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Synthesis of Human Milk Oligosaccharides and Regioselective Ring Opening of Oxabicycles

Phd Thesis – August 2013

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Preface

This thesis describes the results obtained during my PhD study. The work was carried out at the Technical University of Denmark from April 2010 to Juli 2013 with a five month stay at the University of Toronto, Canada from February 2012 through June 2012.

First and foremost, I would like to thank my supervisor Professor Robert Madsen for his help and guidