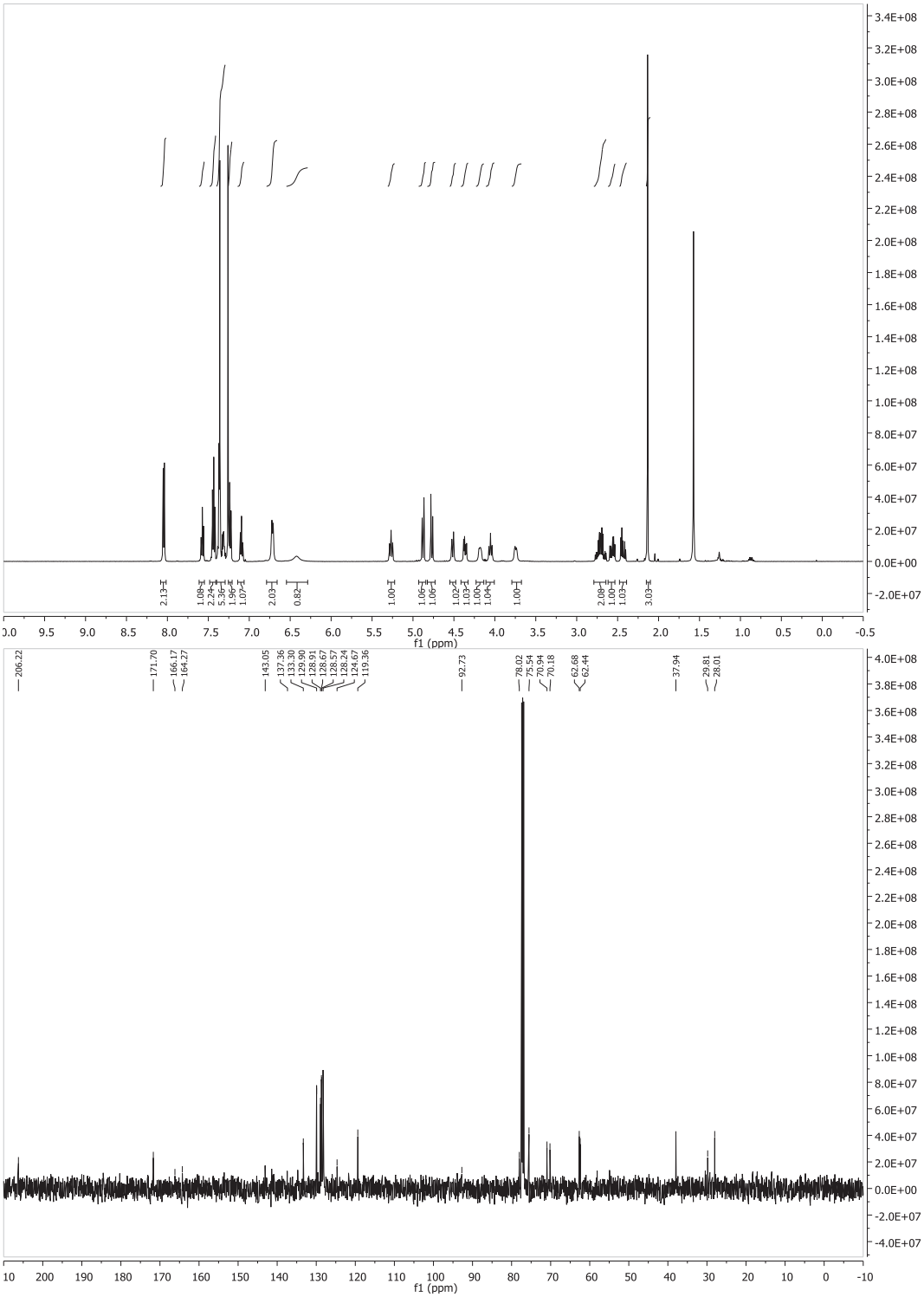


2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-O-levulinoyl-α-D-glucopyranosyl trichloroacetimidate (**65**):

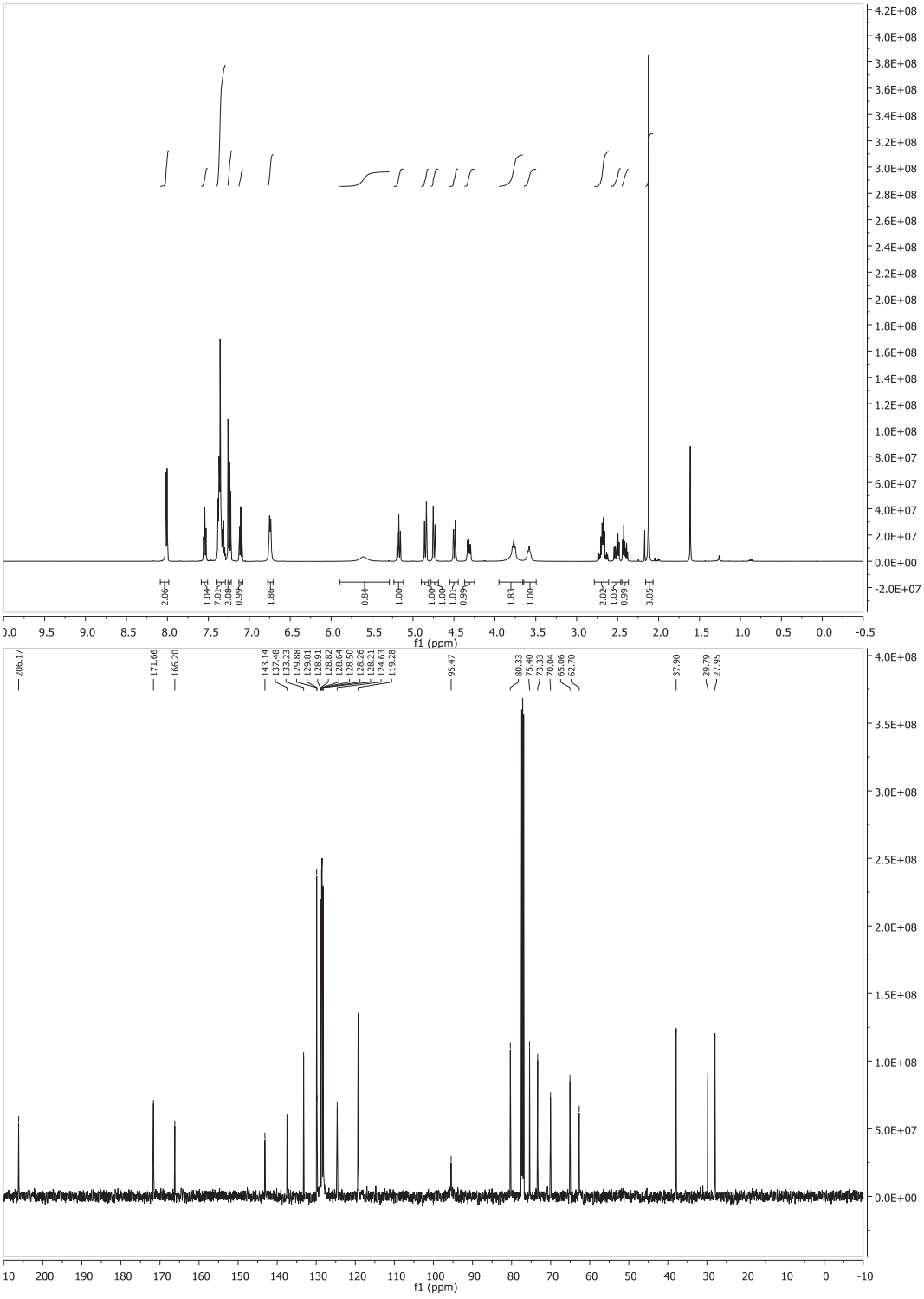


2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-O-levulinoyl-α-D-glucopyranosyl *N*-phenyl trifluoroacetimidate (**66**):

α-anomer:

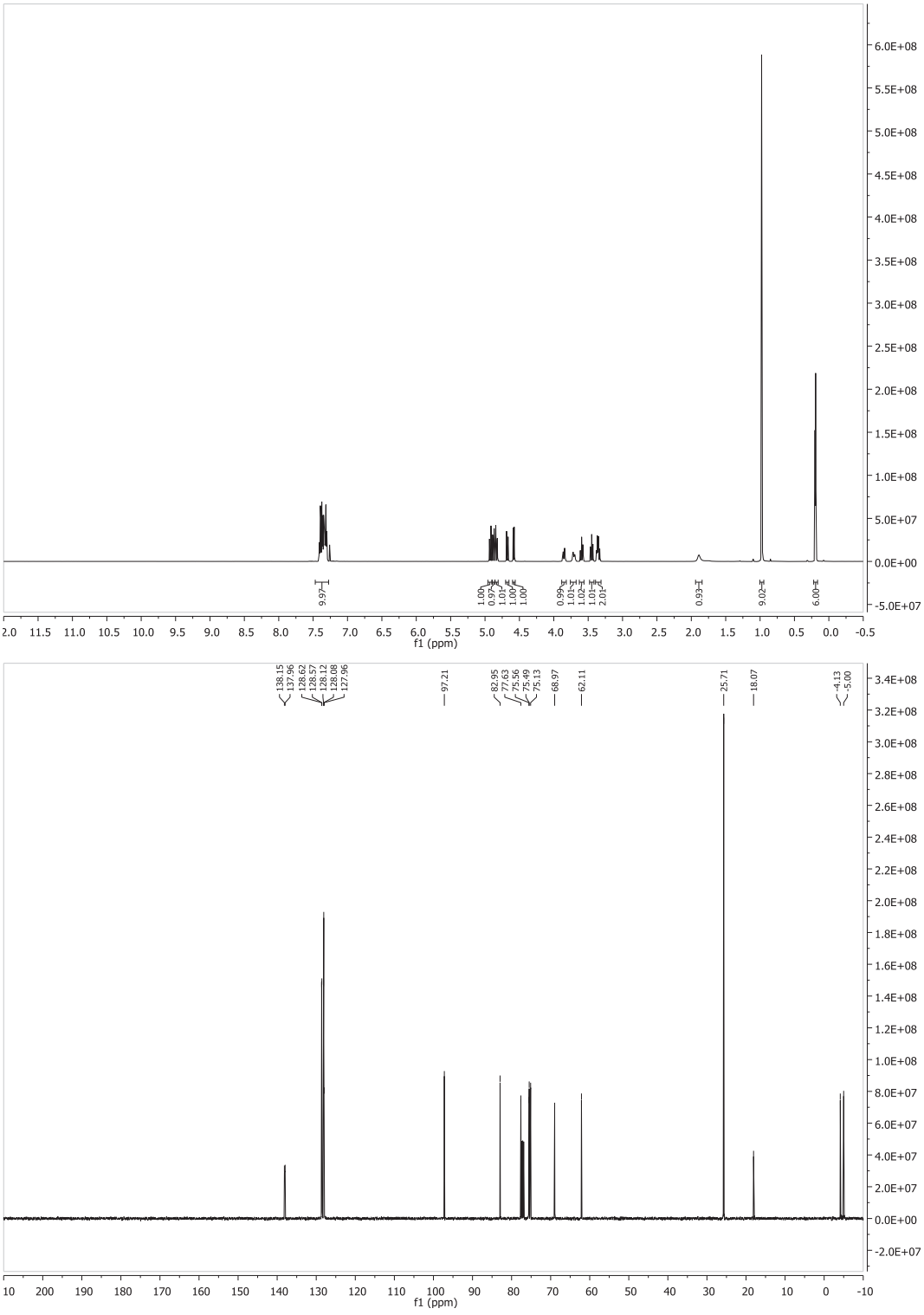


β-anomer:

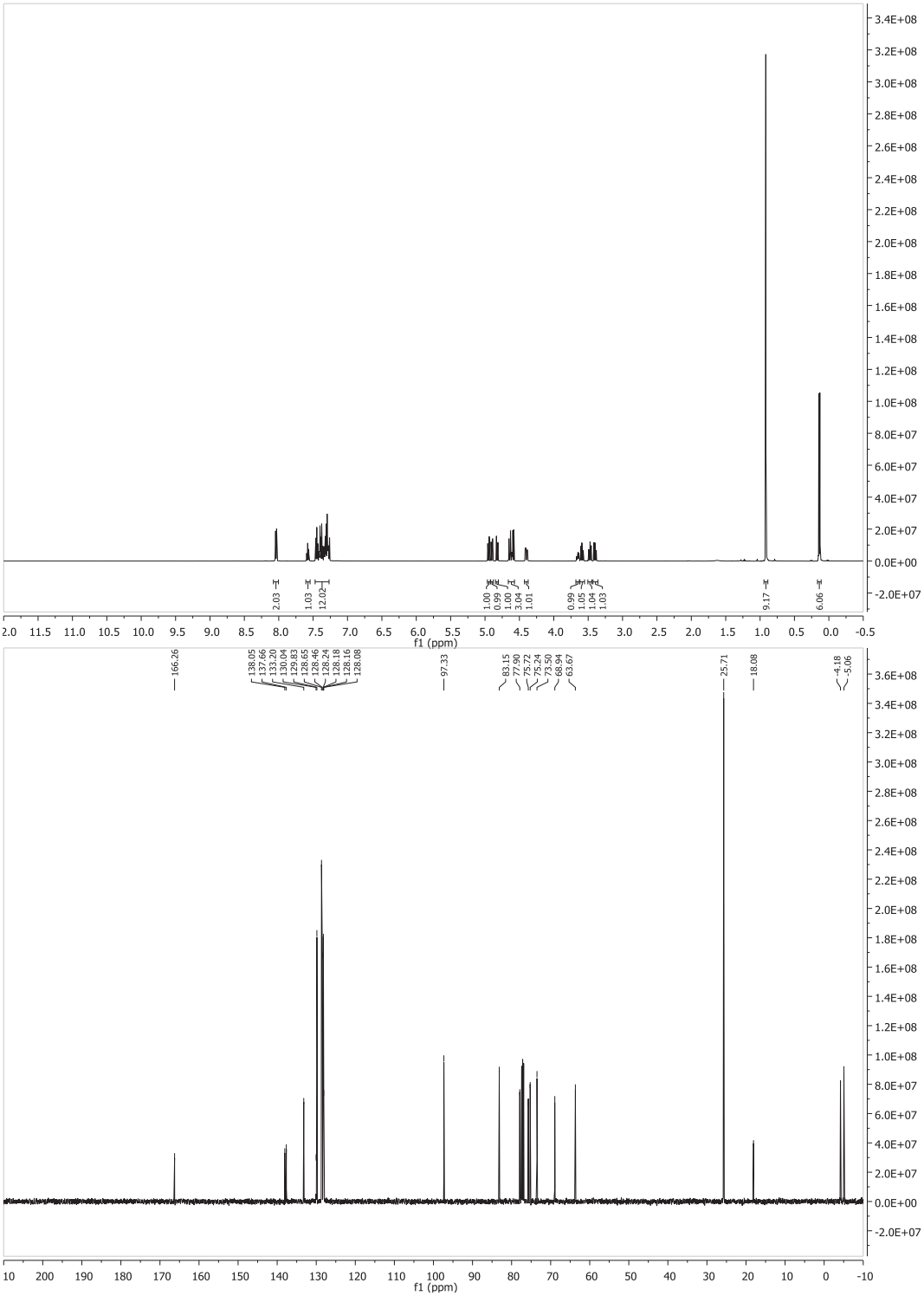

f1 (ppm)
206.17
171.66
166.20
143.14
137.48
133.23
129.88
129.81
128.91
128.82
128.64
128.50
128.26
128.21
124.63
119.28
95.47
80.33
75.40
73.33
70.04
65.06
62.70
37.90
29.79
27.95

4.6.3.1 Synthesis of the Non-Reducing End Building Block of Glucosamine

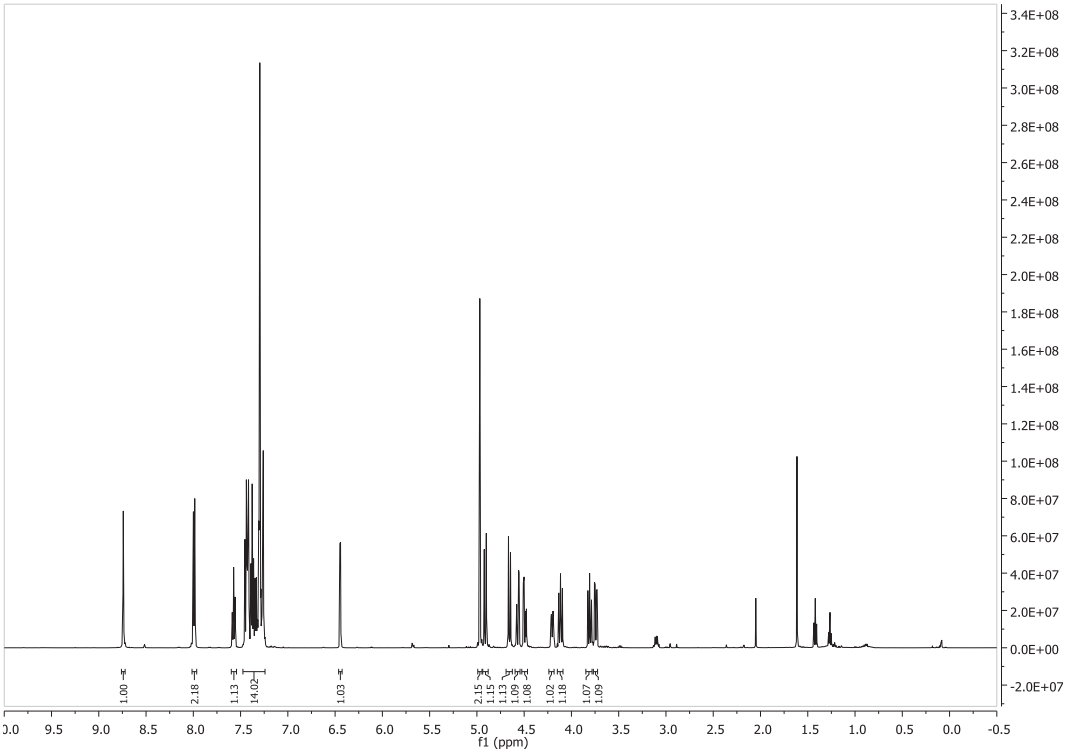
2-Azido-3,4-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy -β-D-glucopyranose (67):



2-Azido-6-O-benzoyl-3,4-O-benzyl-1-O-tert-butyldimethylsilyl-2-deoxy-β-D-glucopyranose (**68**):

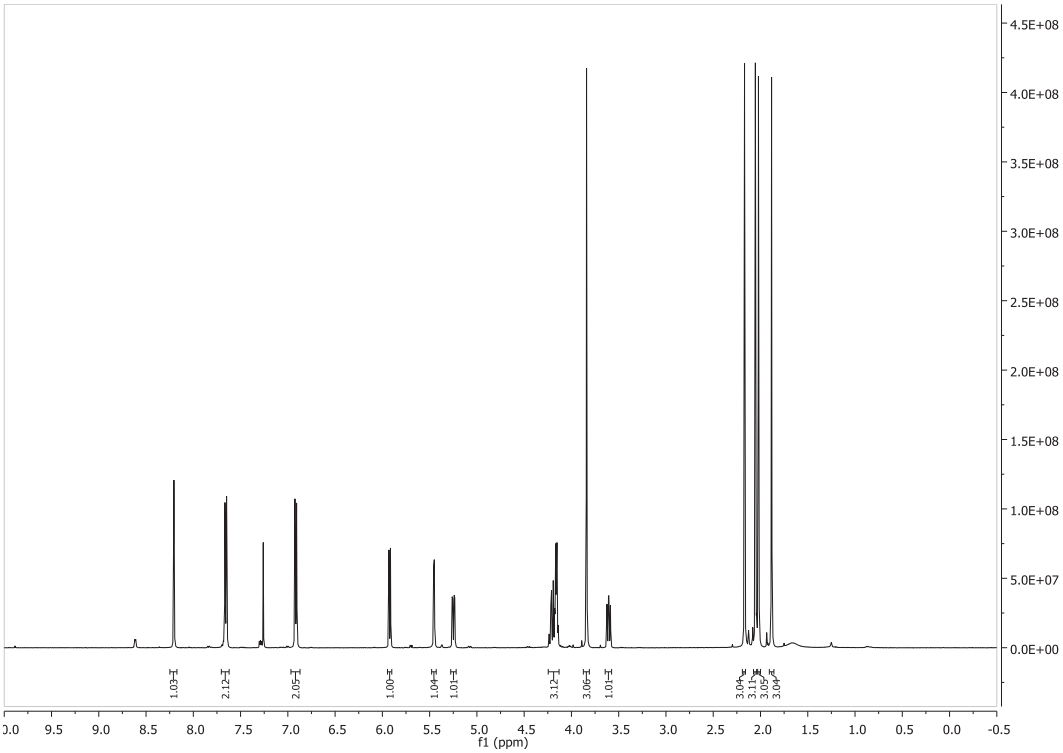


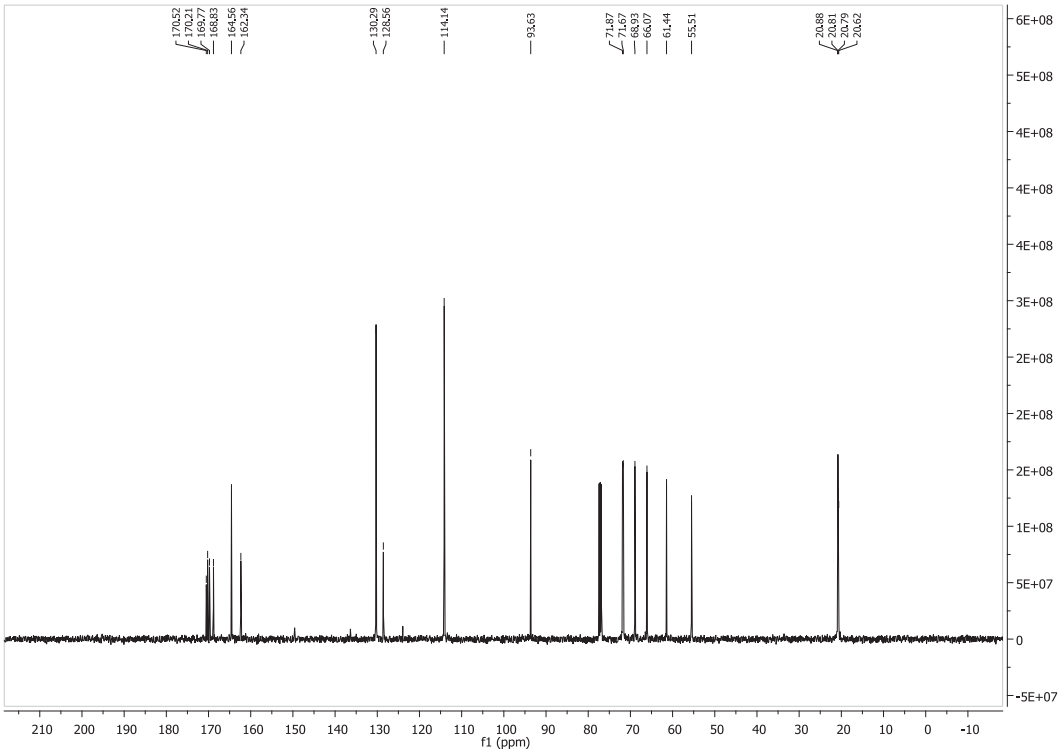
2-Azido-6-*O*-benzoyl-3,4-*O*-benzyl-1-*O*-tert-butyldimethylsilyl-2-deoxy–α-D-glucopyranosyl trichloroacetimidate (70):



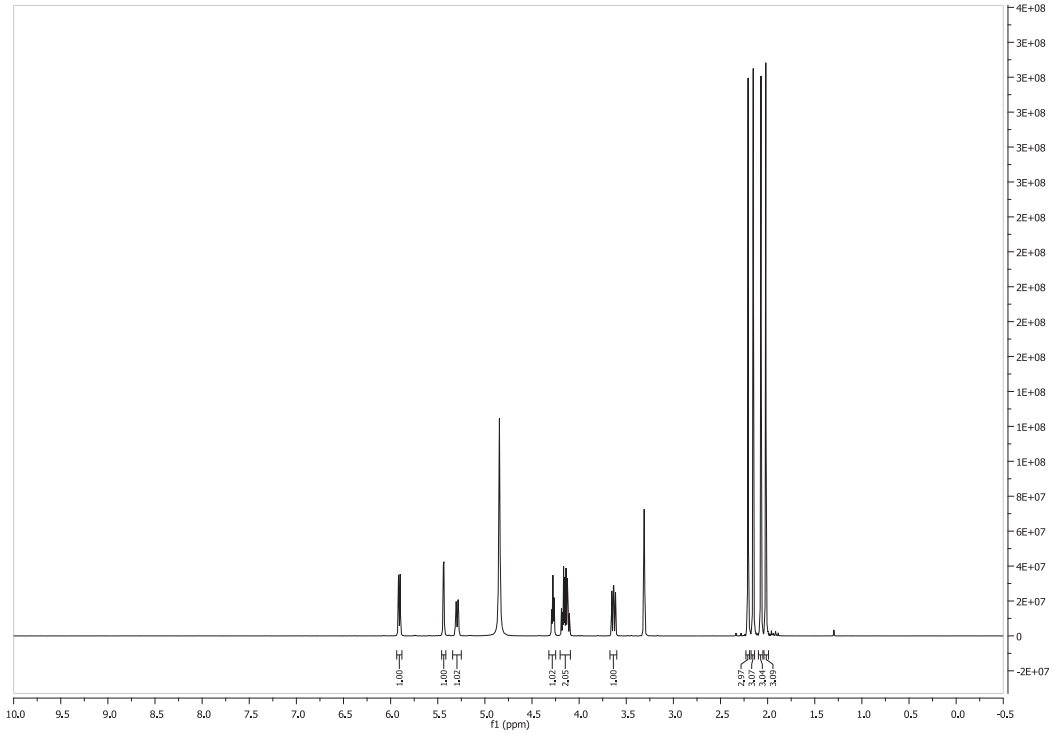
4.6.4 Synthesis of galactosamine building blocks

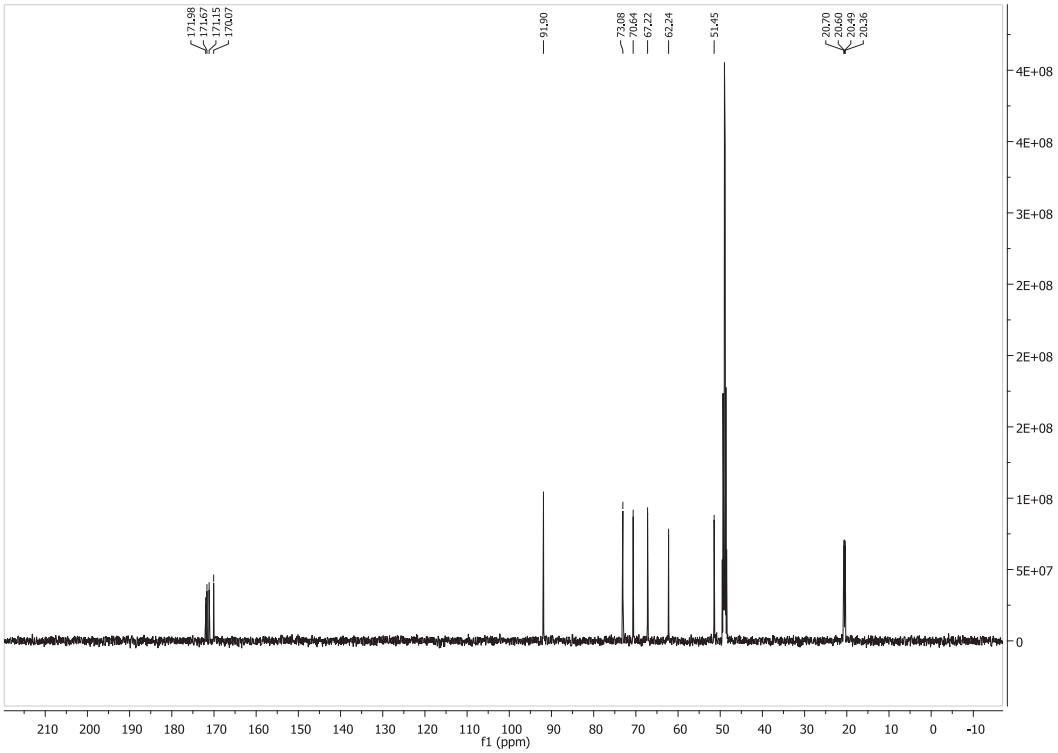
1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-*p*-methoxyphenylimino-D-galactopyranose (72):



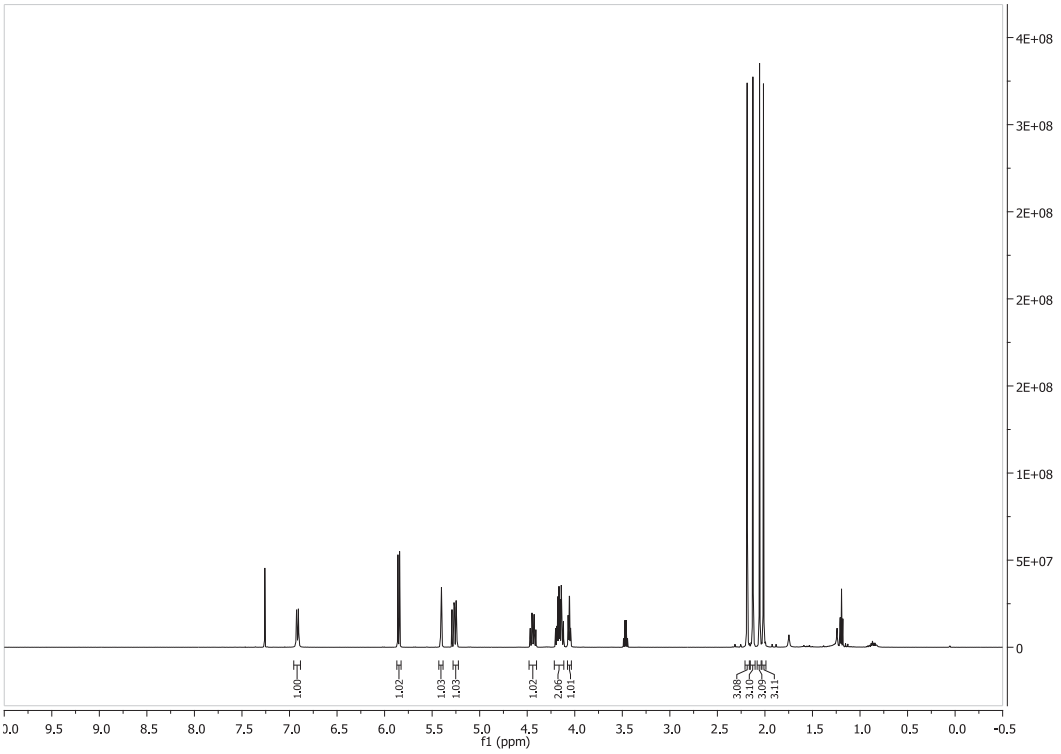


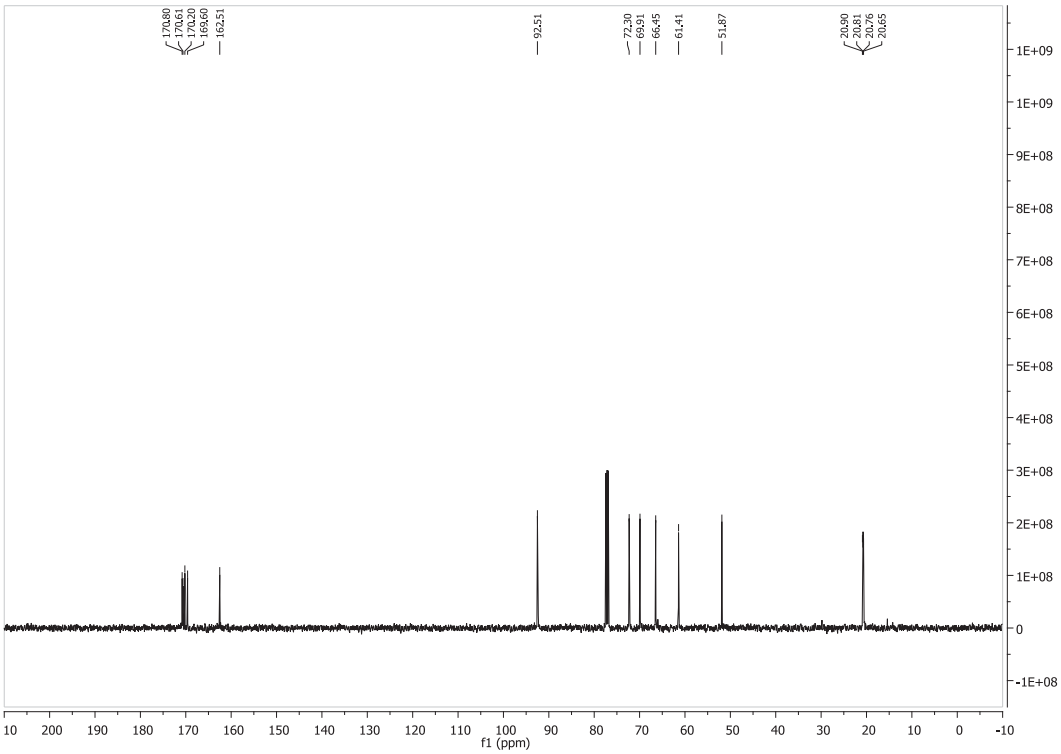
1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-D-galactopyranose (**73**):



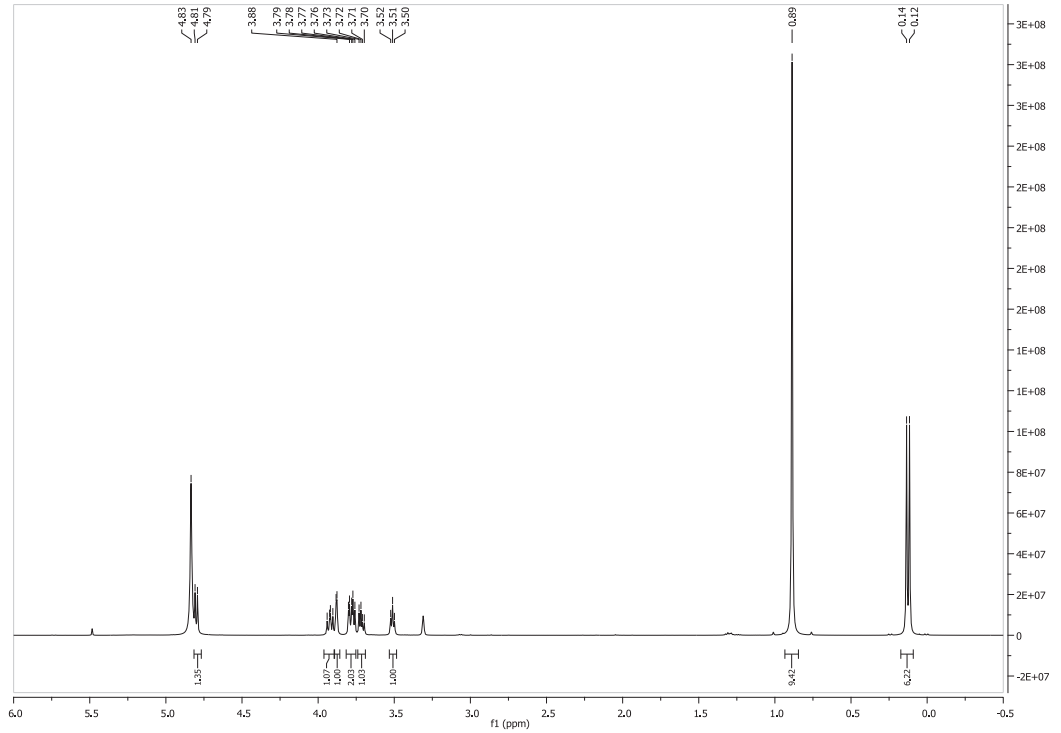


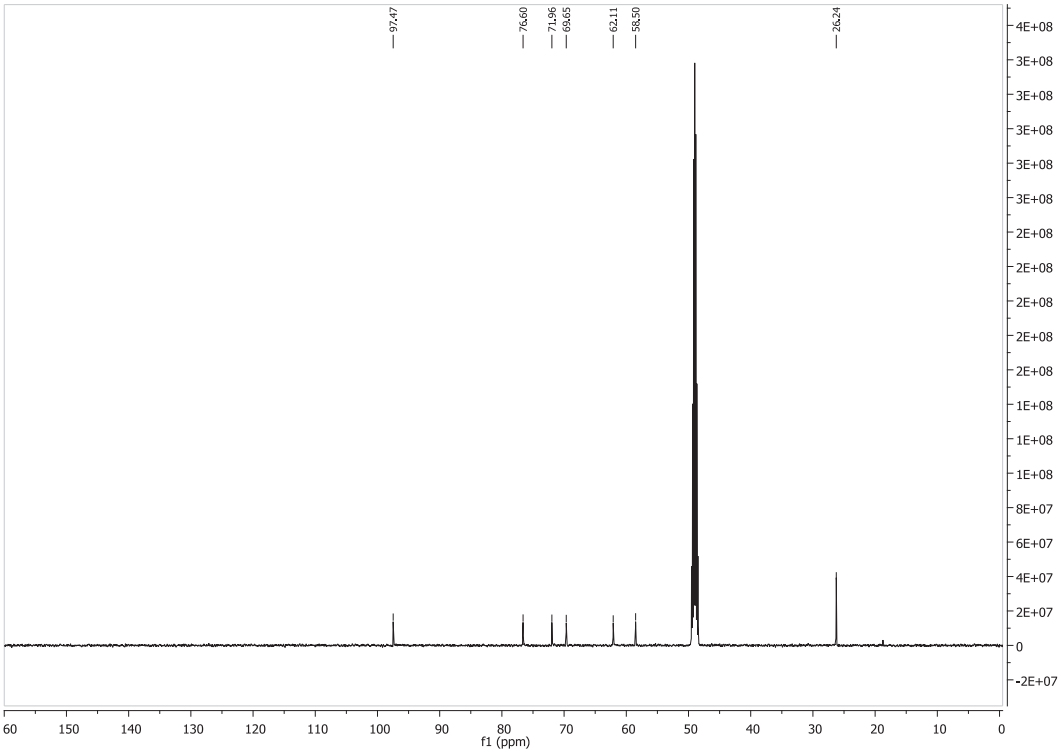
1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-trichloroacetamidate-D-galactopyranose (**74**):



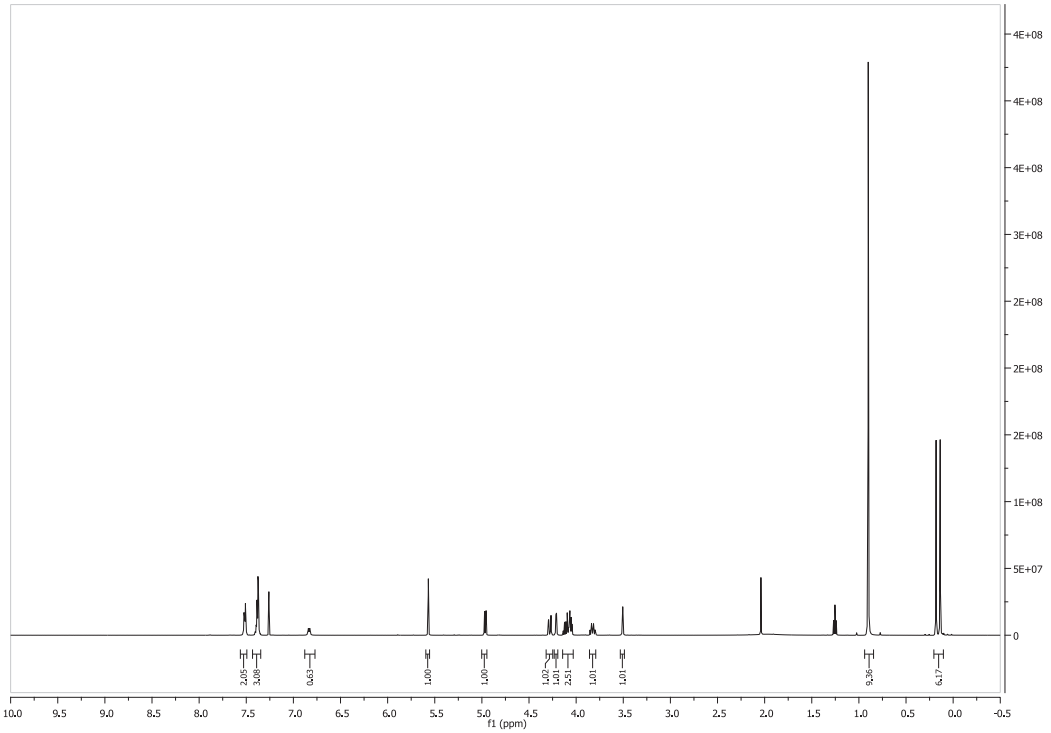


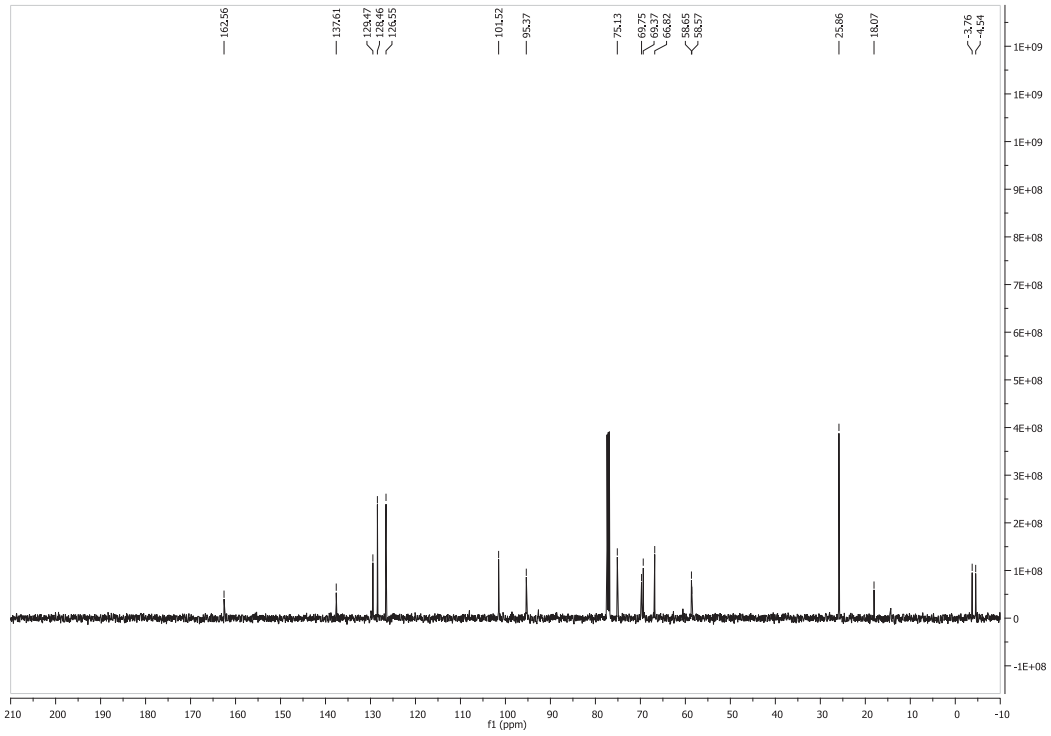
1-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranose (75):



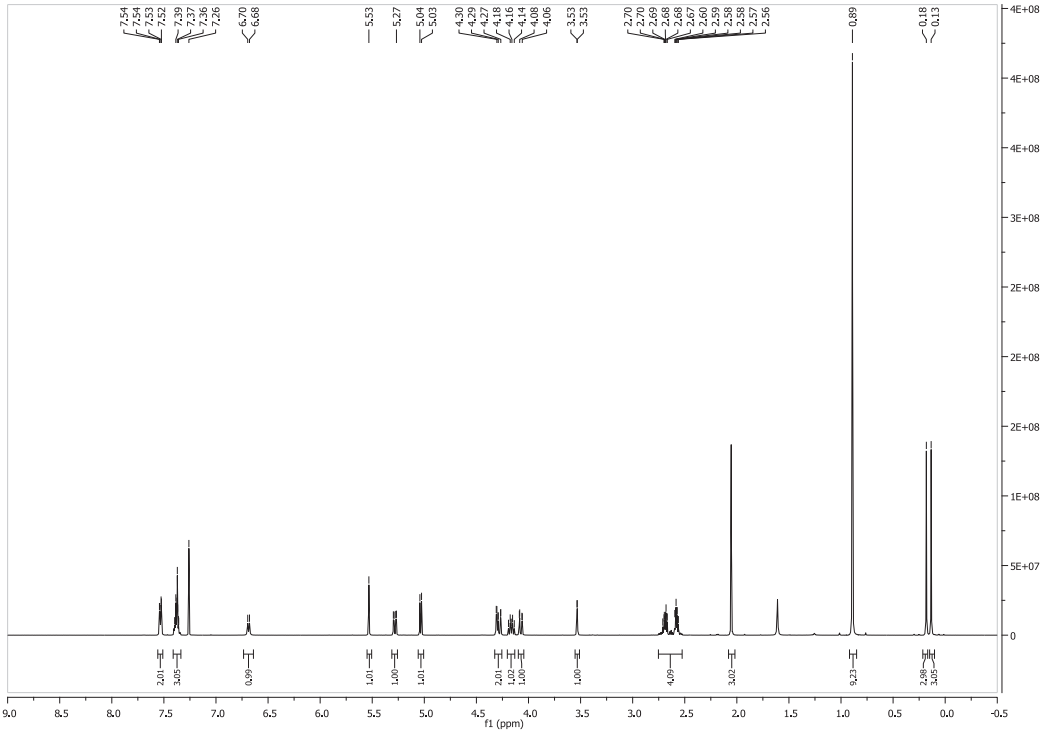


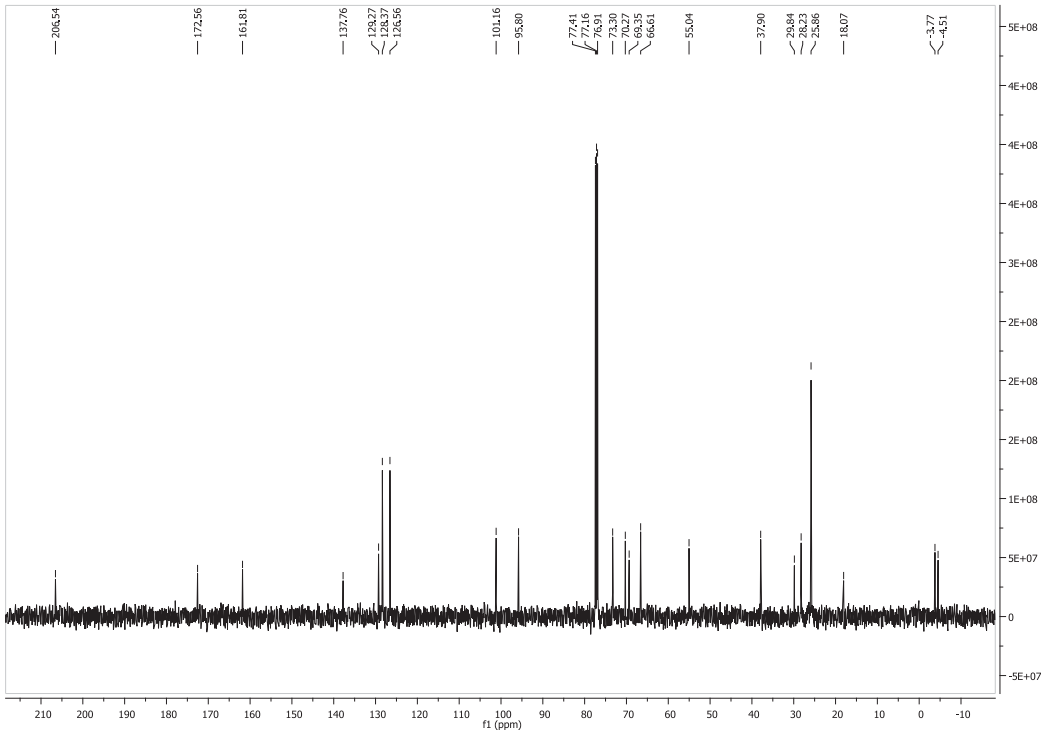
4,6-Benzylidene-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranose (**76**):



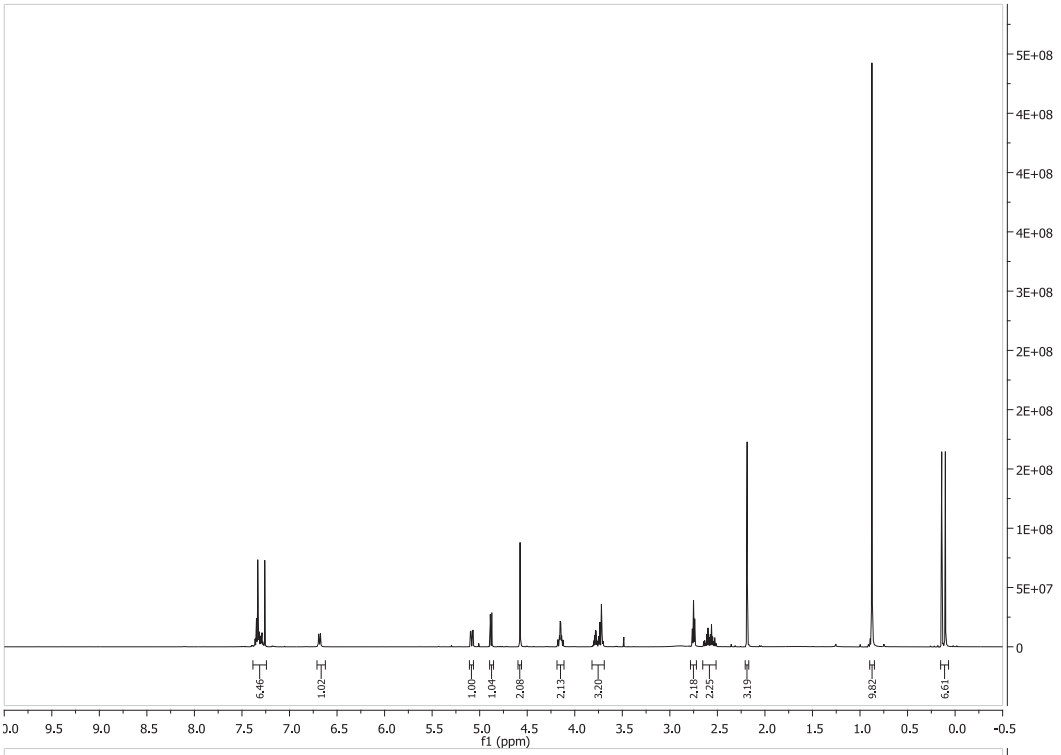


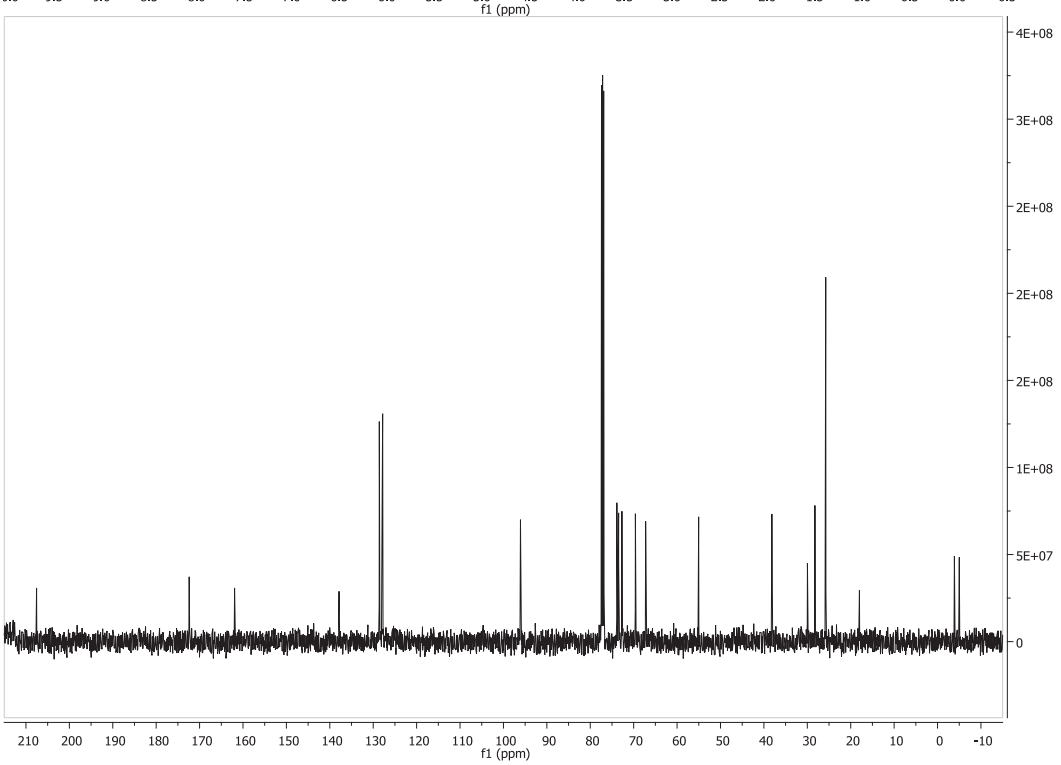
4,6-Benzylidene-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (**77**):



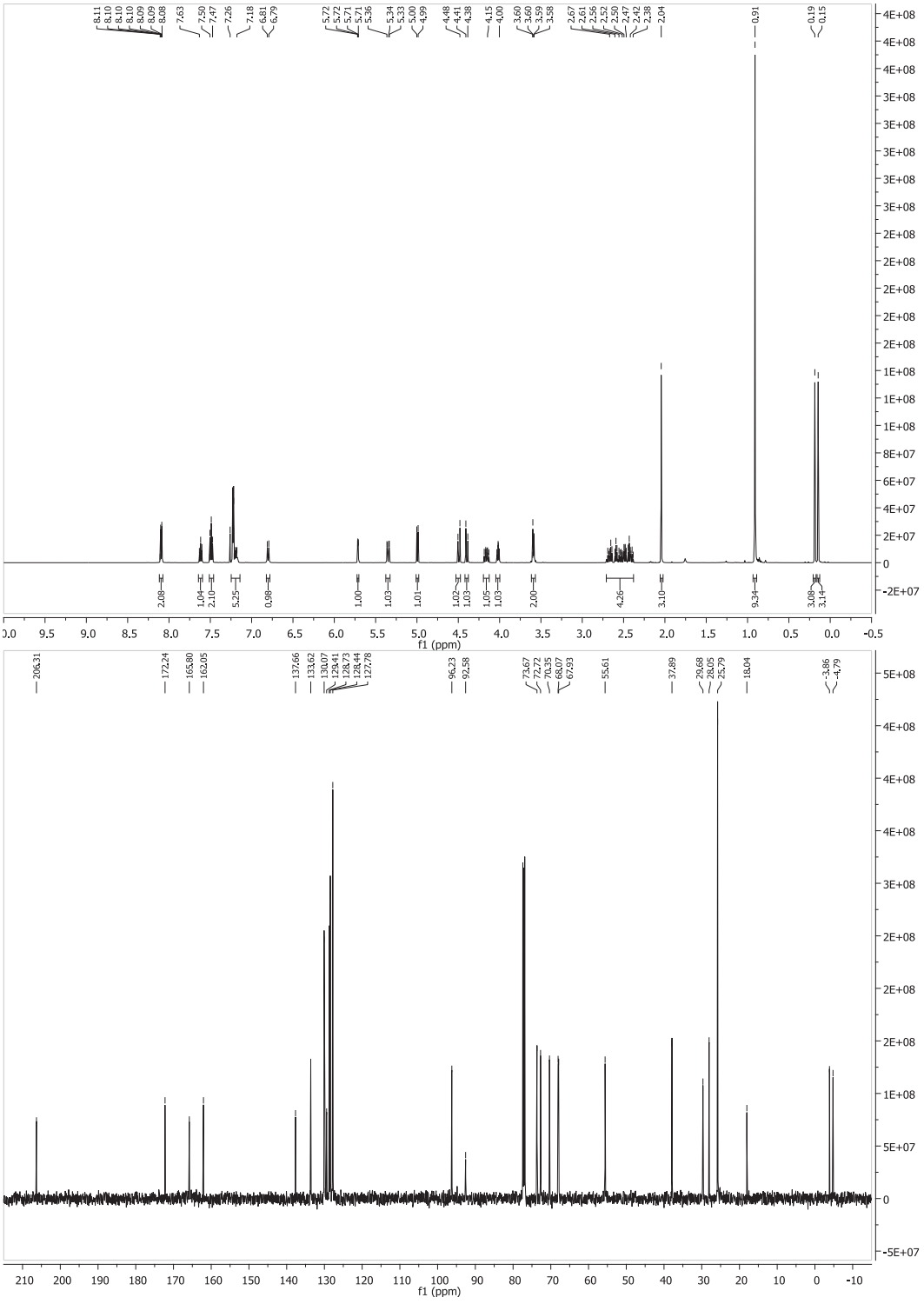


6-*O*-Benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (**78**):

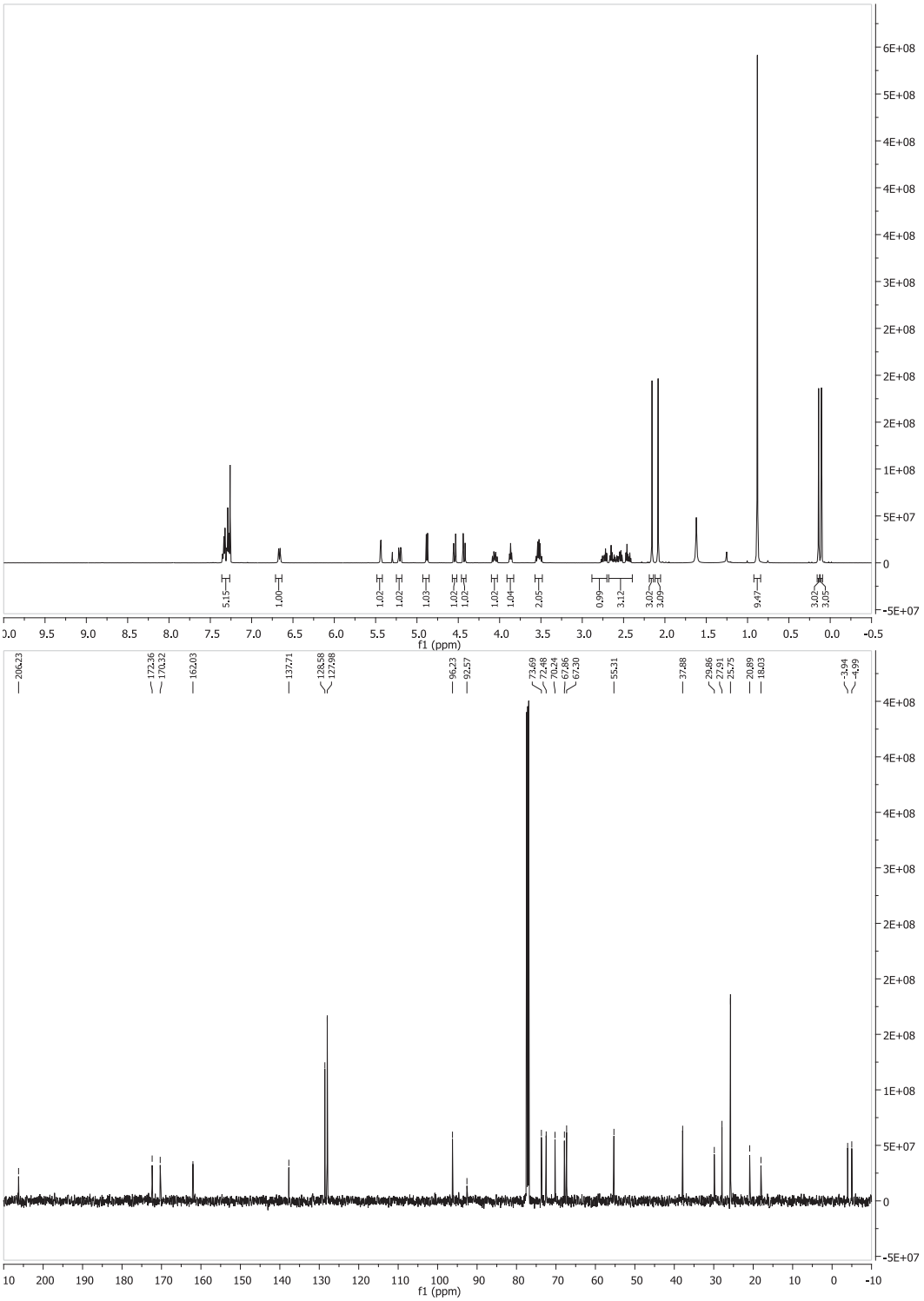




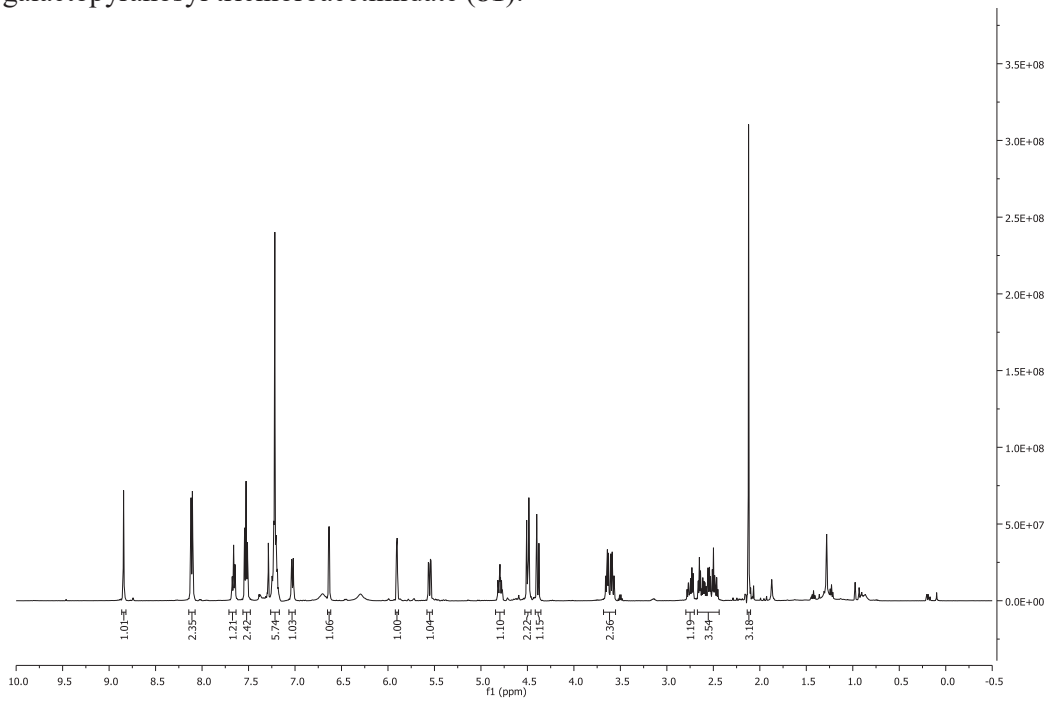
4-*O*-Benzoyl-6-*O*-benzyl-1-*O*-*tert.*-butyldimethylsilyl-2-deoxy-3-*O*-levulinyl-2-trichloroacetamido-D-galactopyranose (**79**):



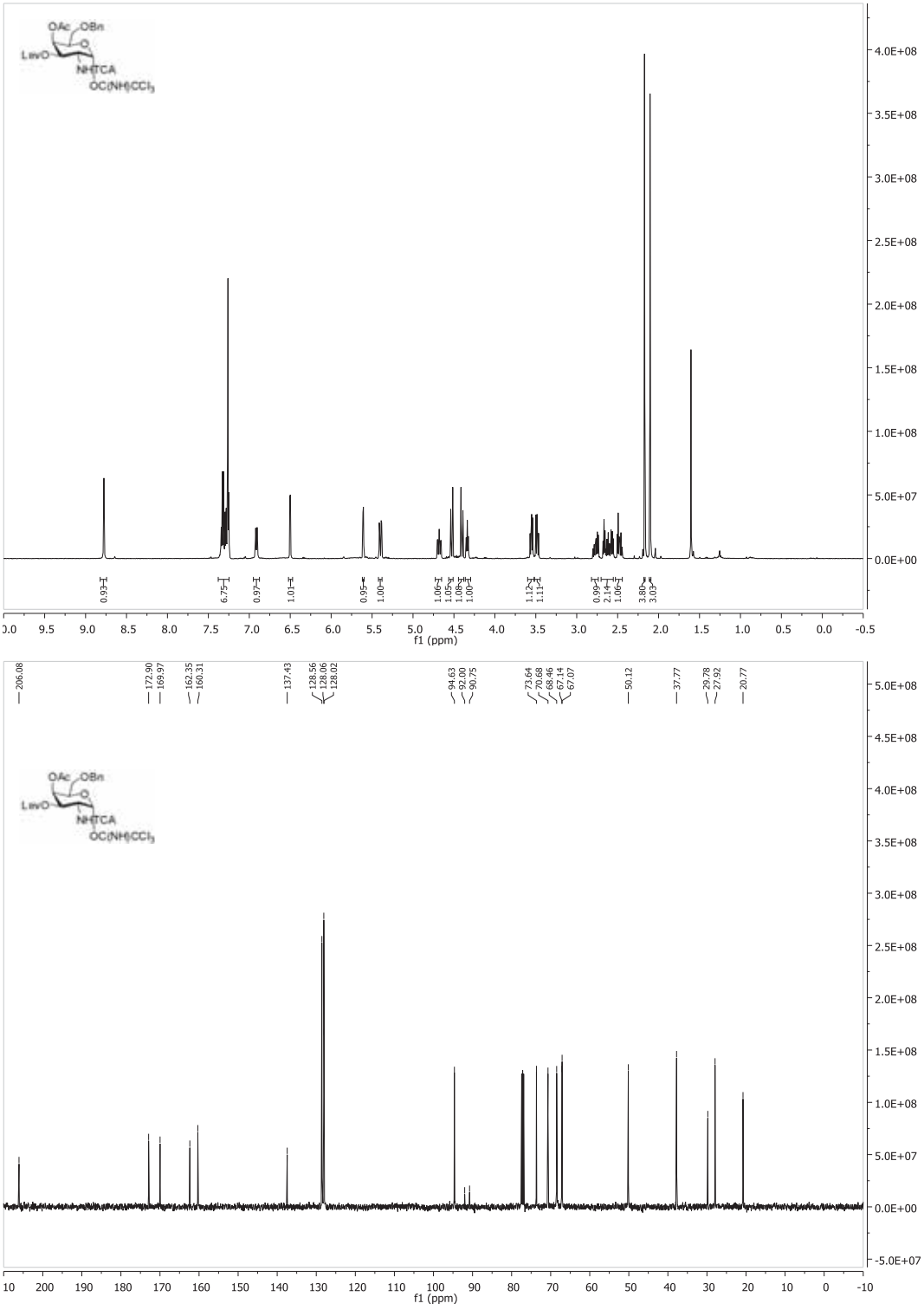
4-*O*-Acetyl-6-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (**80**):



4-*O*-Benzoyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-α-d-galactopyranosyl trichloroacetimidate (**81**):

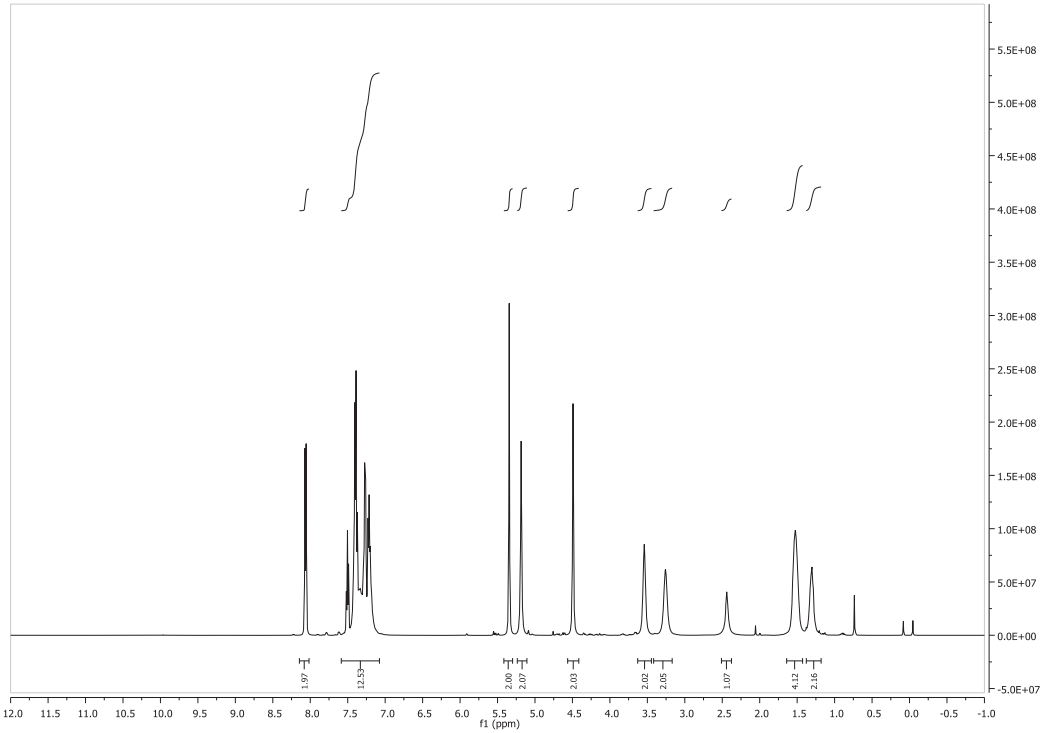


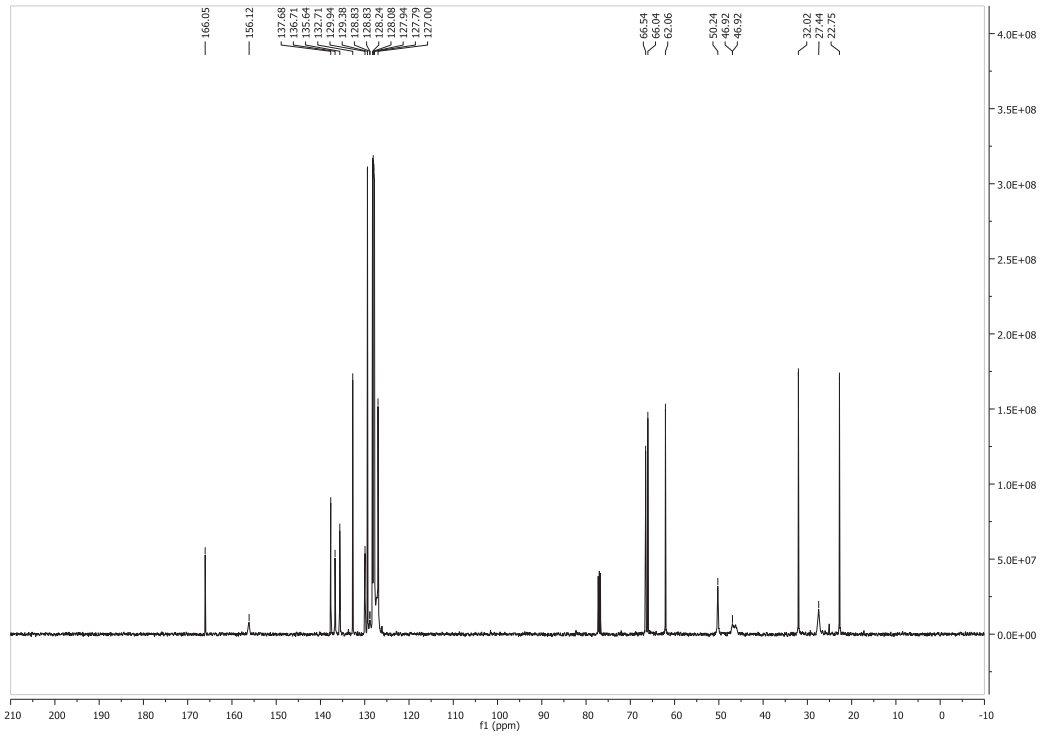
4-*O*-Acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-α-D-galactopyranosyl trichloroacetimidate (**82**):



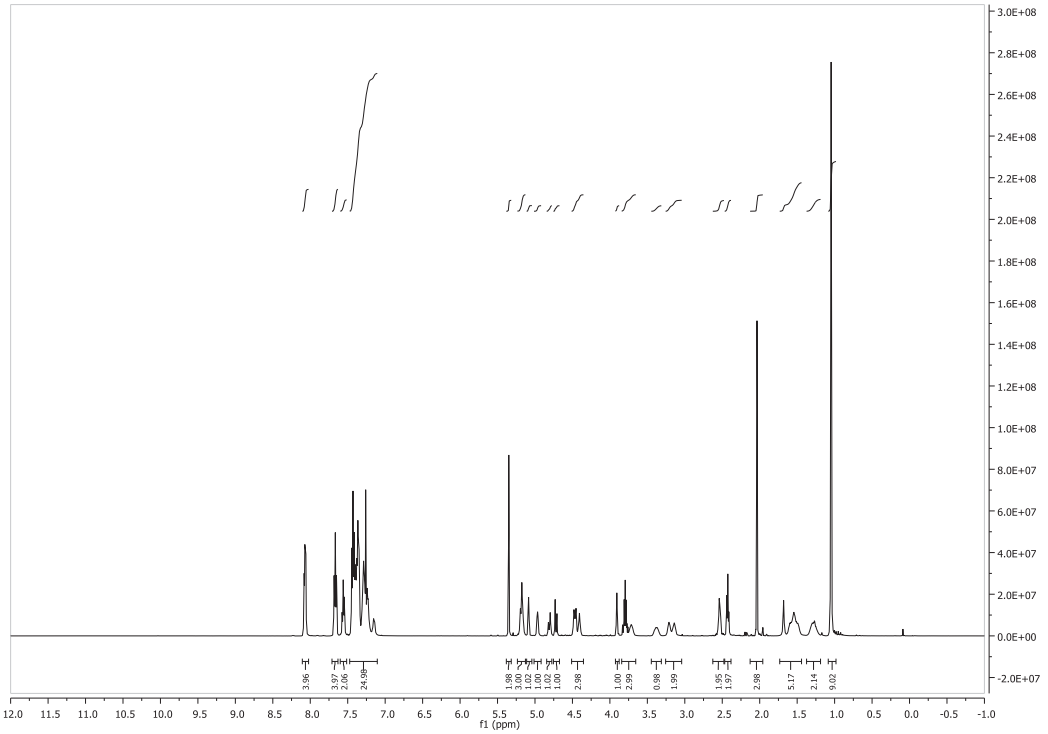
4.7.1 Glycosylation of the linker

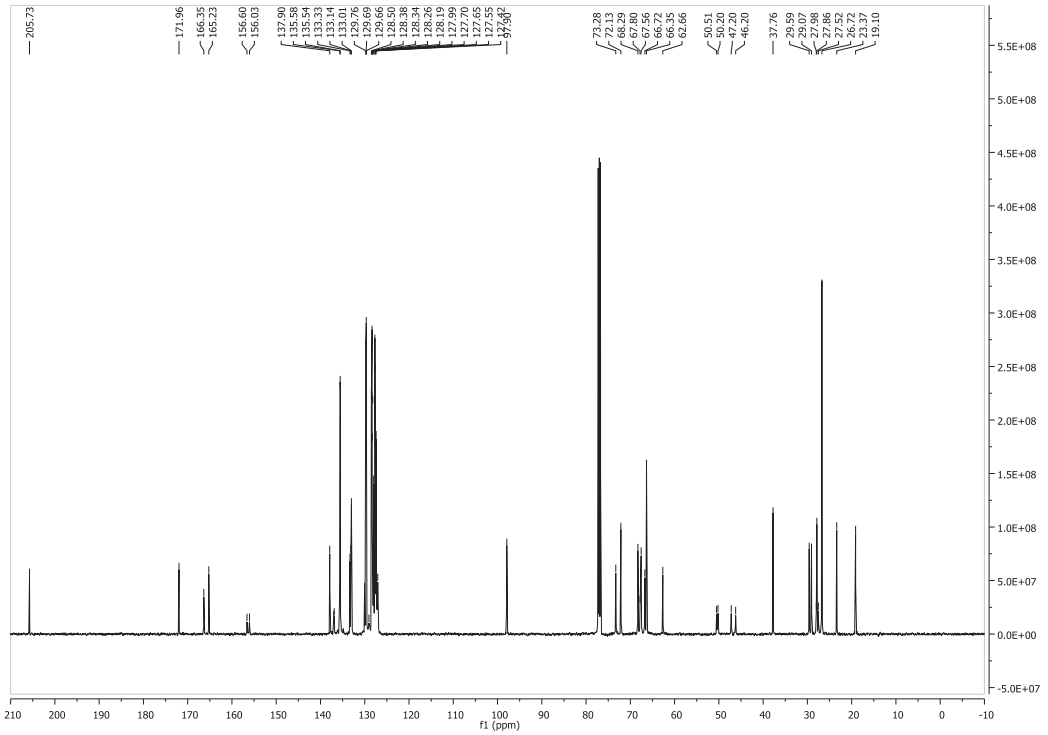
4-((Benzyl(5-hydroxypentyl)carbamoyloxy)methyl)benzyl benzoate (83):



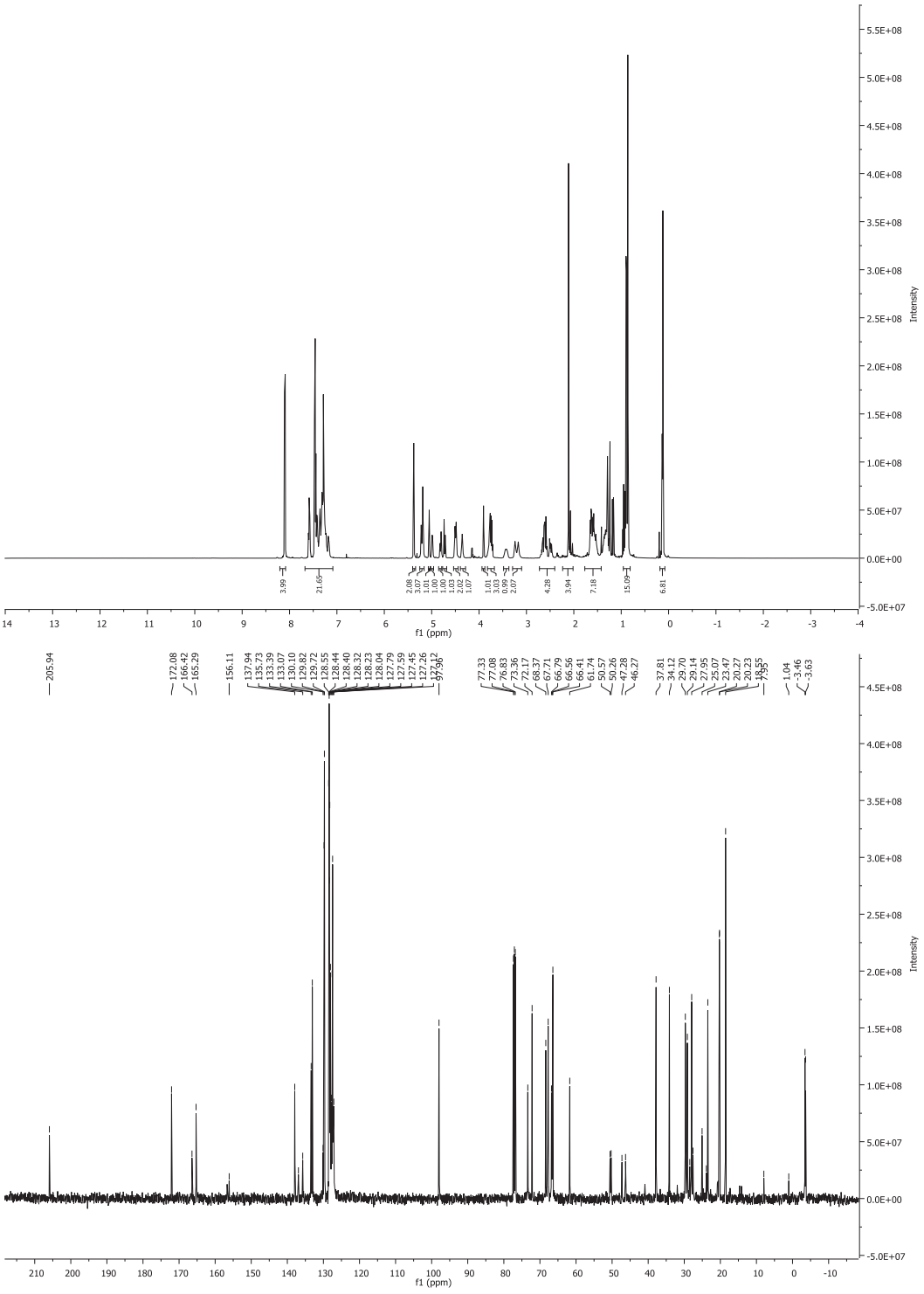


4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(3-*O*-benzyl-2-*O*-benzoyl-4-*O*-levulinoyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**84**):

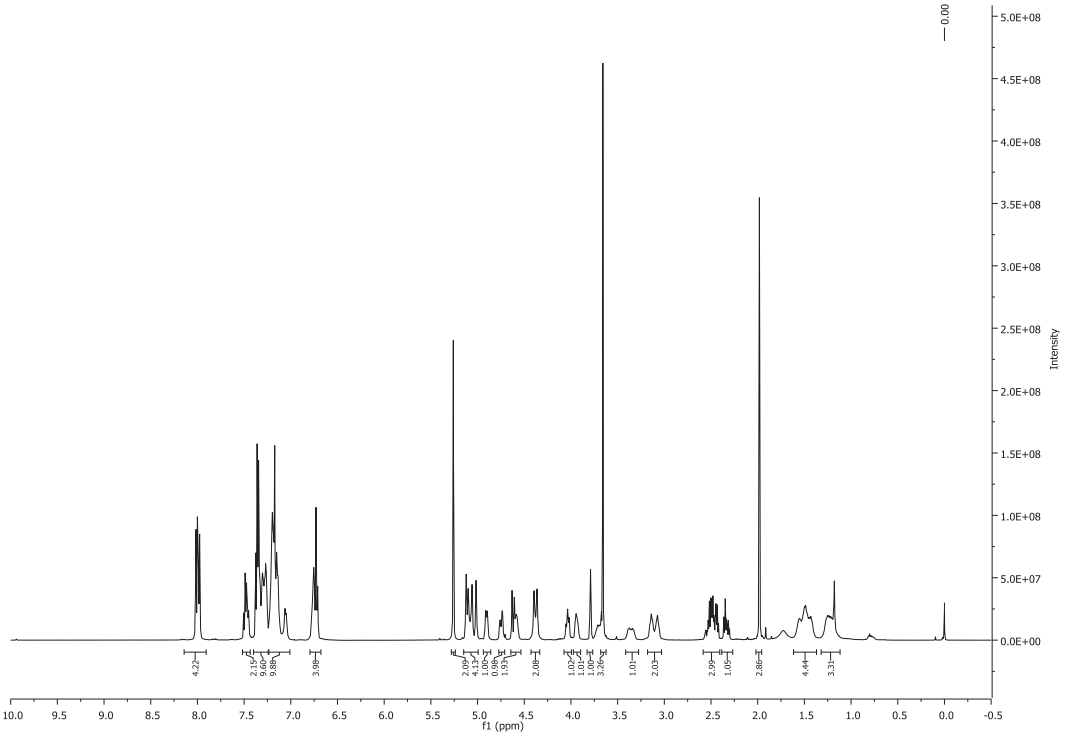


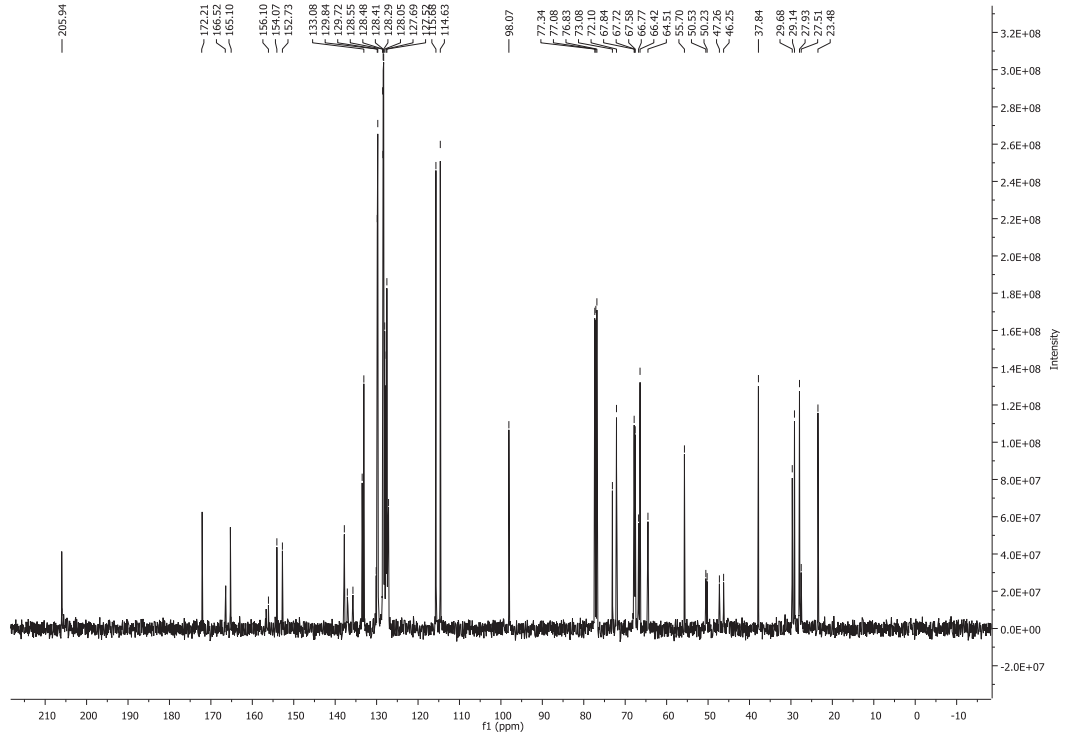


4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-dimethylthexylsilyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)pentyl) carbamate (**85**):

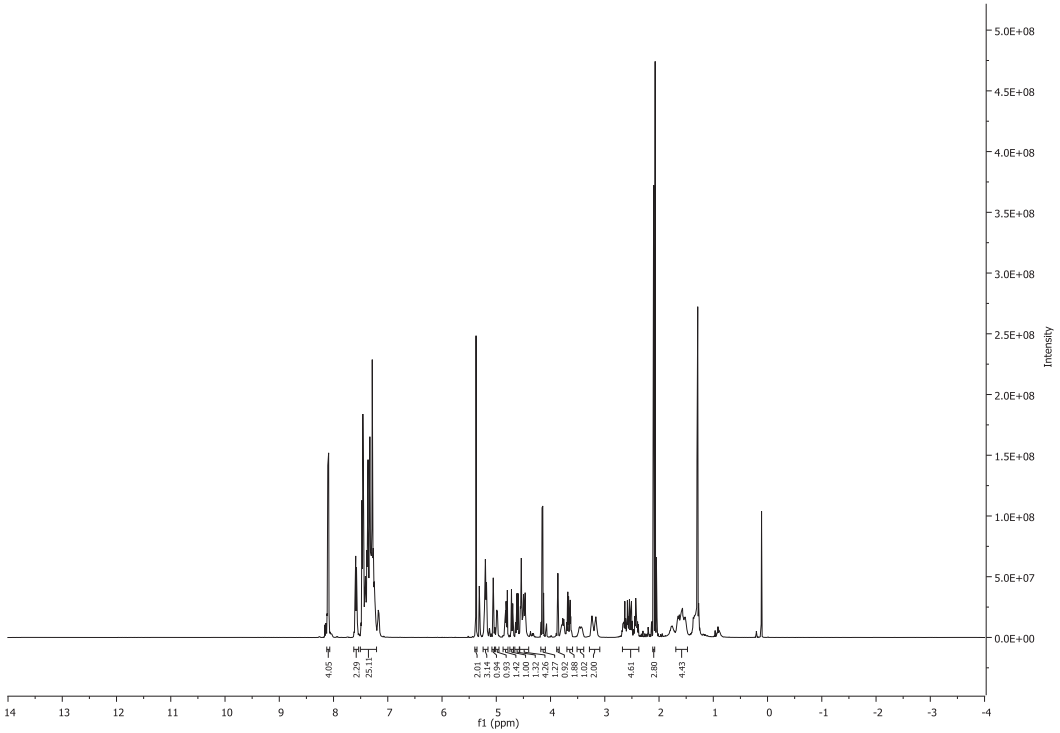


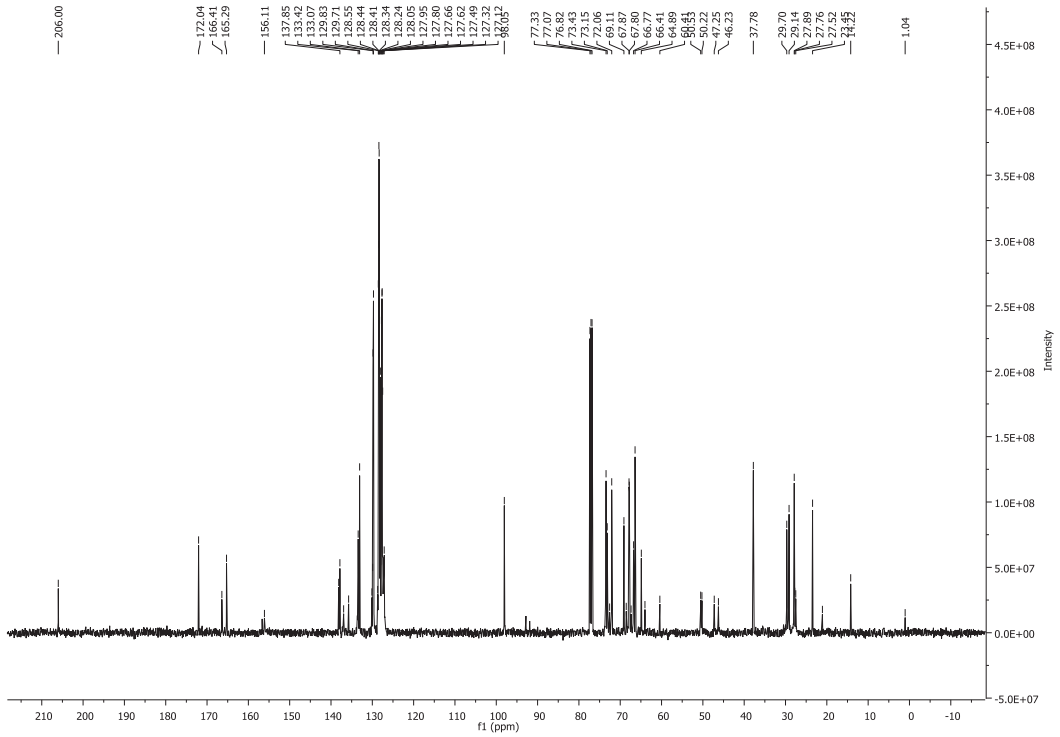
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (**86**):



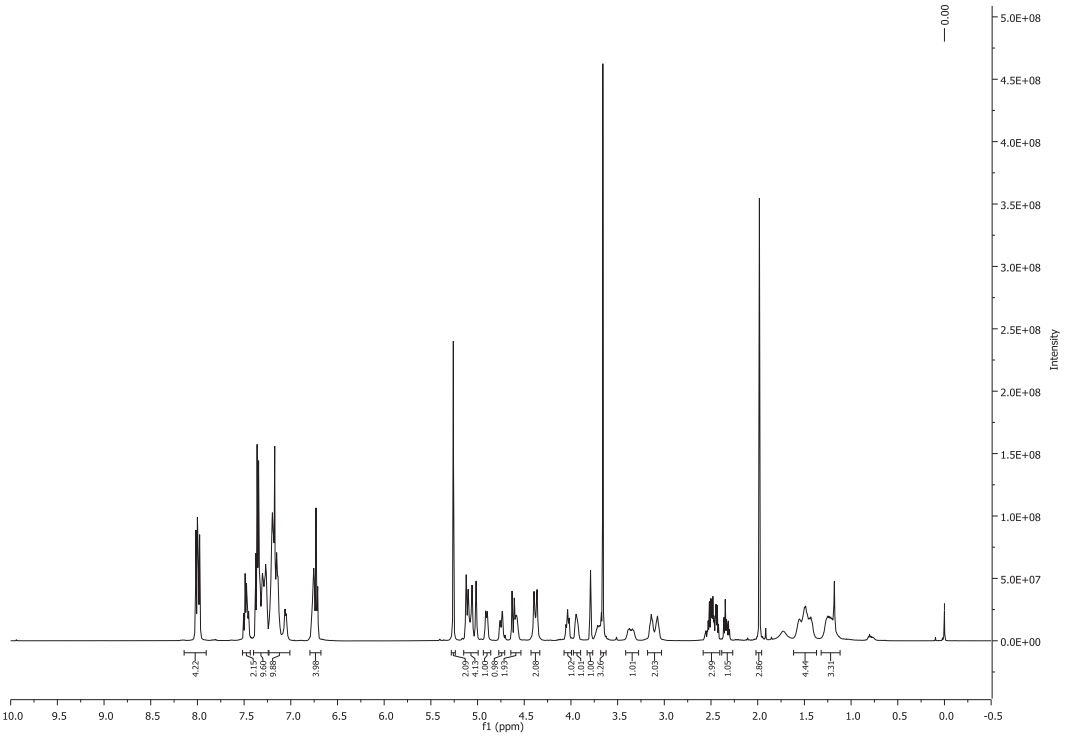


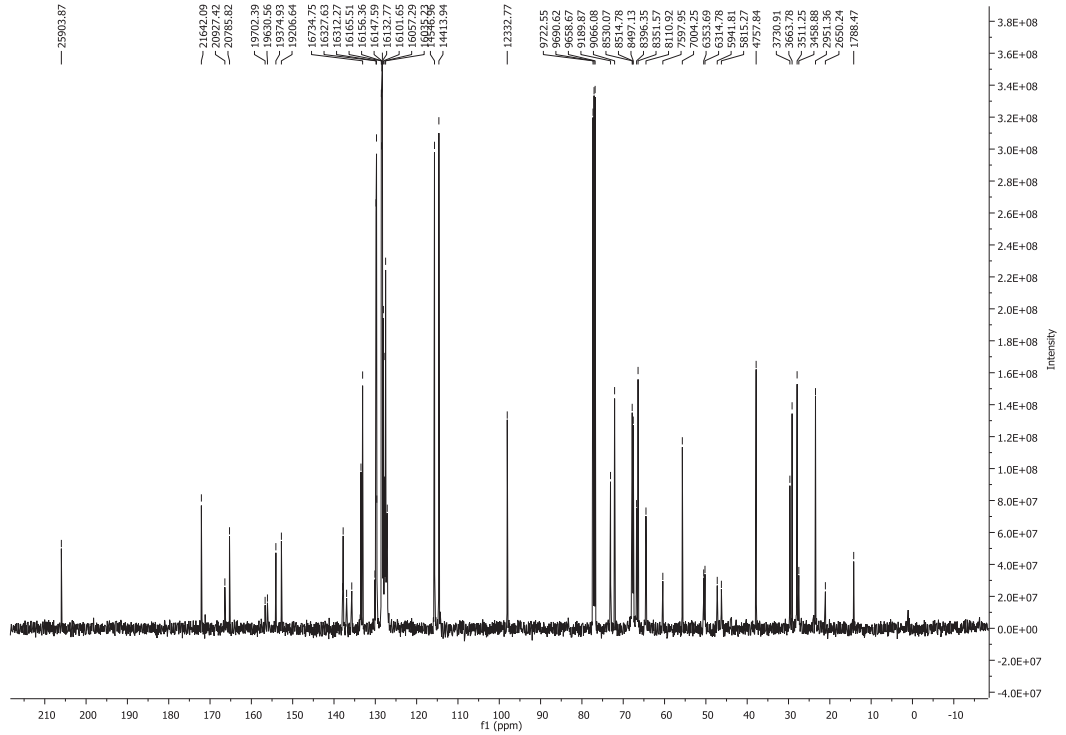
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)pentyl) carbamate (**87**):



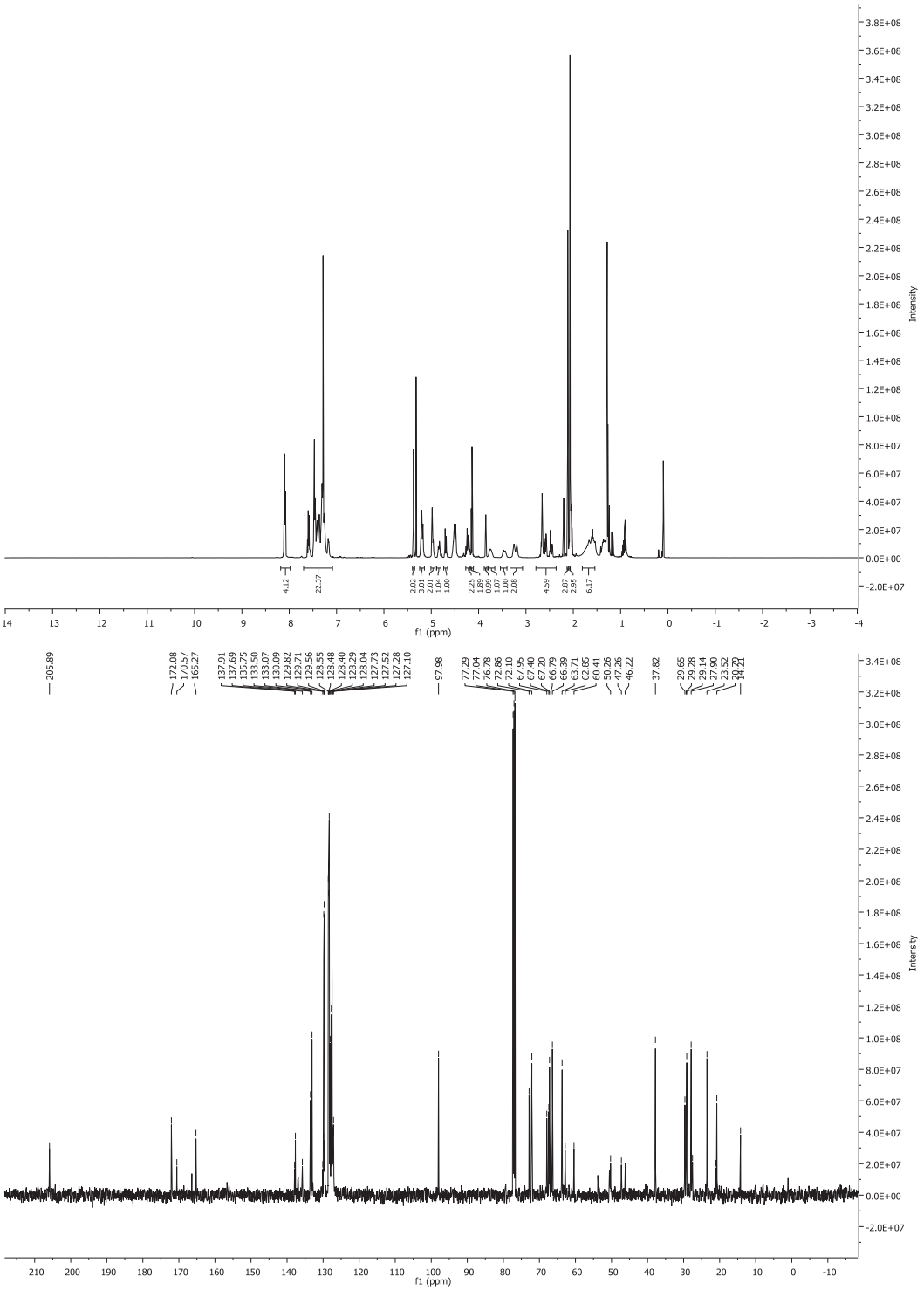


4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*para*-methoxybenzyl-α-L-idopyranosyloxy)pentyl) carbamate (**88**):

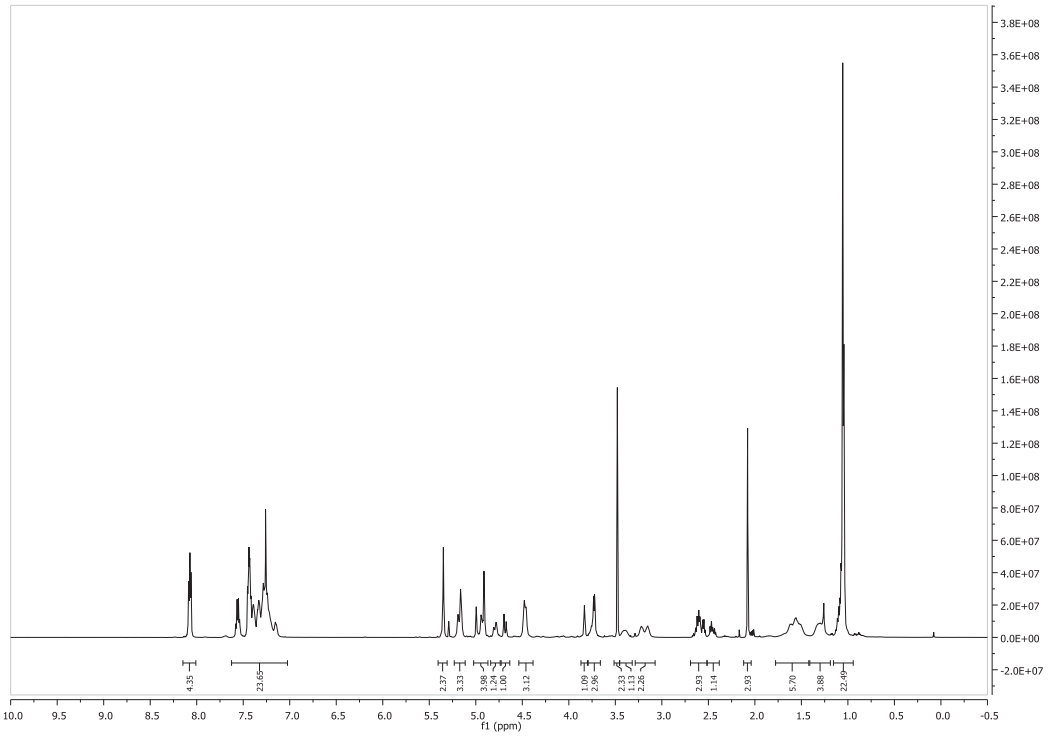


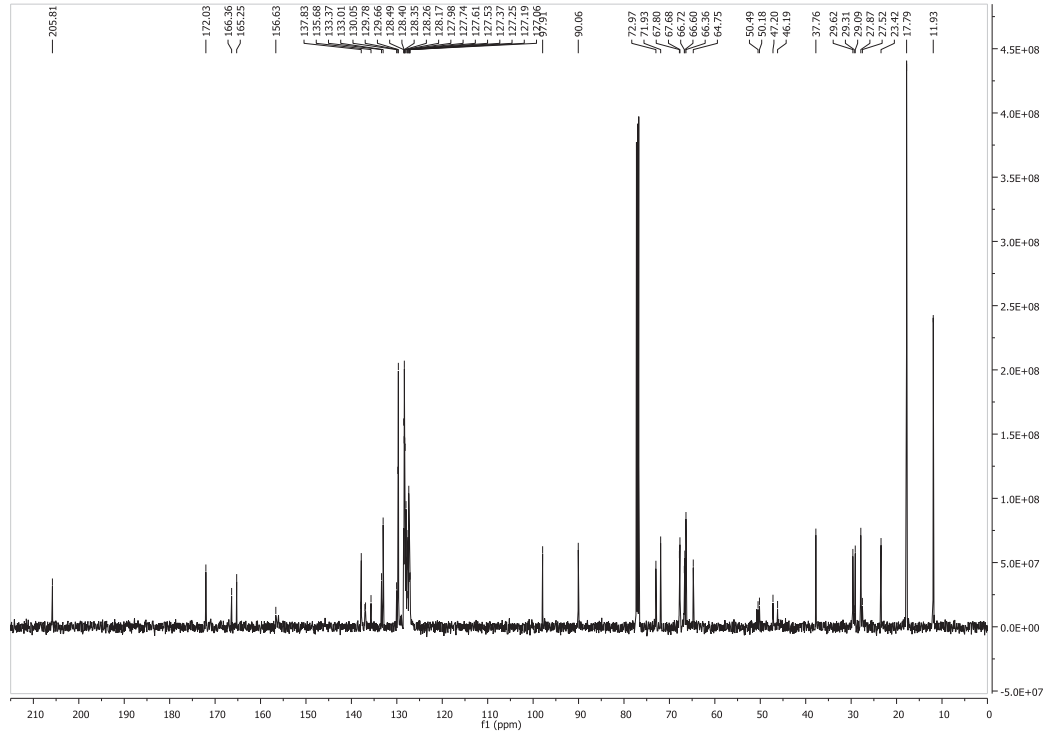


4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(6-*O*-acetyl-2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)pentyl) carbamate (**89**):

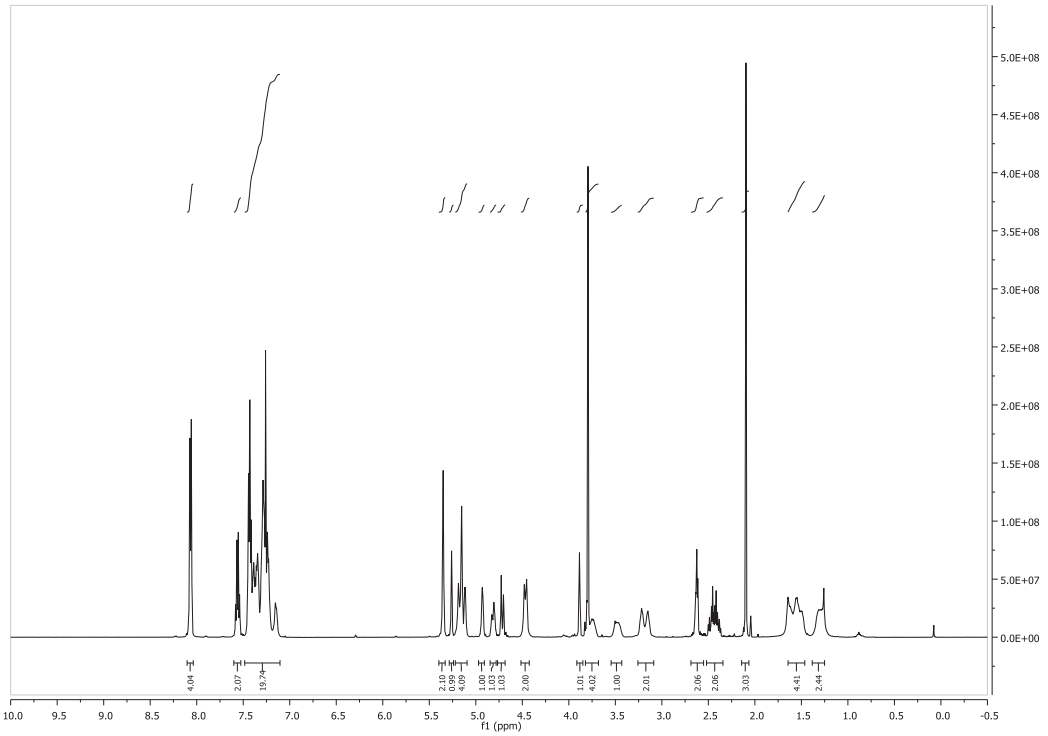


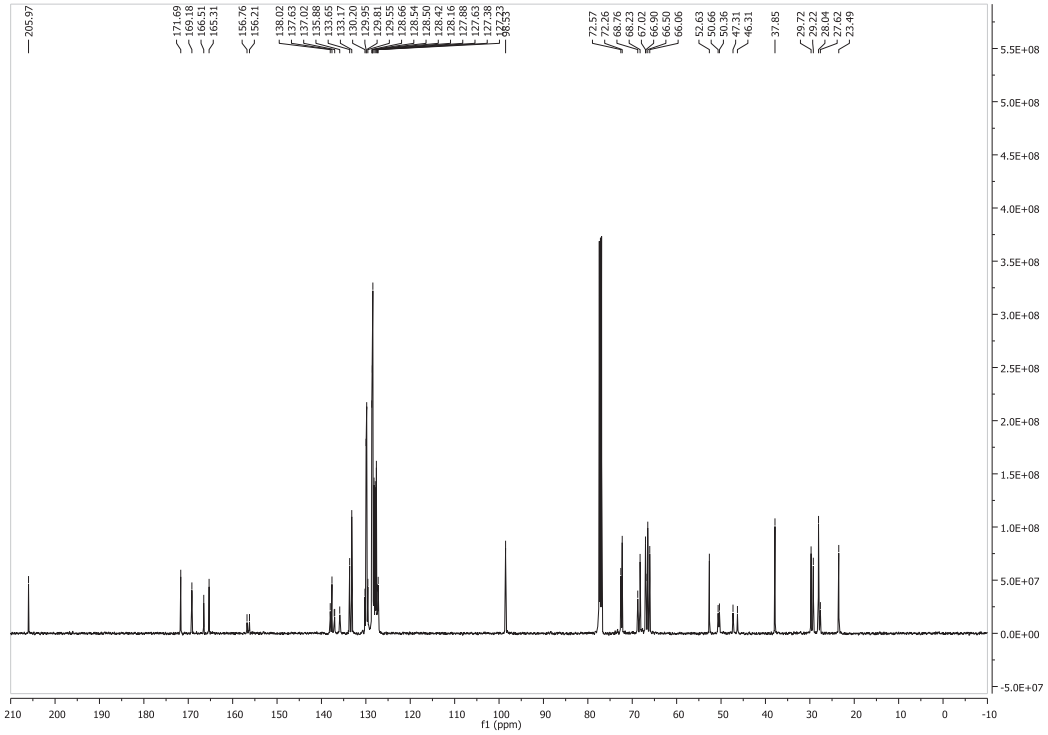
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-tri-*iso*-propylsiloxymethyl-α-L-idopyranosyloxy)pentyl) carbamate (**90**):



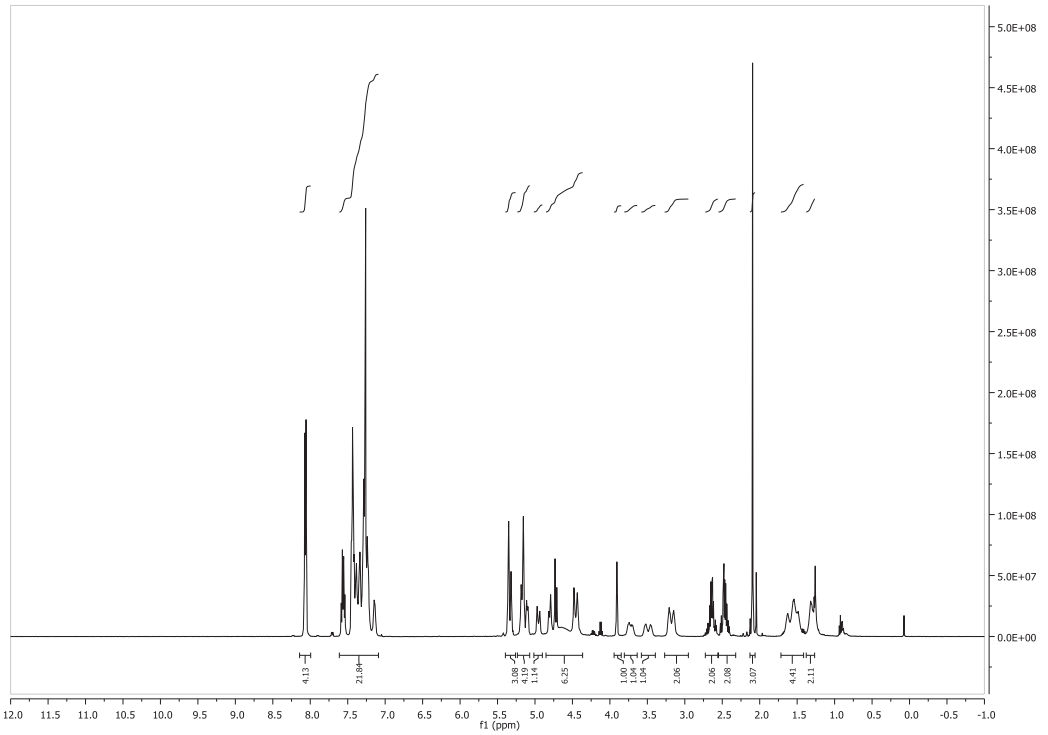


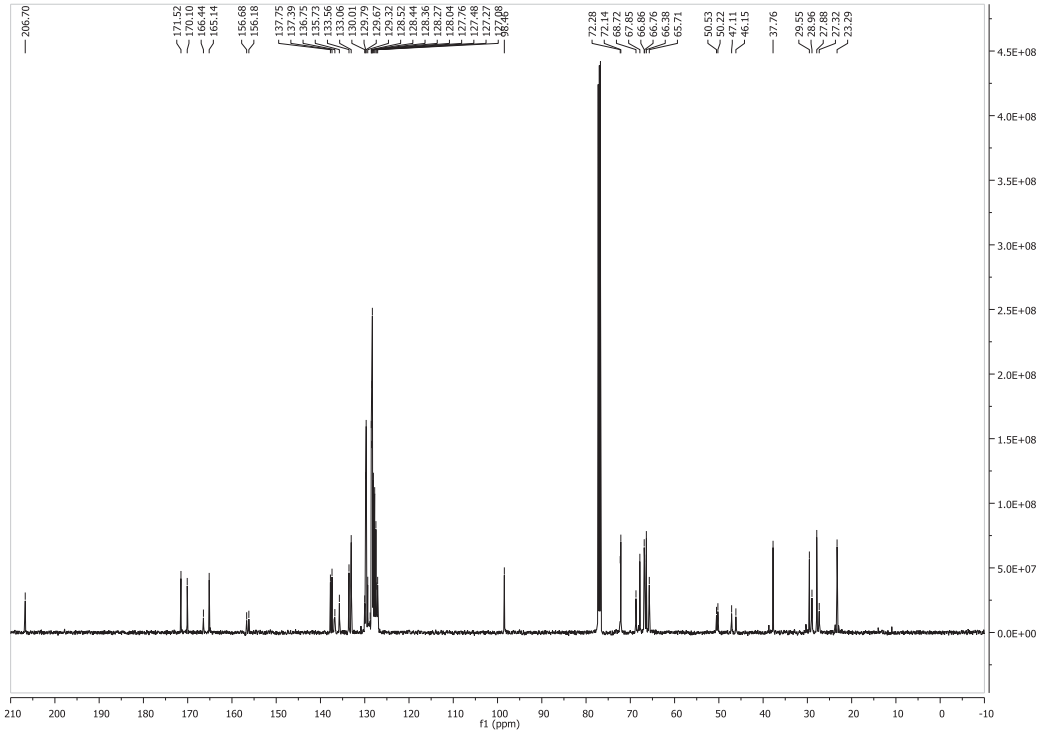
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**91**):





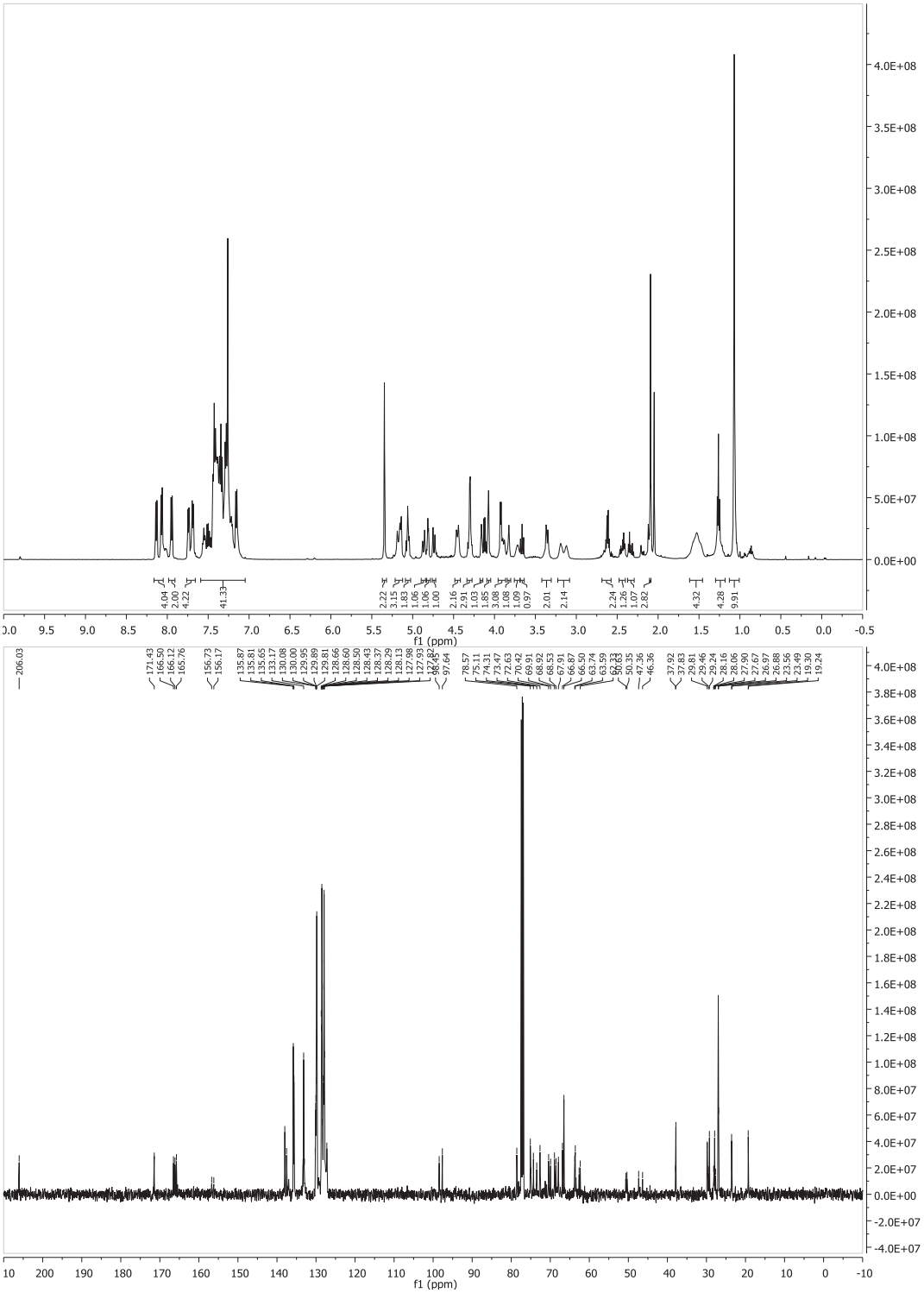
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-((3-*O*-benzyl-2-*O*-benzoyl-4-*O*-levulinoyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**92**):



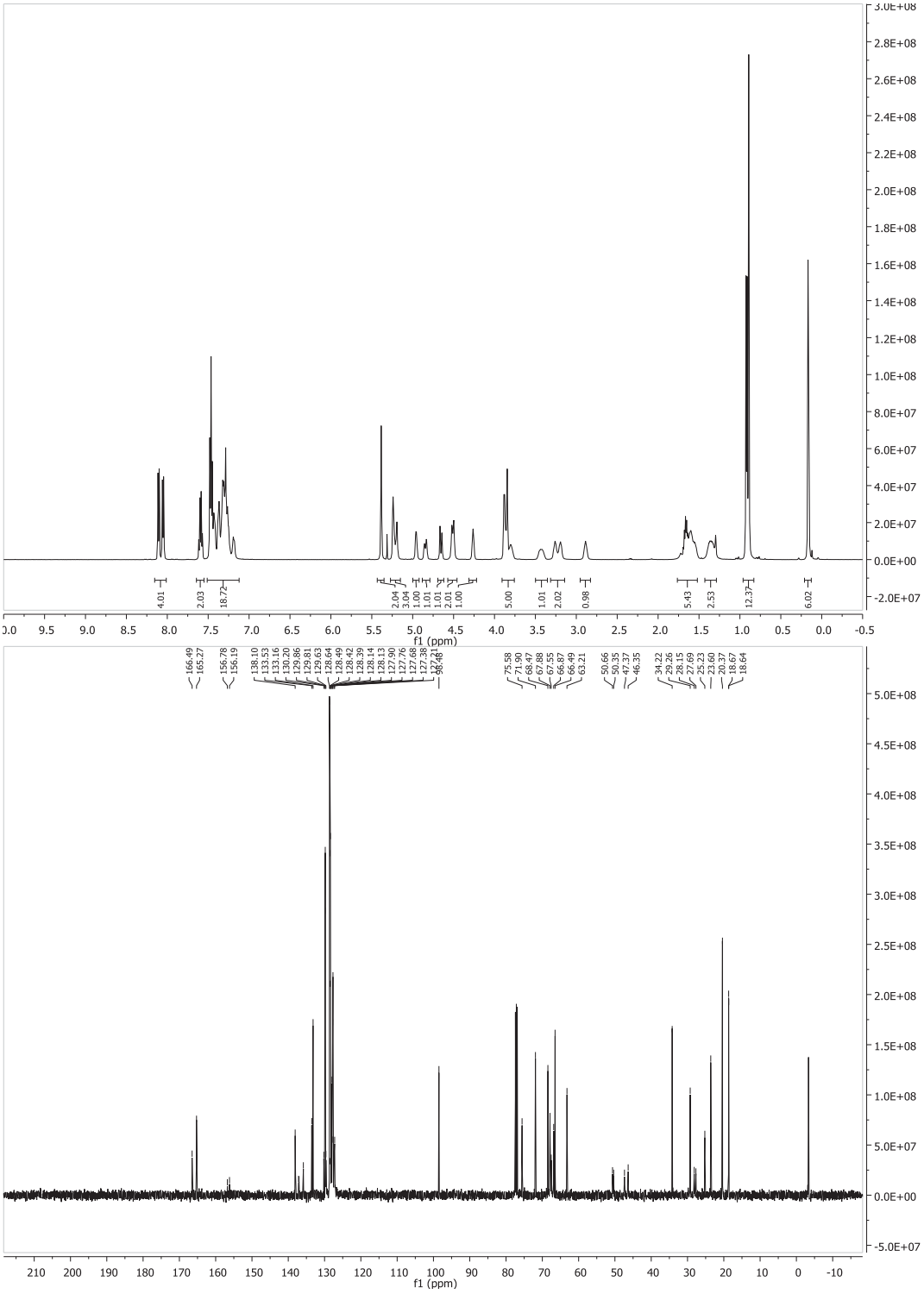


4.7.2 Disaccharide synthesis

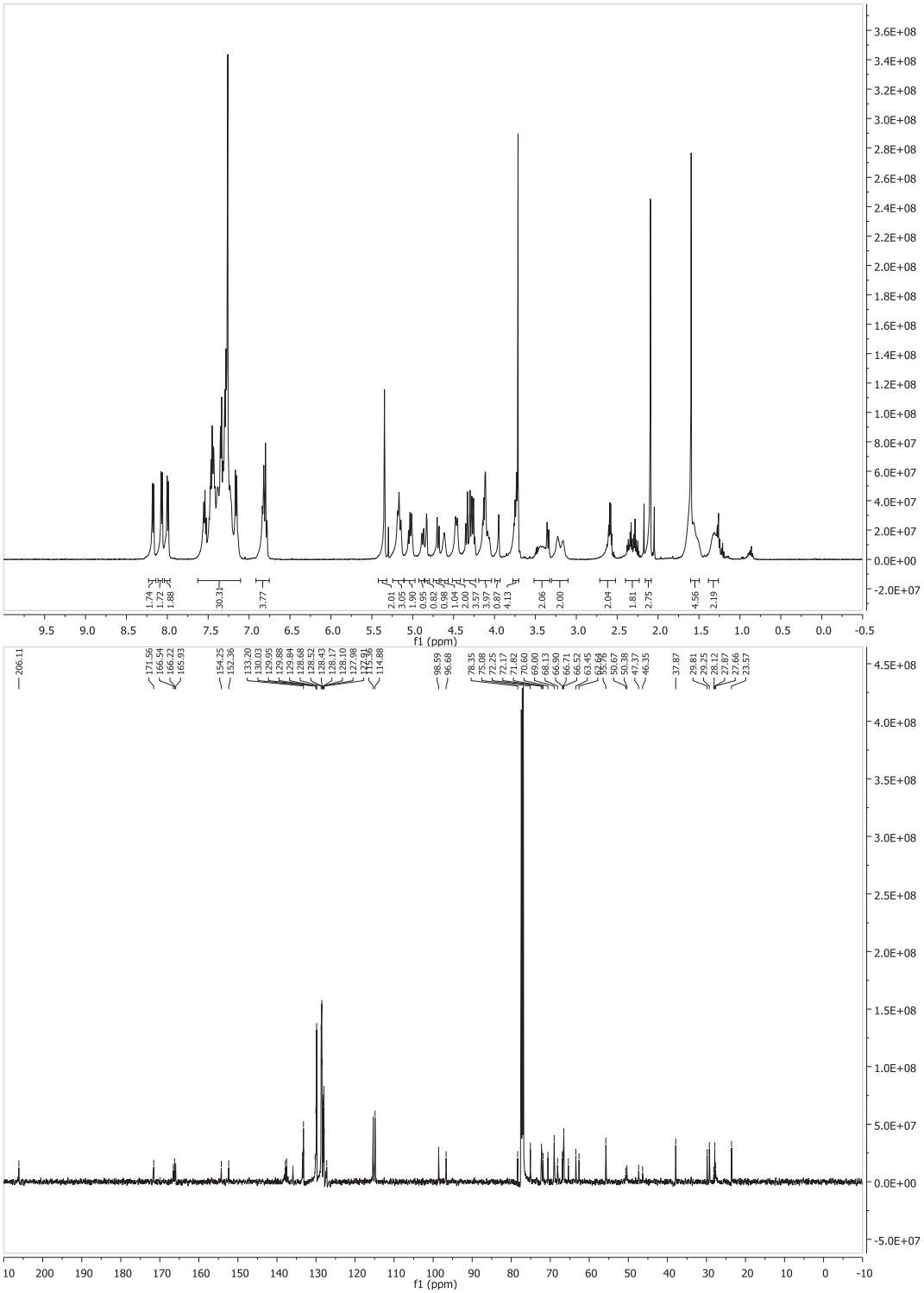
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**101**):



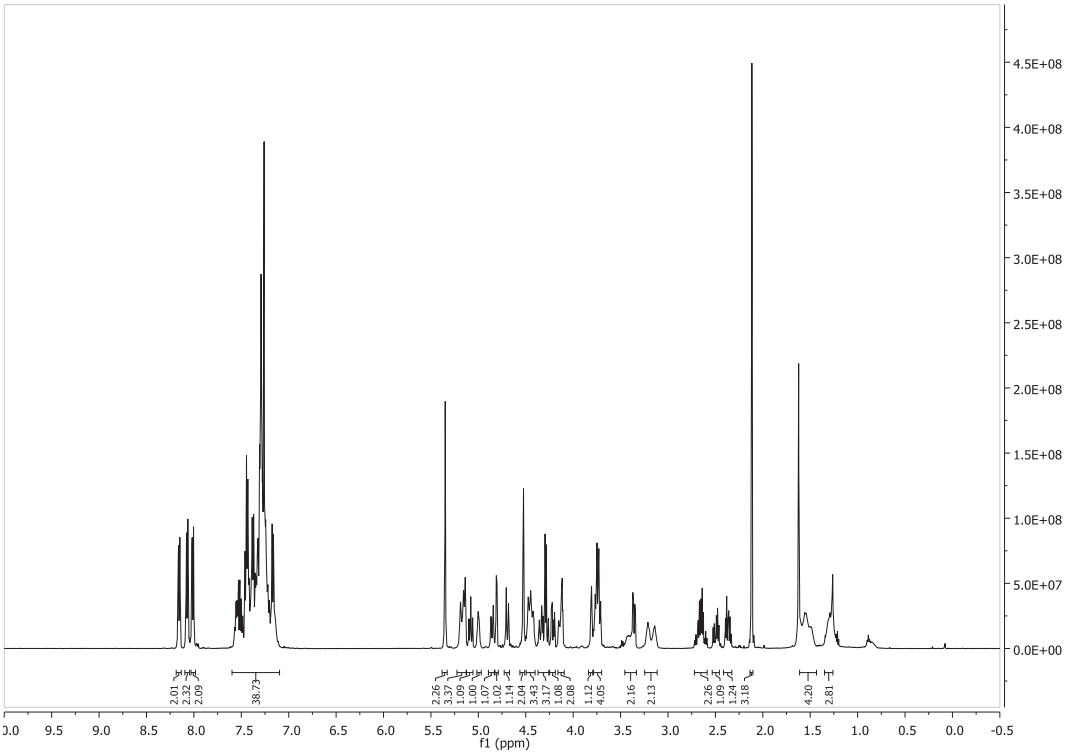
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-6-*O*-dimethylthexylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**102**):

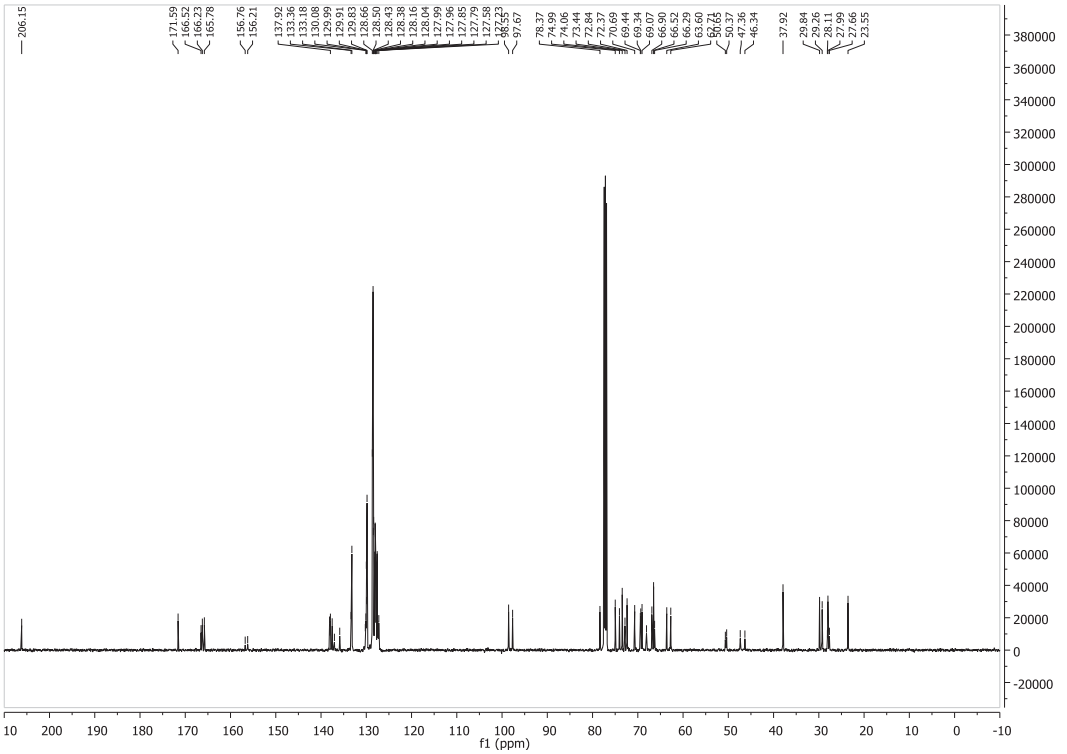


4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate **(103):**

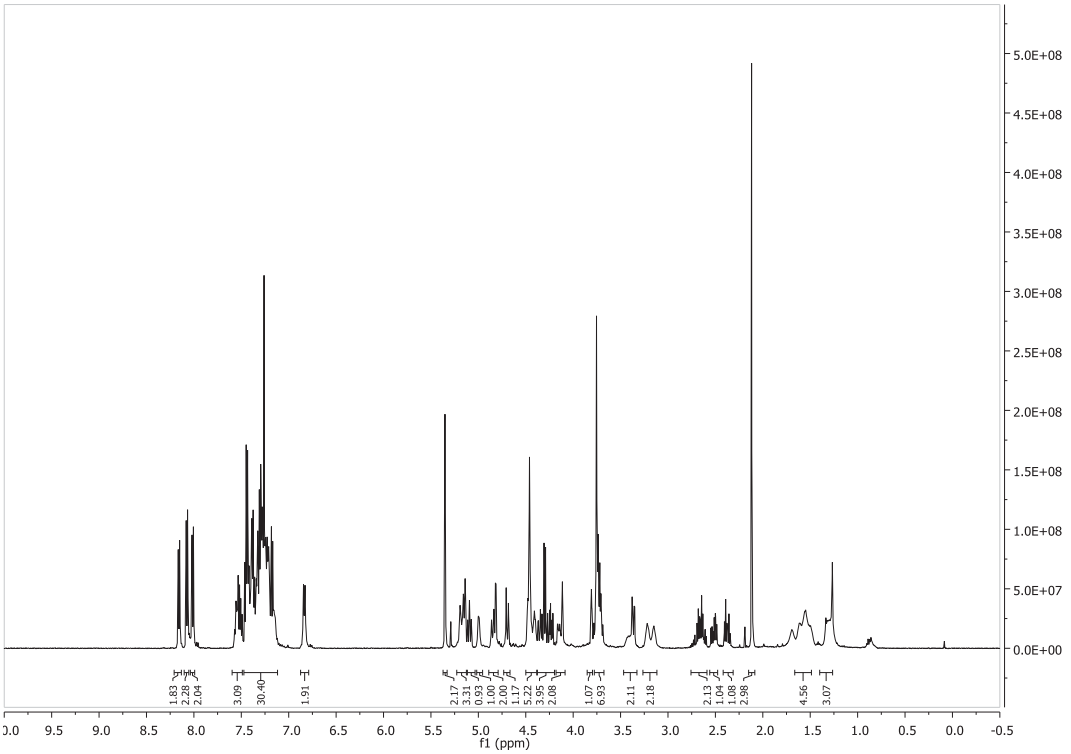


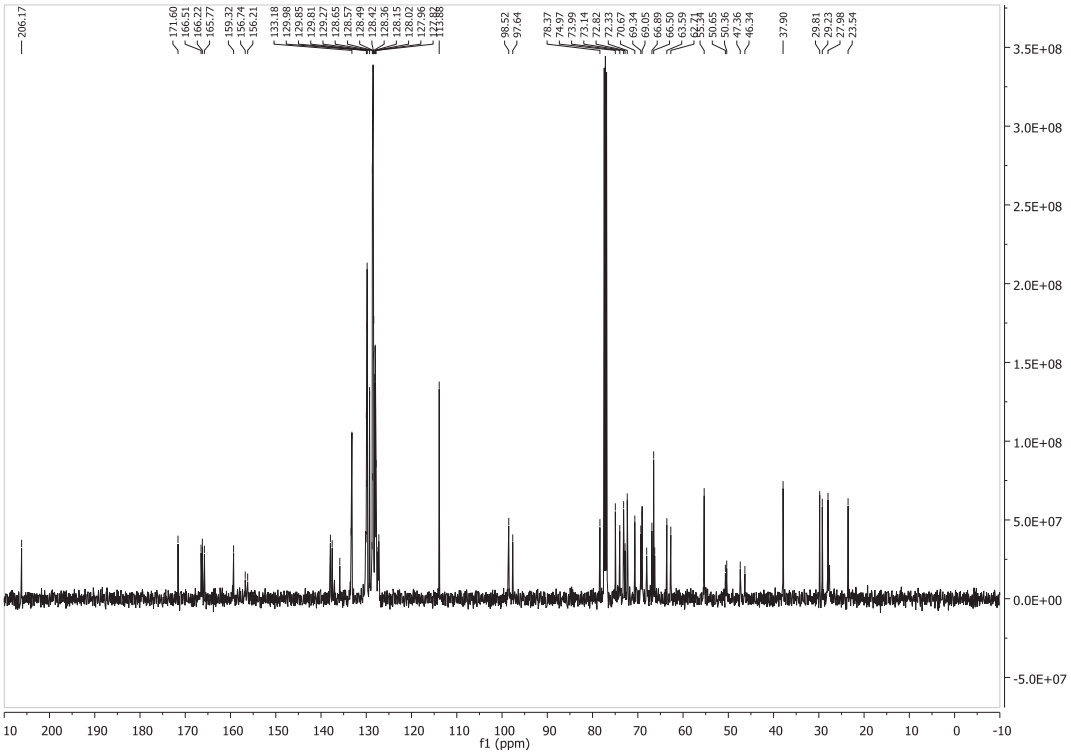
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-3,6-di-*O*-benzyl-2-*O*-benzoyl-α-L-idopyranosyloxy)pentyl) carbamate (**104**):



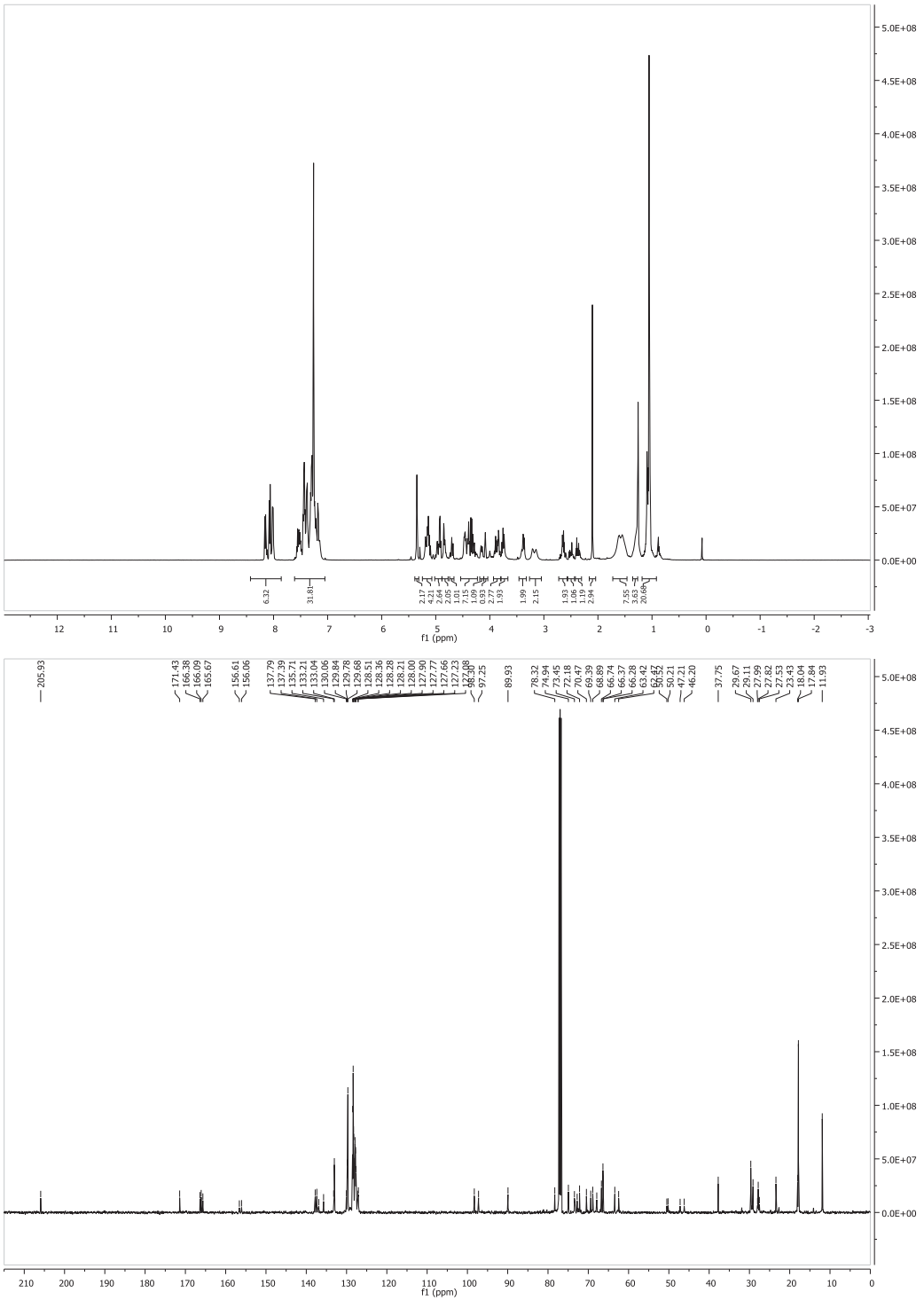


4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-6-*O*-p-methoxybenzyl-α-L-idopyranosyloxy)pentyl) carbamate (**105**):

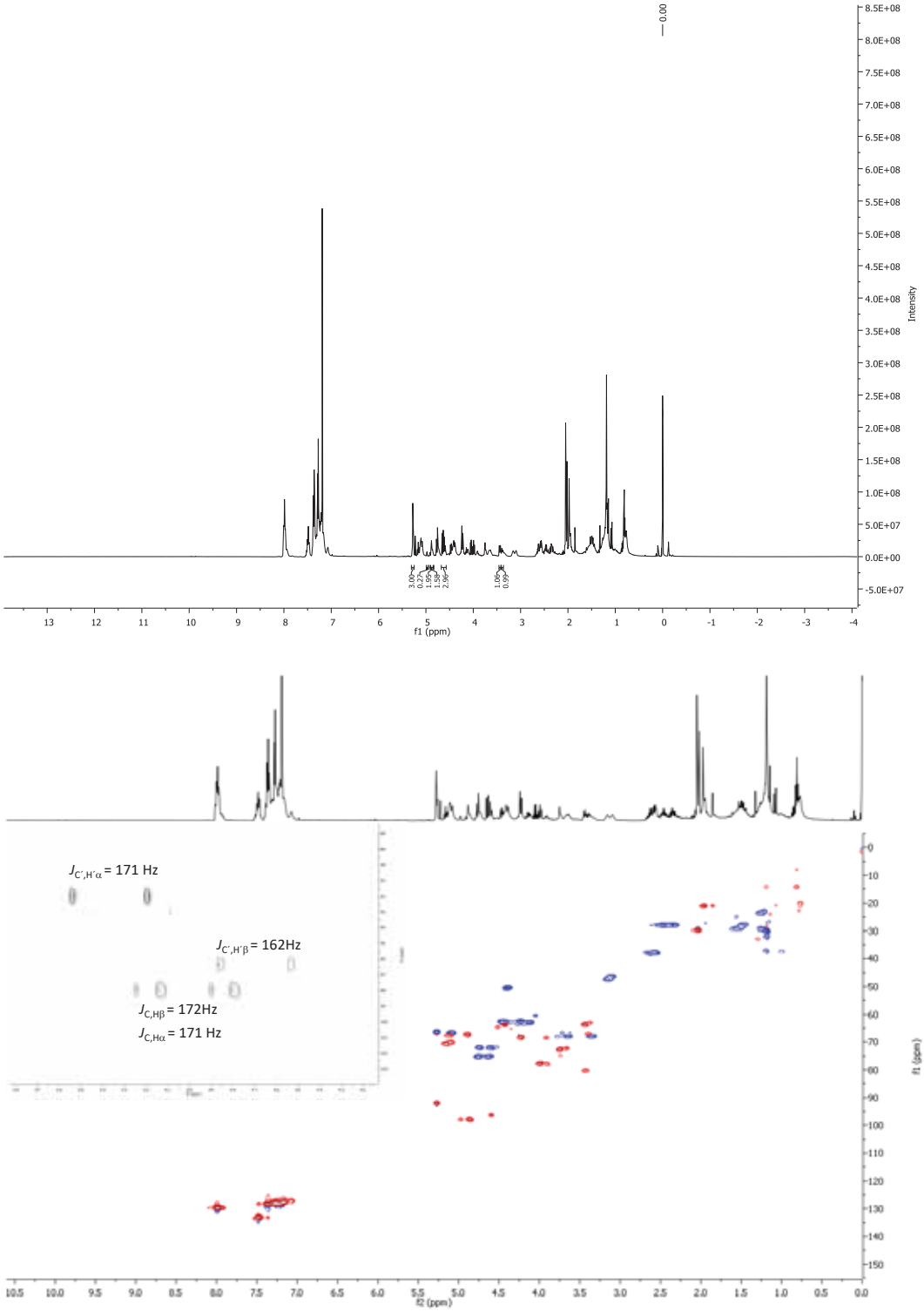




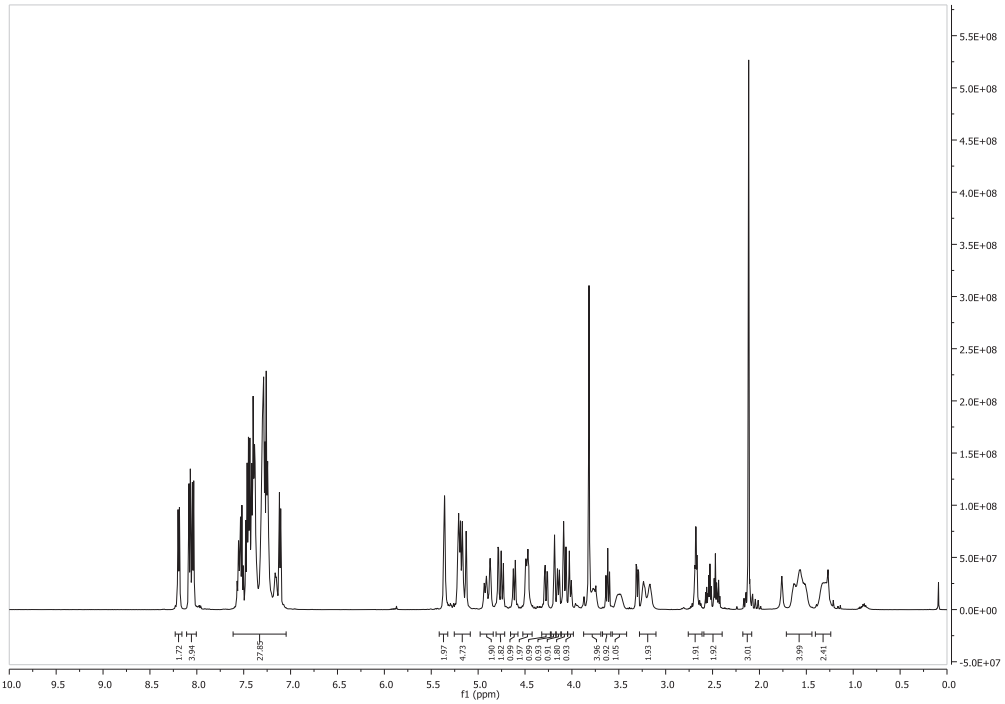
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-tri-*iso*-propylsiloxymethyl-α-L-idopyranosyloxy)pentyl) carbamate (**106**):

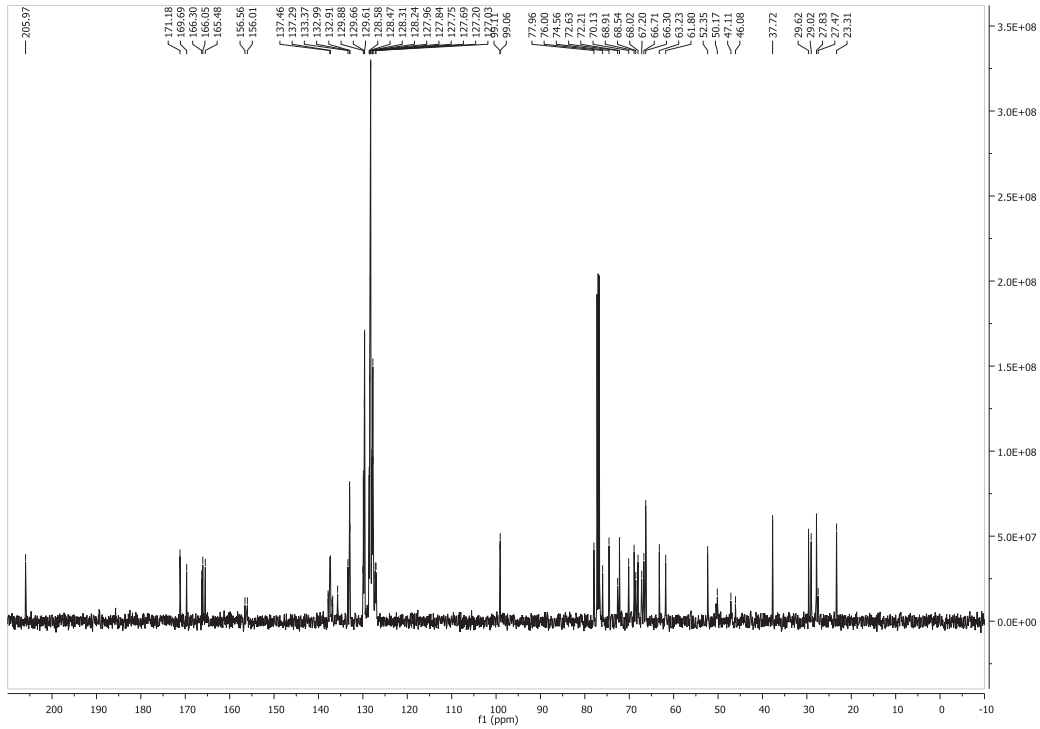


4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-6-*O*-acetyl-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)pentyl) carbamate (**107**):

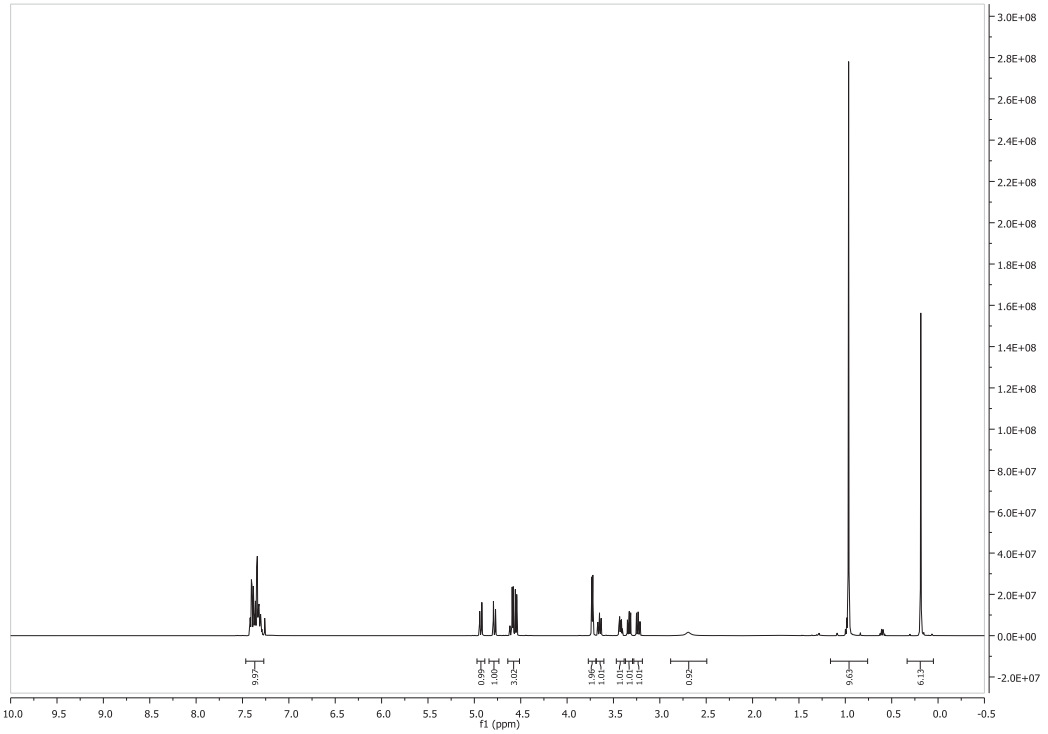


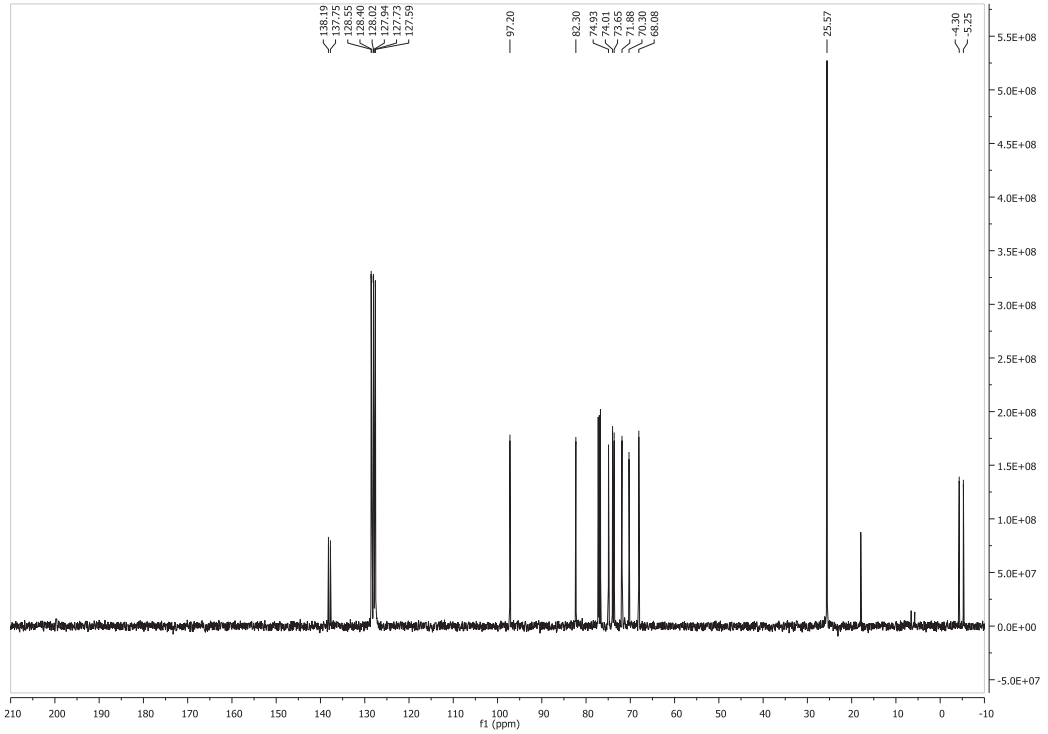
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**108**):



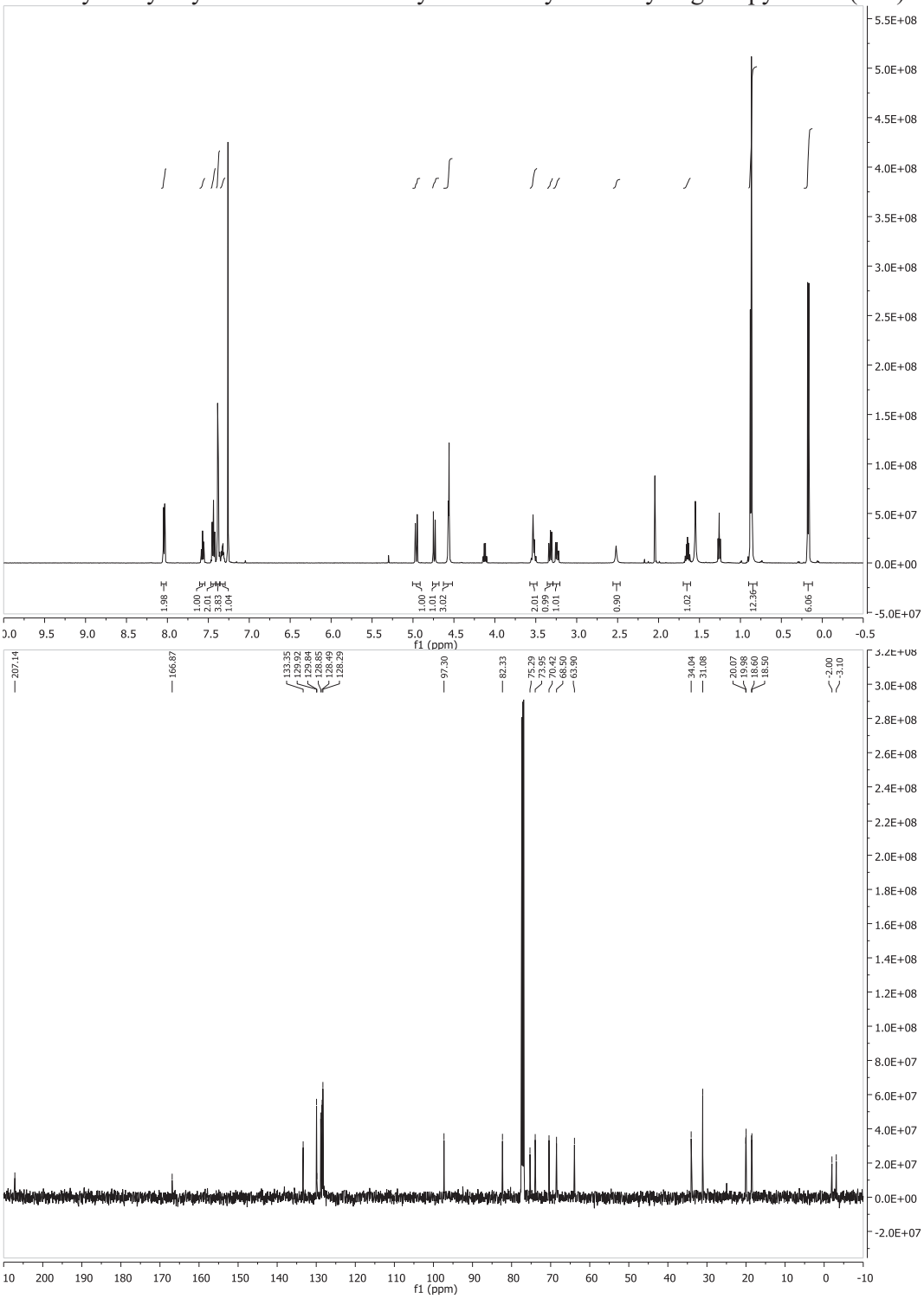


tert-Butyldimethylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranose (**109**):

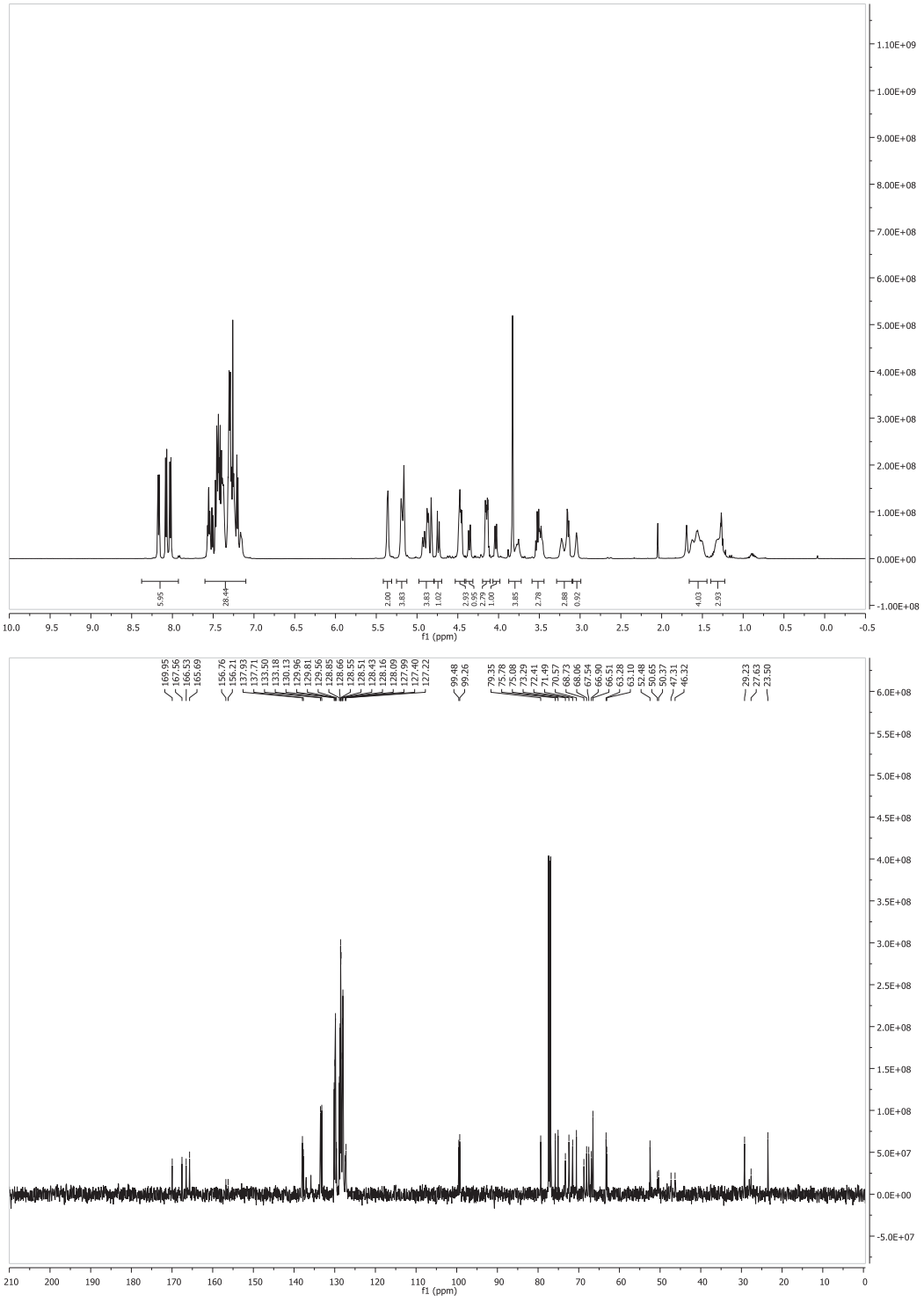




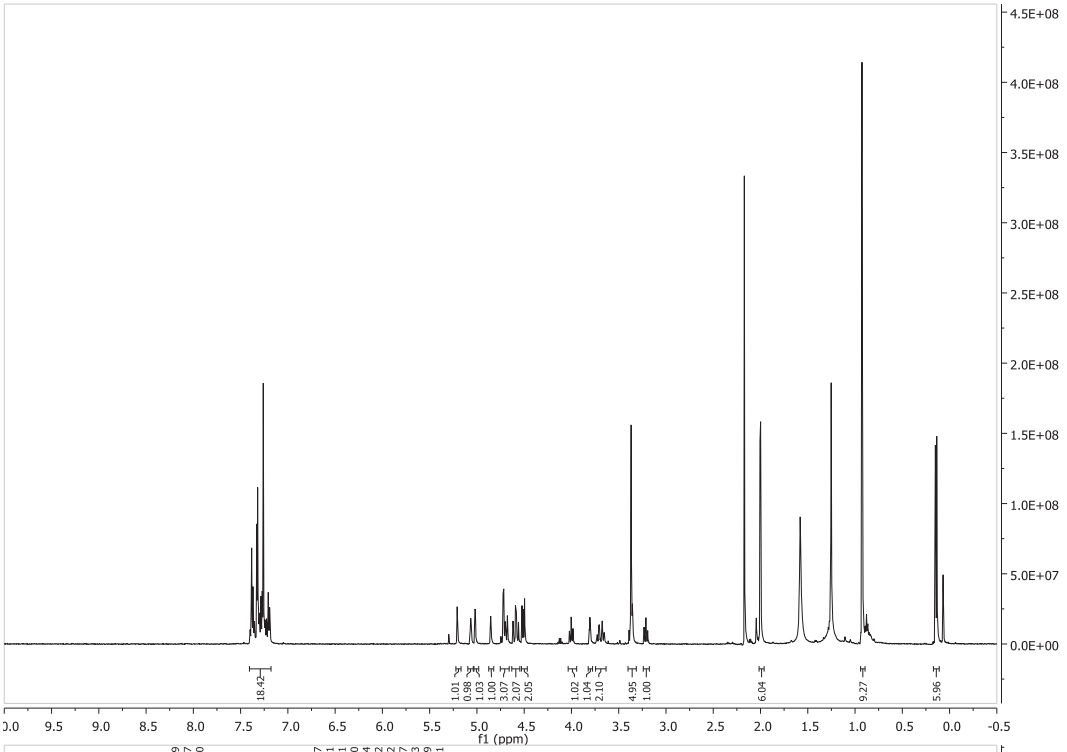
Dimethylthexylsilyl 2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-D-glucopyranose (**110**):

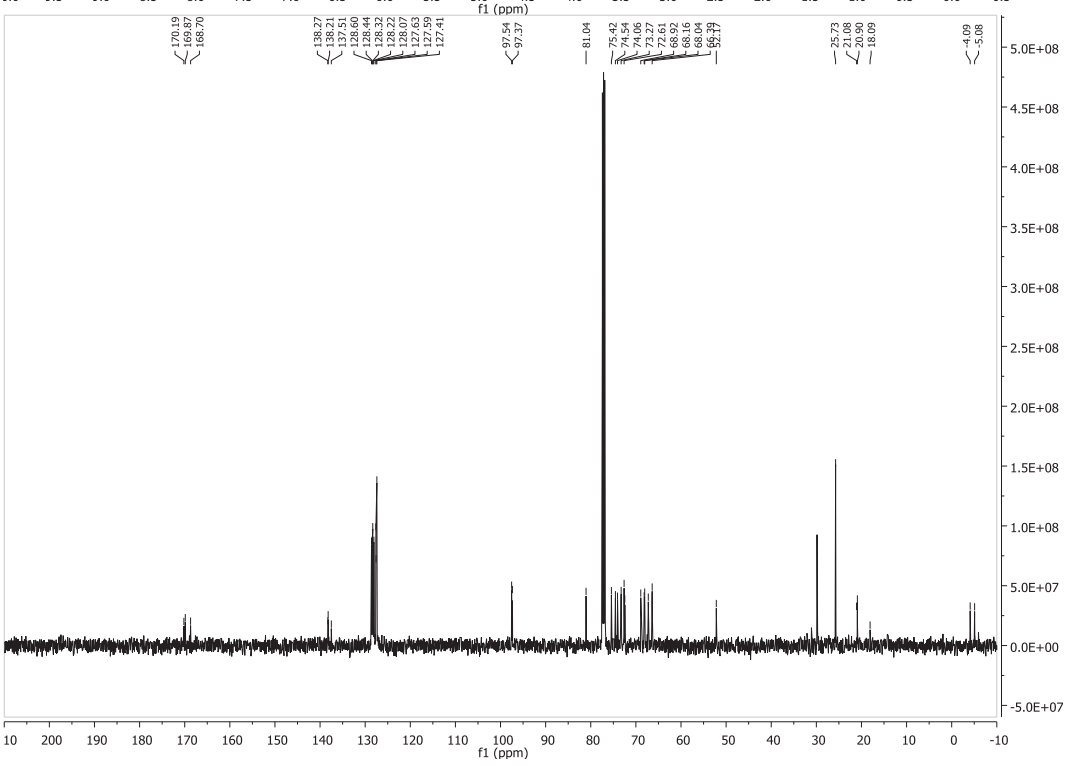


4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (**55**):

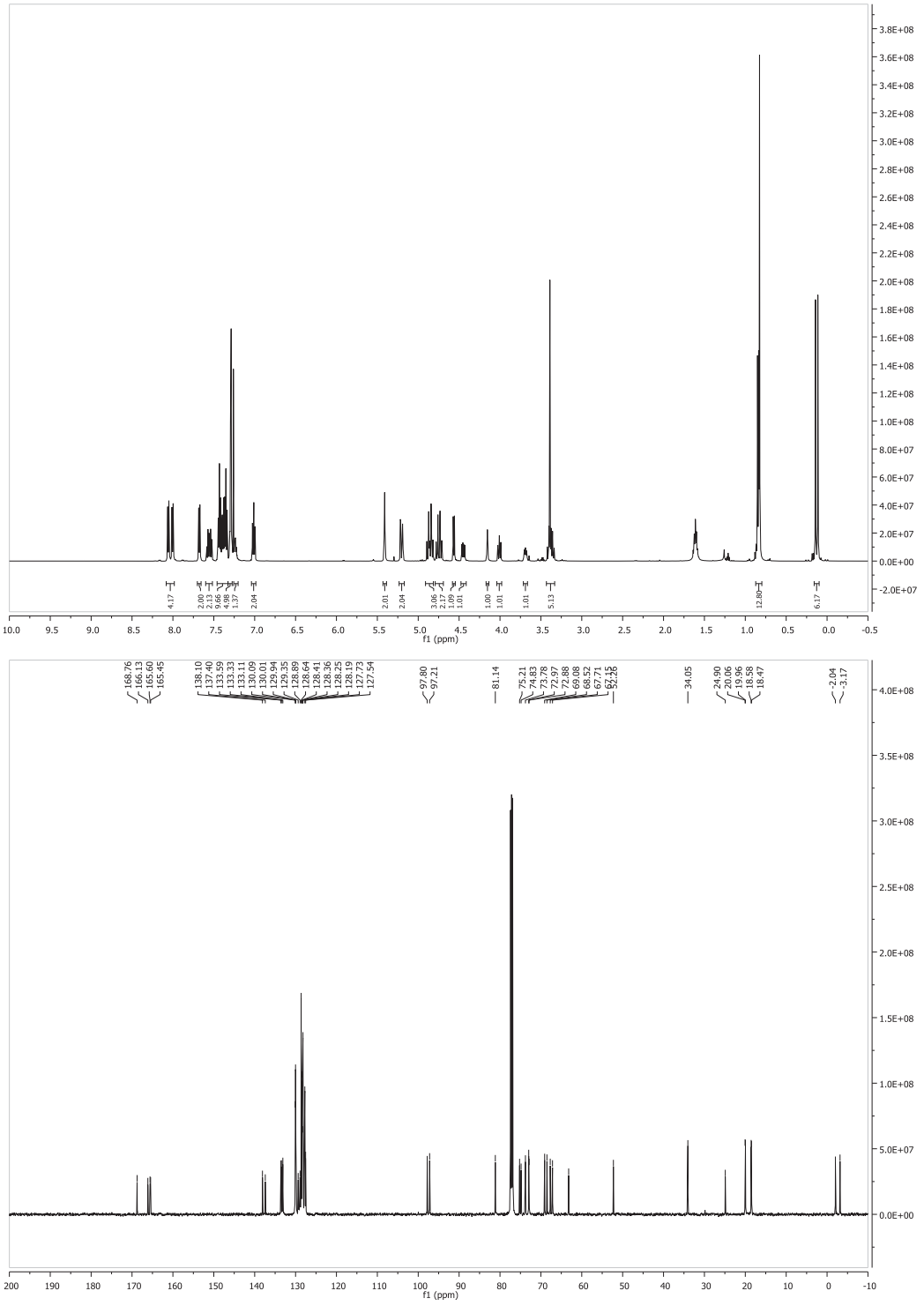


Tert-Butyldimethylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl)uronate)-D-glucopyranose (**111**):


18.42
1.01
0.98
1.03
1.00
2.07
2.05
1.02
1.04
2.10
4.95
1.00
6.04
9.27
5.96
f1 (ppm)

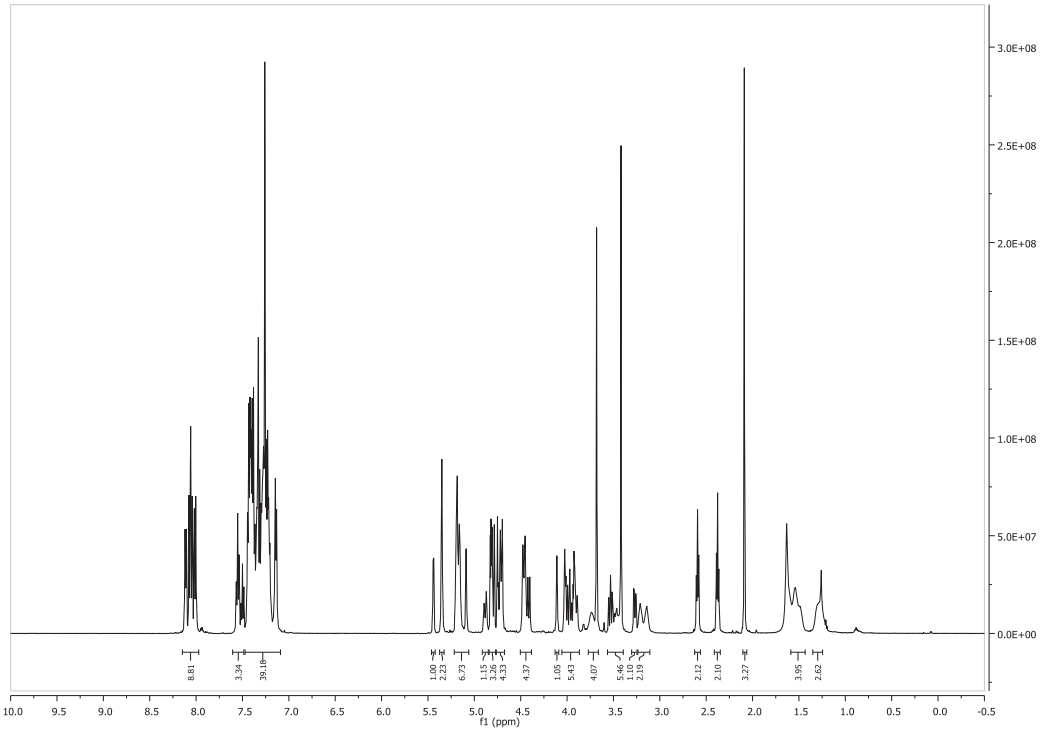

170.19
169.87
168.70
138.27
138.21
137.51
128.60
128.44
128.33
128.22
128.07
127.63
127.59
127.41
97.54
97.37
81.04
75.42
74.54
74.06
73.27
72.61
68.92
68.16
68.04
66.39
52.17
25.73
21.08
20.90
18.09
-4.09
-5.08
f1 (ppm)

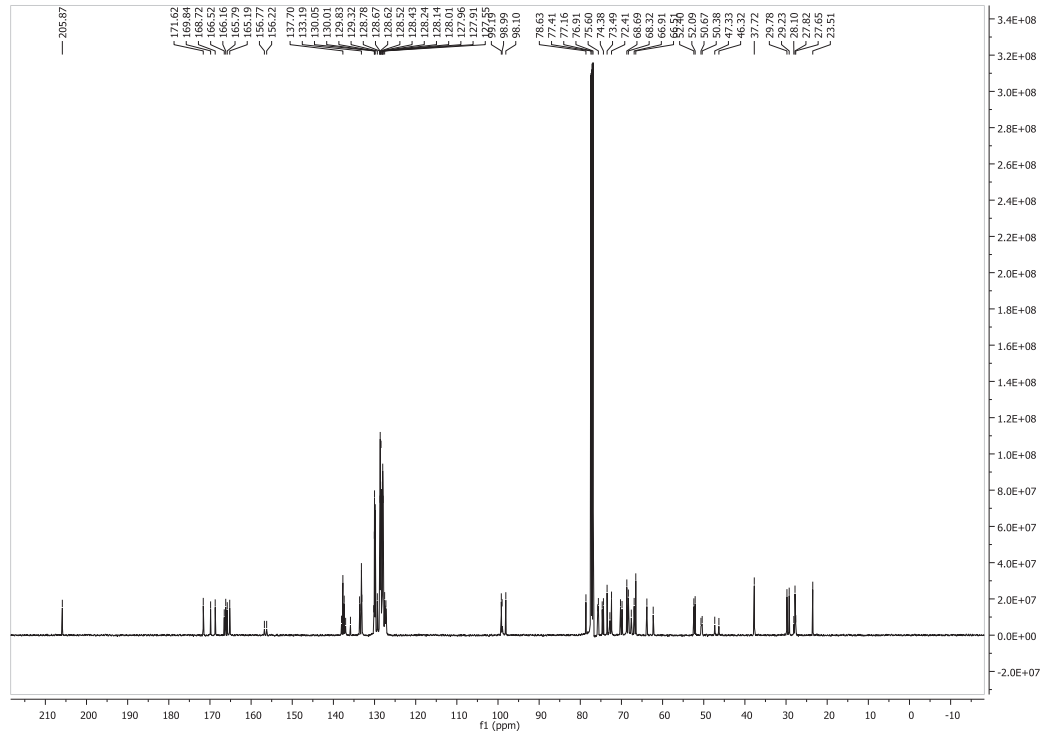
Dimethylthexylsilyl 2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(methyl (2,4-di-*O*-benzoyl-3-O-benzyl-α-L-idopyranosyl)uronate)-D-glucopyranose (**113**):



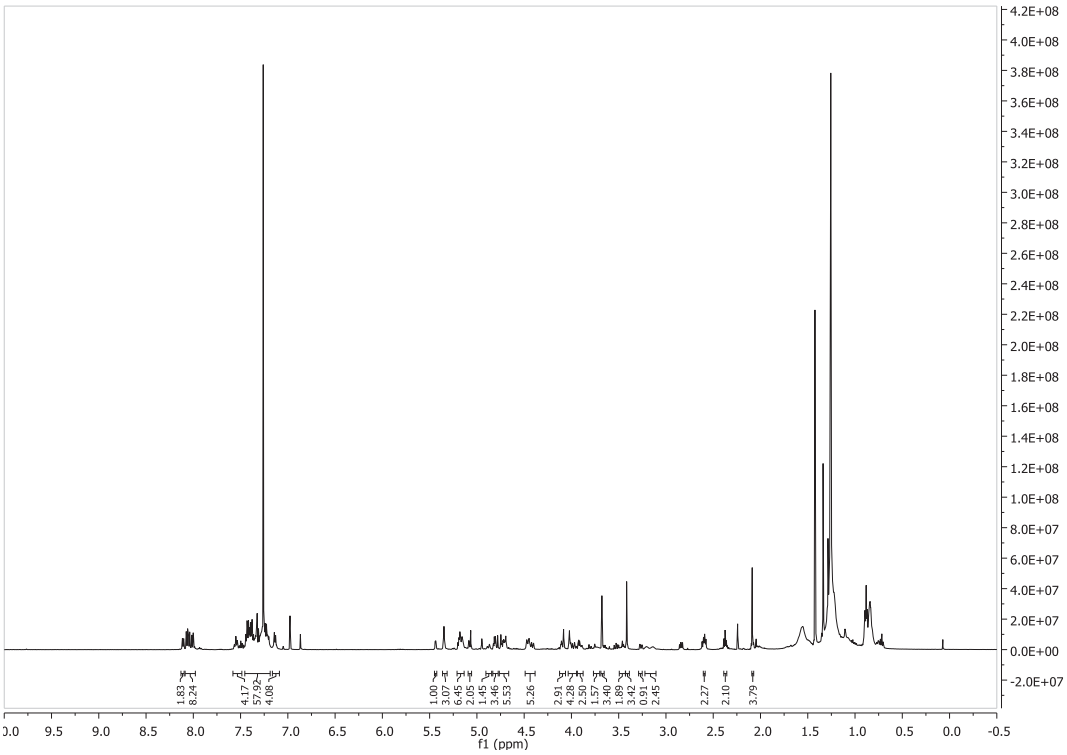
4-(Phenylcarboxymethyl)benzyl *N*-benzyl *N*-(5-(methyl (4-*O*-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-O-(methyl(2,4-di-*O*-benzoyl-3-O-benzyl-D-L-

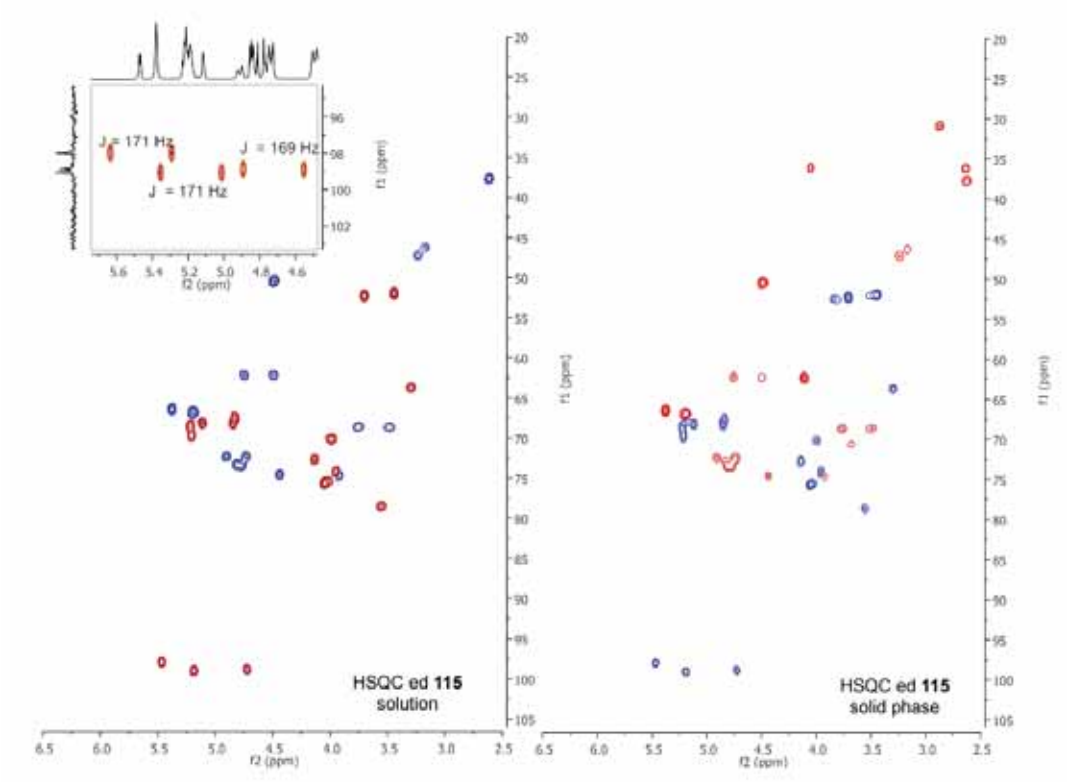
idopyranosyl)uronate)-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-D-L-idopyranosyloxy)uronate)pentyl) carbamate (**115**):





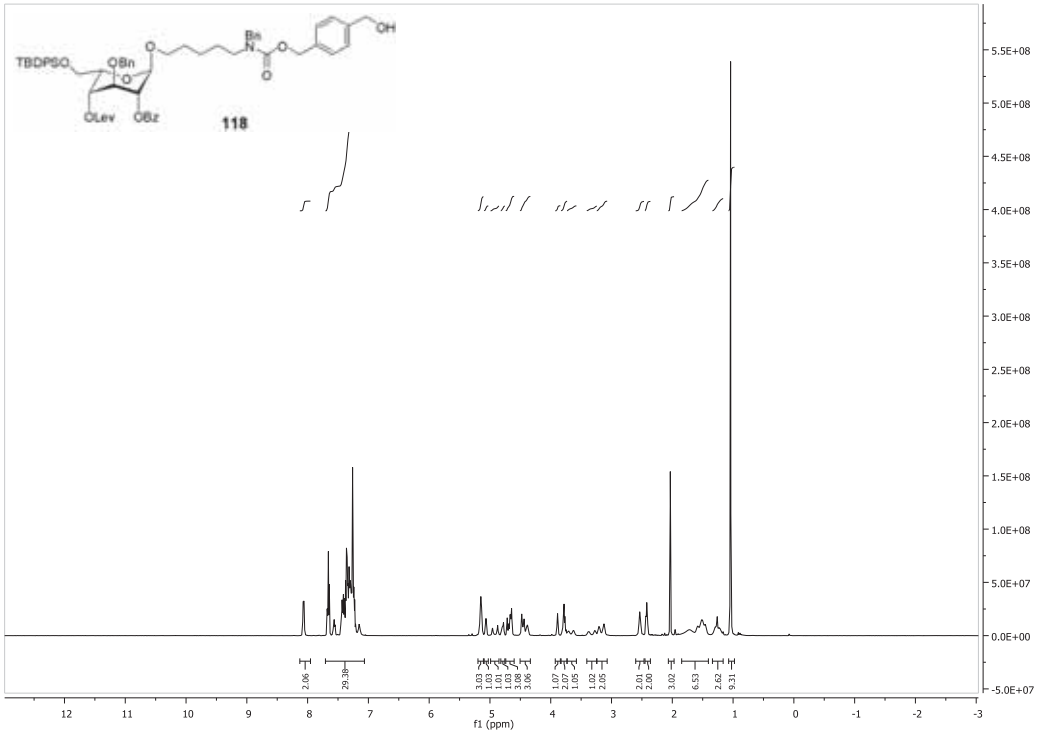
^{1}H NMR data of compound **115** cleaved from the resin:

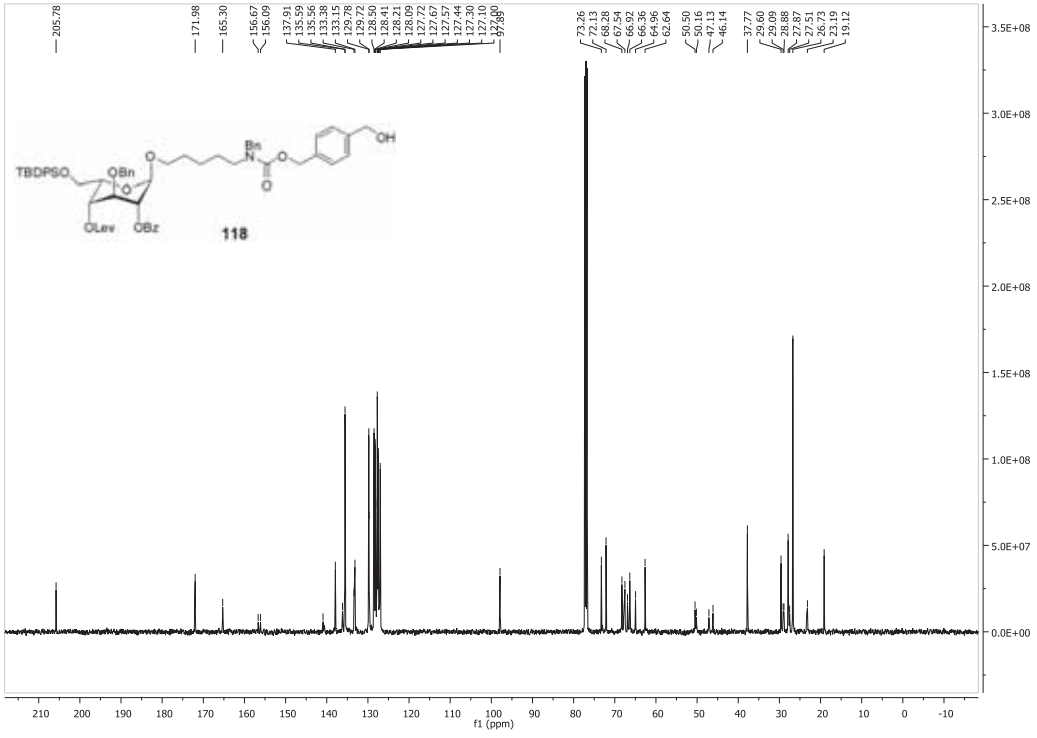




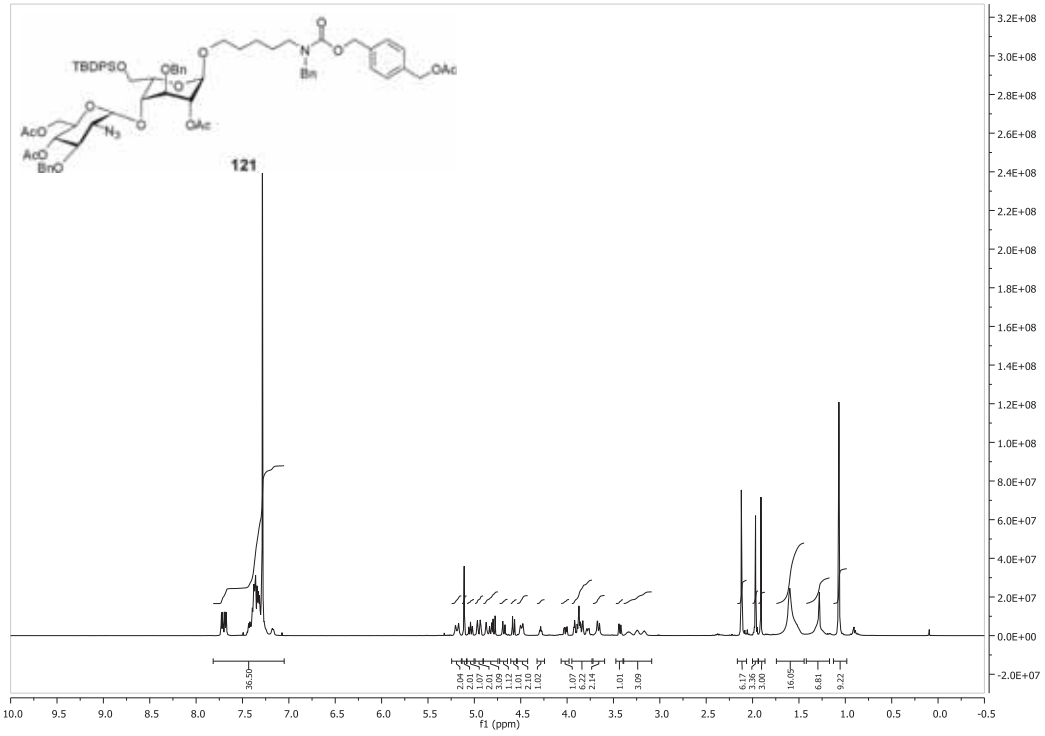
HSQC edited and HSQC-J coupled NMR (500MHz, $CDCl_3$) of compound **115**.

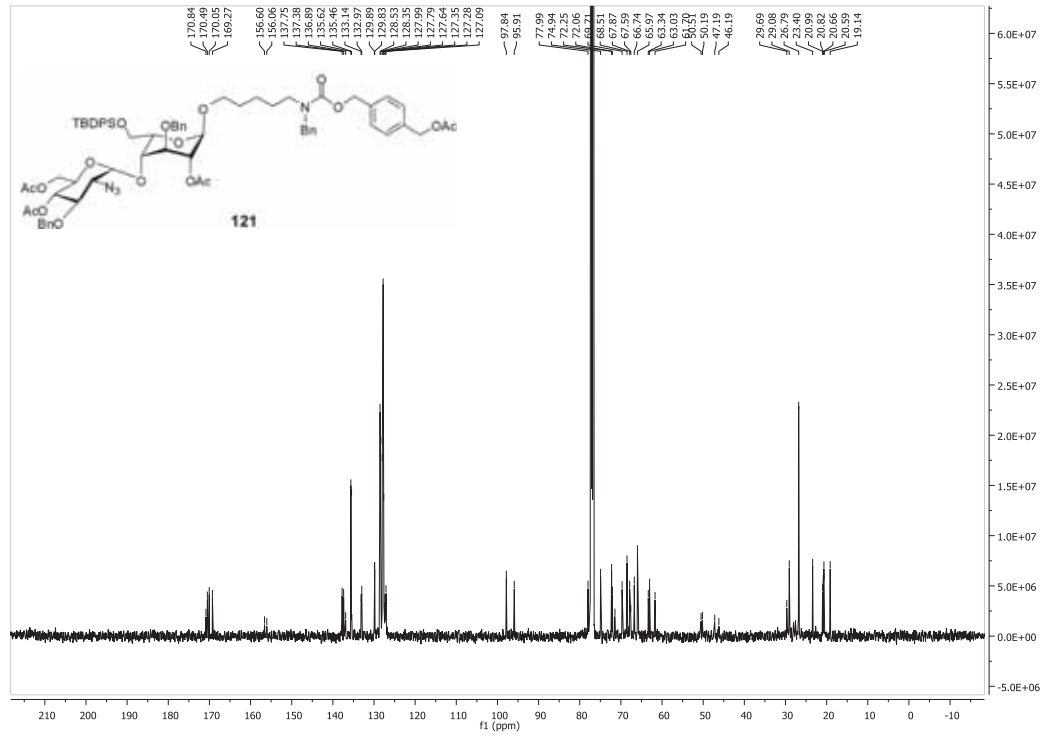
4-(Hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**118**):


TBDPSO
OBn
OLev
OBz
Bn
N
O
OH
118
f1 (ppm)

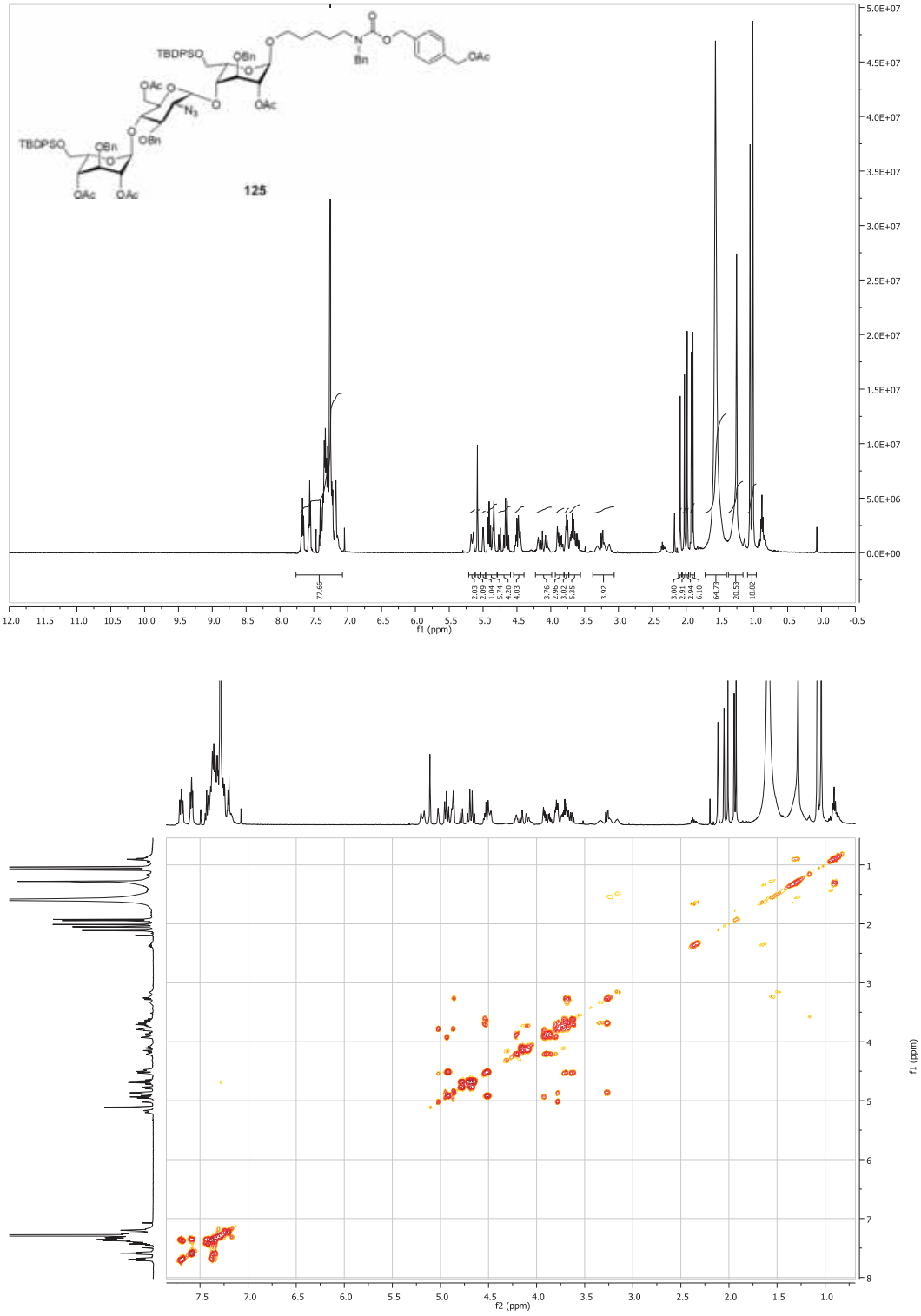

205.78
171.98
165.30
156.67
156.09
137.91
135.56
133.38
133.15
129.78
129.72
128.50
128.41
128.21
128.09
127.72
127.67
127.57
127.44
127.30
127.10
127.00
97.89
73.26
72.13
68.28
67.54
66.92
66.36
64.96
62.64
50.50
50.16
47.13
46.14
37.77
29.60
29.09
28.88
27.87
27.51
26.73
23.19
19.12
TBDPSO
OBn
OLev
OBz
Bn
N
O
OH
118
f1 (ppm)

4-(Acetoxymethyl)benzyl N-benzyl N-(5-(2-O-acetyl-3-O-benzyl-4-O-(4,6-O-di-acetyl-2-azido-3-O-benzyl-2-deoxy-α-D-glucopyranosyl)-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**121**):

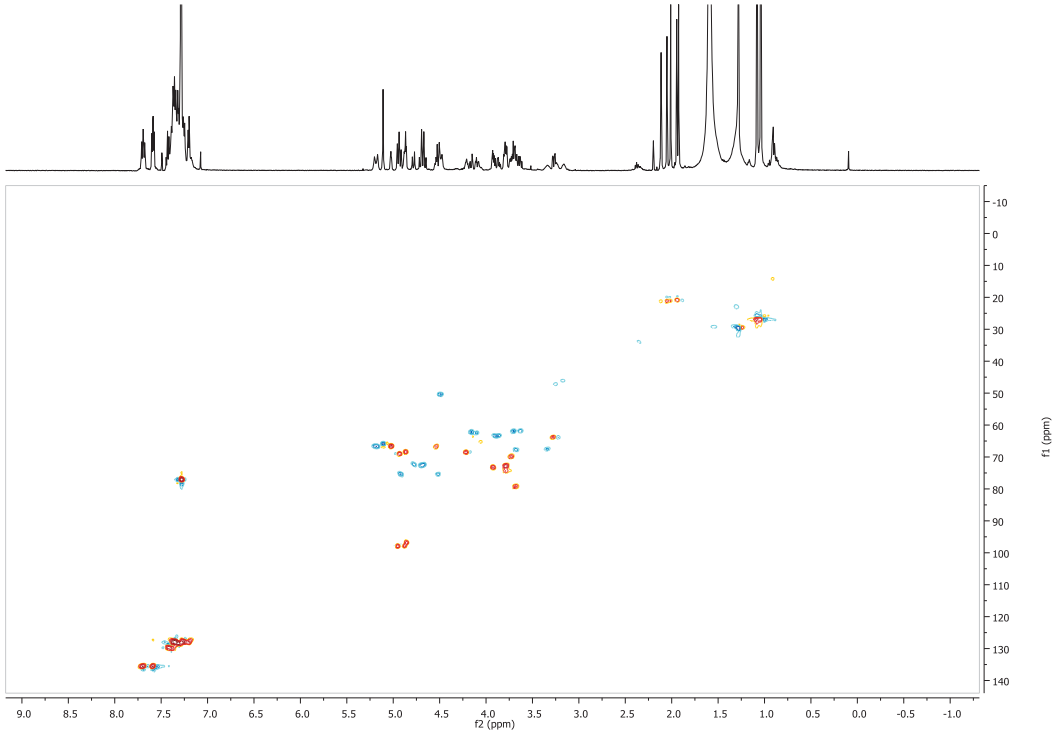




4-(Acetoxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-acetyl-3-*O*-benzyl-4-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-(2,4-di-*O*-acetyl-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyl)-2-deoxy-α-D-glucopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (**125**):

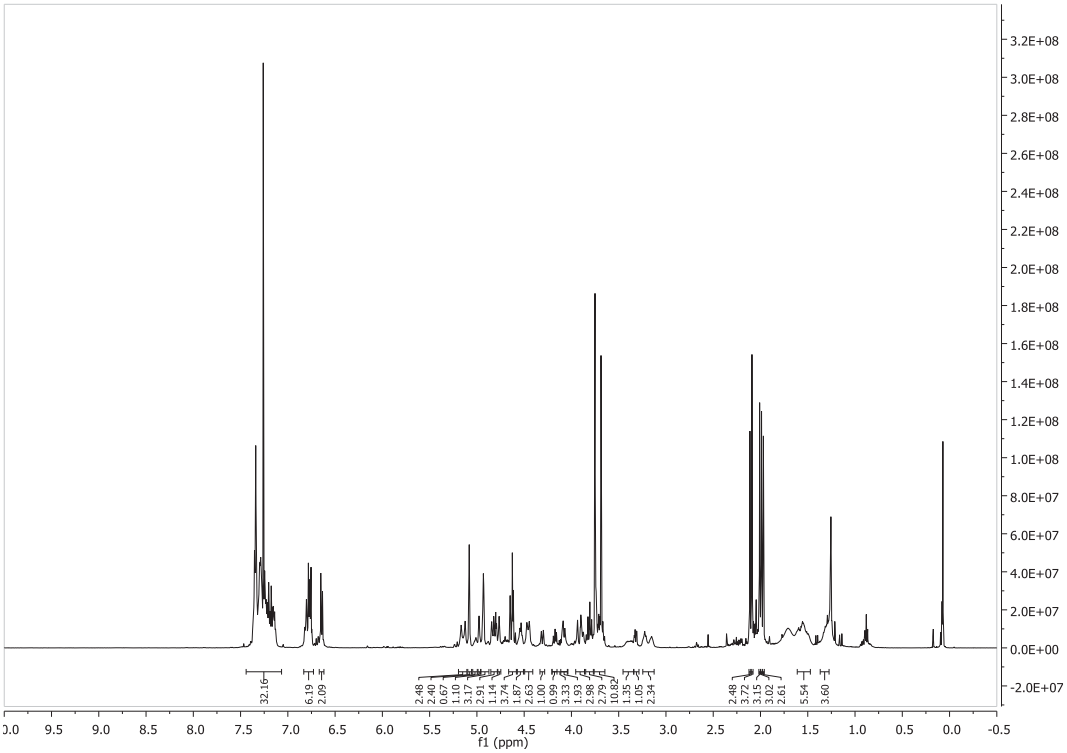


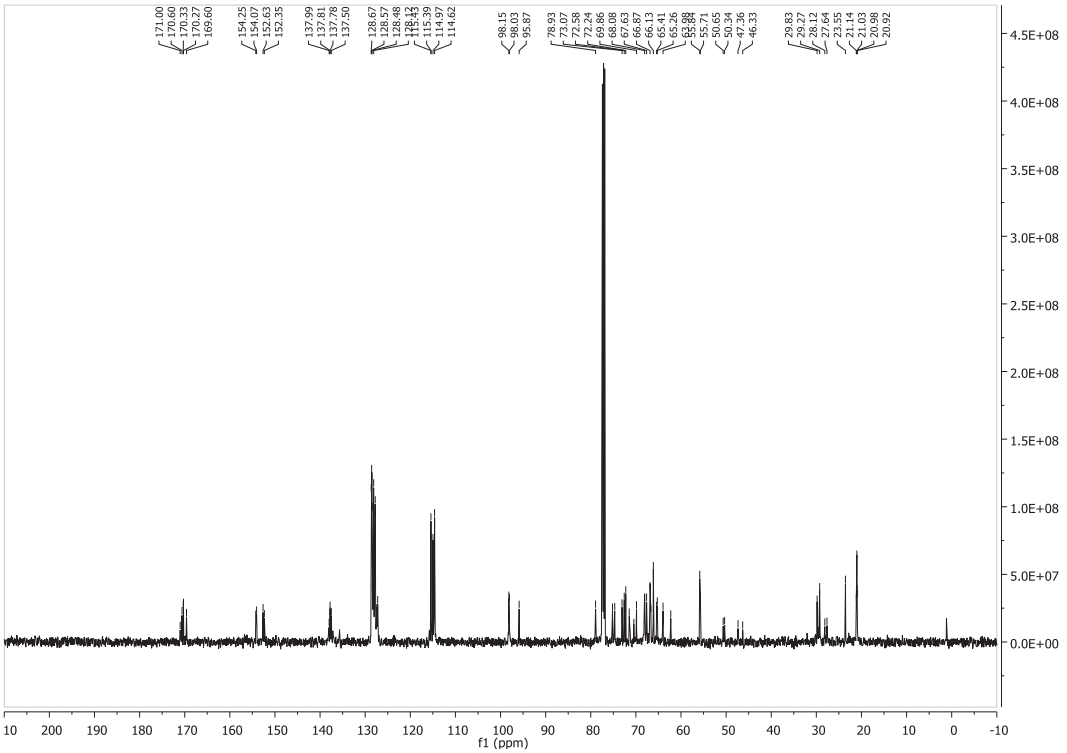
COSY spectrum of compound **125**

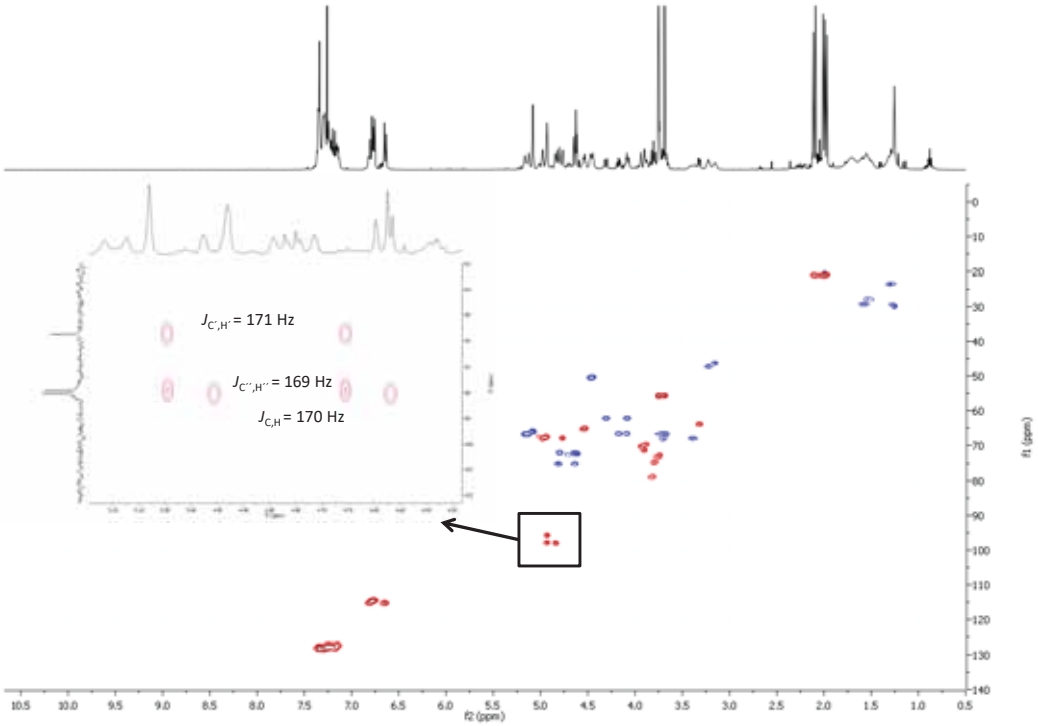


HSQC spectrum of compound **125**

4-(Acetoxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-acetyl-4-*O*-(6-*O*-acetyl-2-azido-4-*O*-(2,4-di-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (**156**):

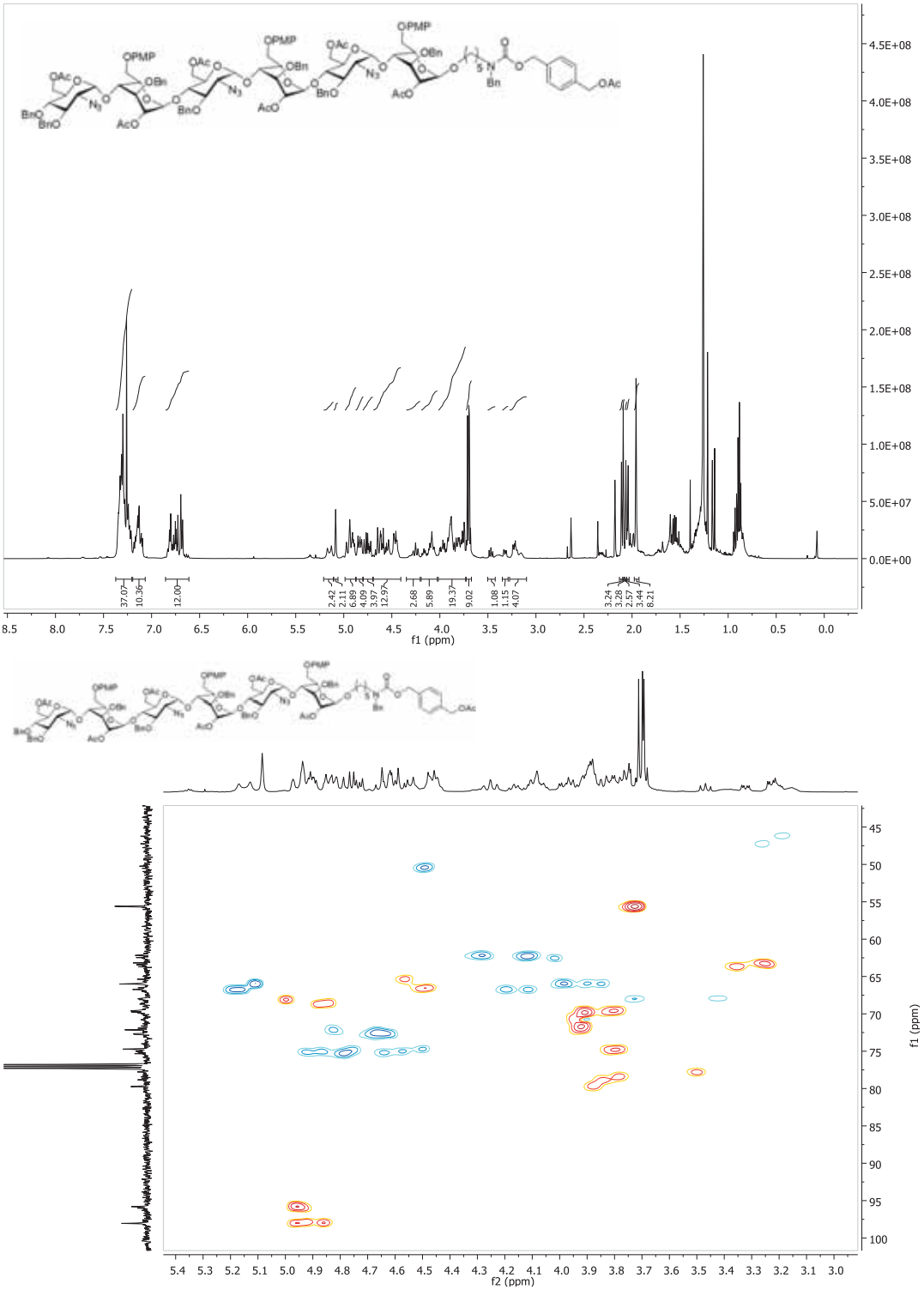


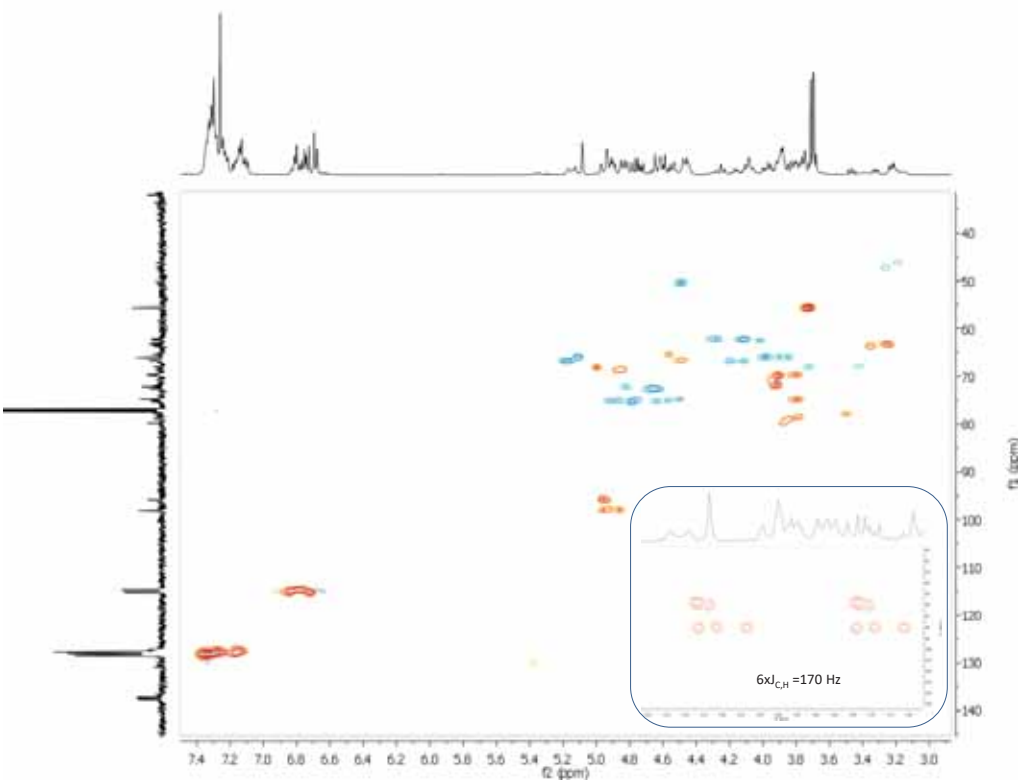




HSQC edited and HSQC-J coupled NMR (500MHz, $CDCl_3$) of compound **156**.

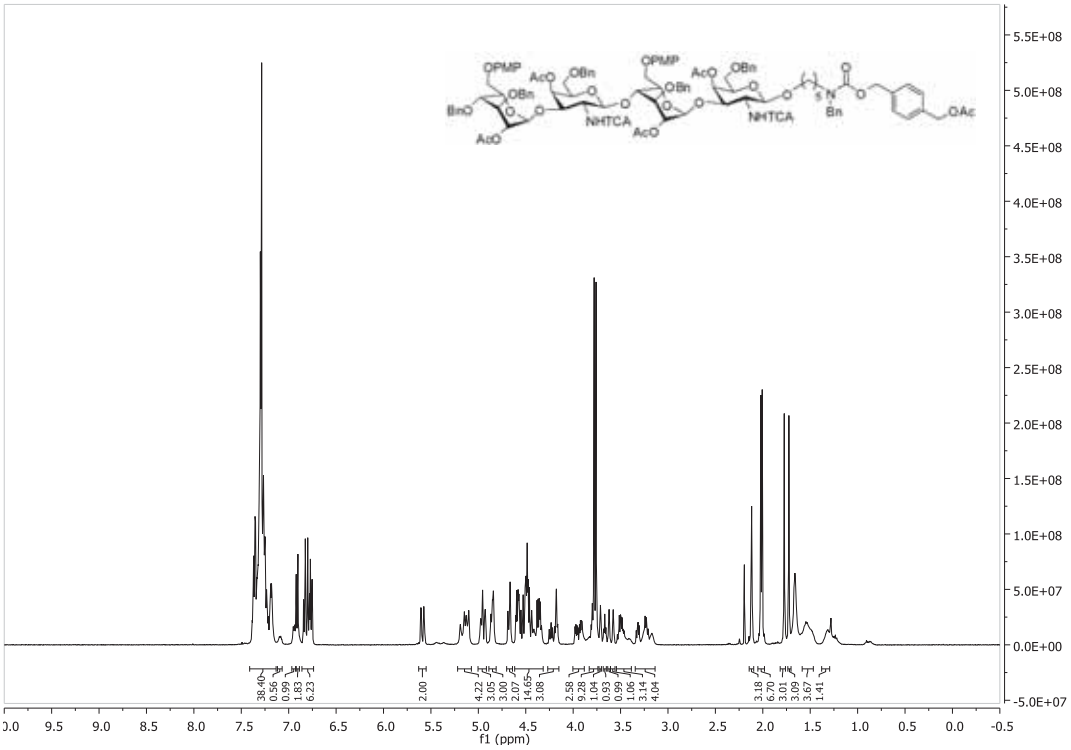
4-(Acetoxymethyl)benzyl *N*-benzyl *N*-[5-((6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl(-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (**163**):

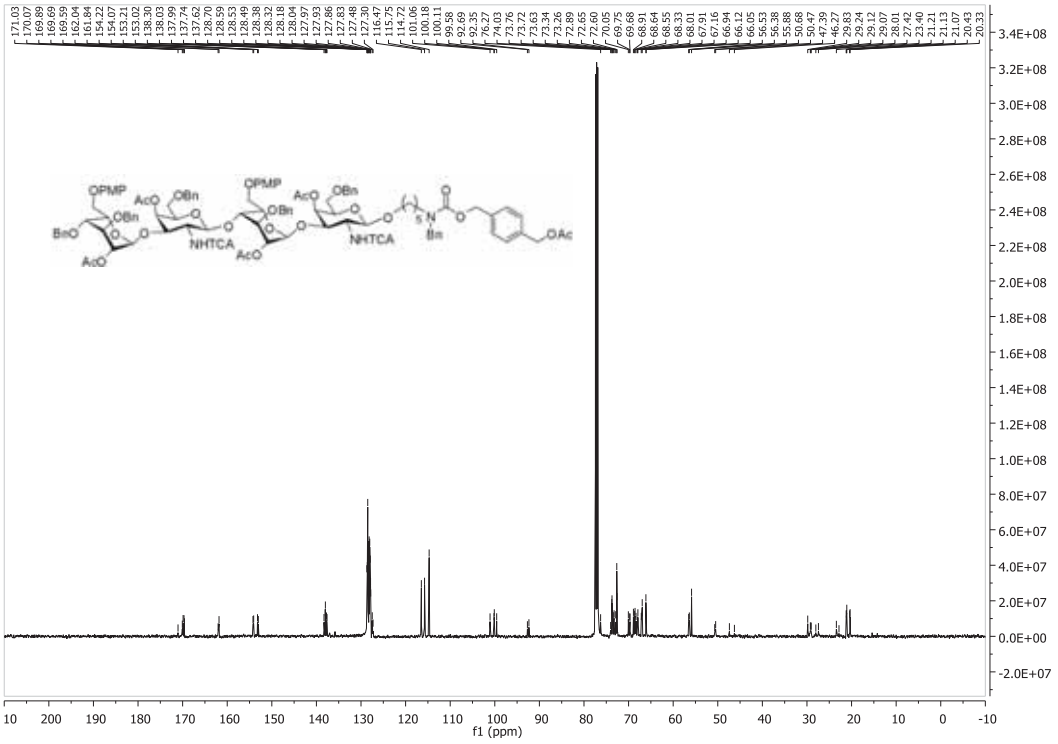


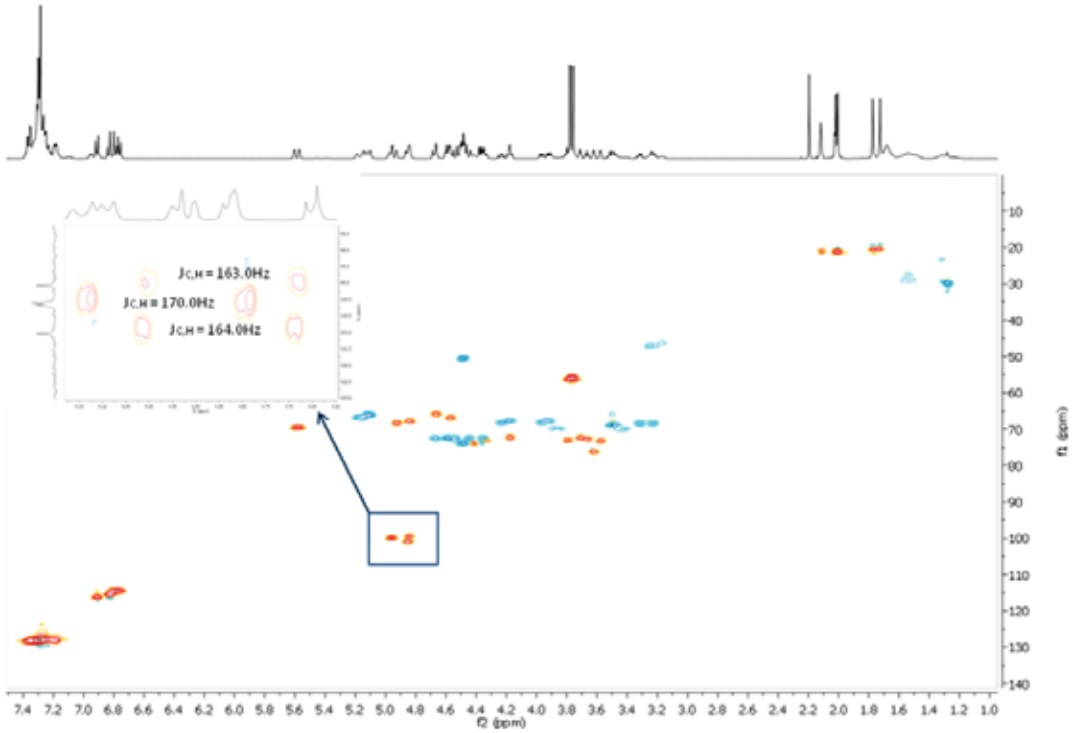


HSQC edited and HSQC-J coupled NMR (500MHz, $CDCl_3$) of compound **163**.

4-(Acetoxymethyl)benzyl N-benzyl-N-[5-((2-*O*-acetyl-3,4-di-*O*-benzyl-6-*O*-p-methoxyphenyl-α-l-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-d-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-l-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-d-galactopyranosyloxy)pentyl]carbamate (**190**):

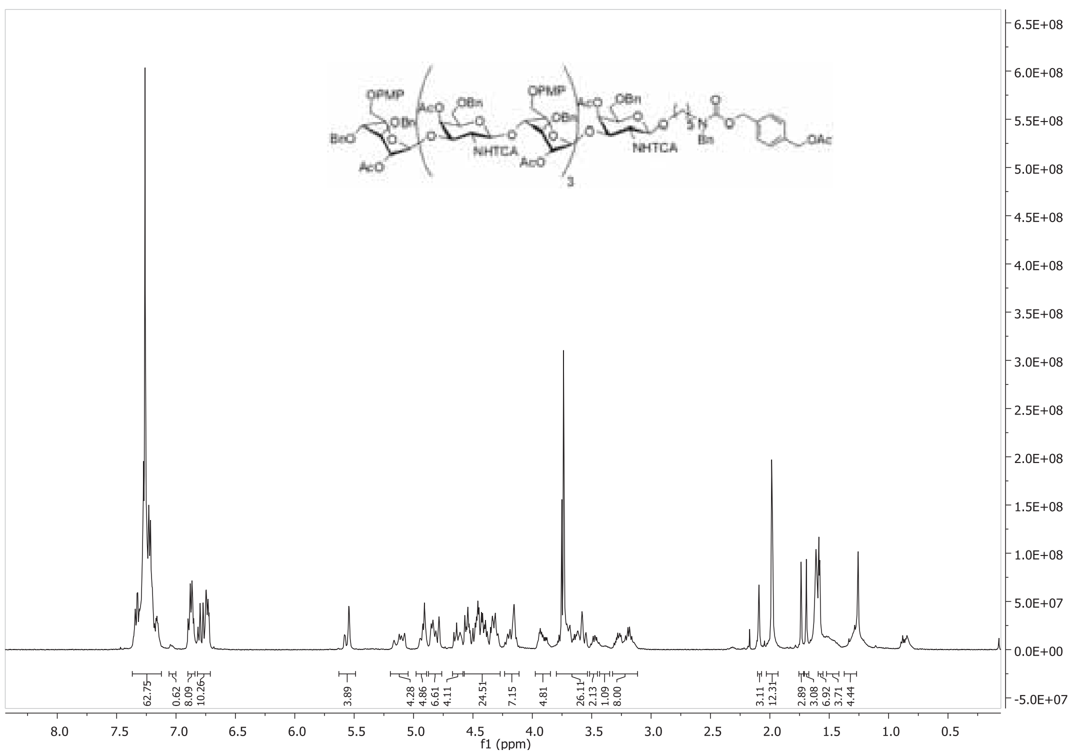


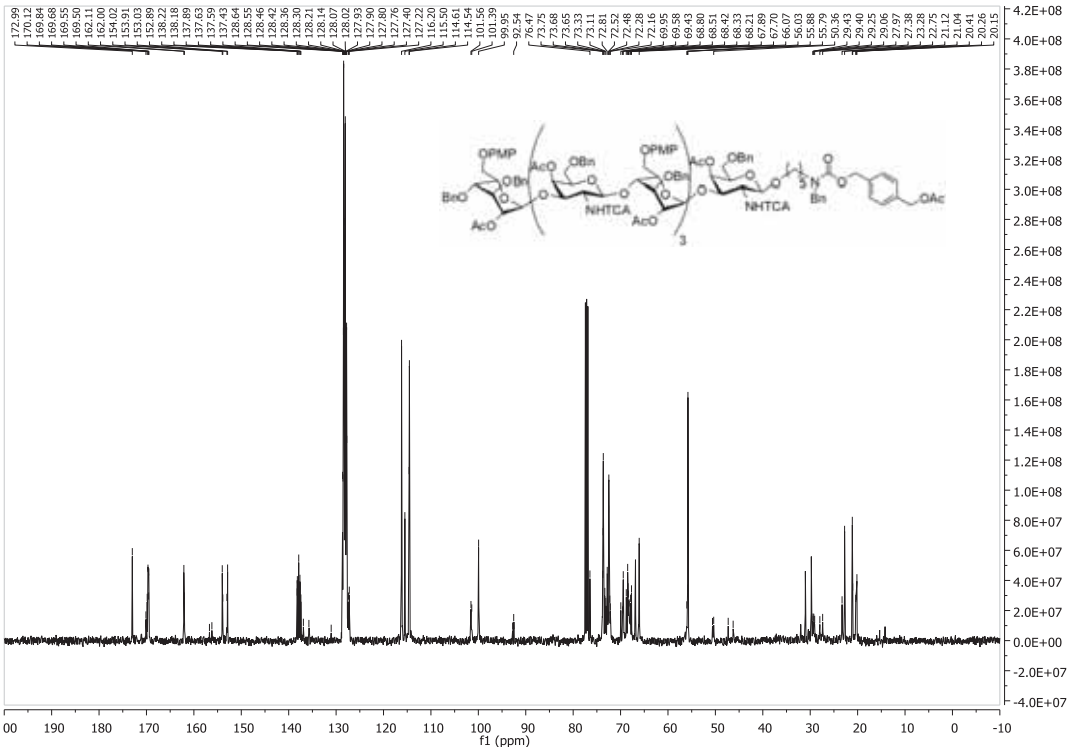




HSQC edited and HSQC-J coupled NMR (500MHz, $CDCl_3$) of compound **190**.

4-(Acetoxymethyl)benzyl N-benzyl-N-[5-((2-*O*-acetyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate (**201**):





HSQC edited and HSQC-J coupled NMR (500MHz, $CDCl_3$) of compound **201**.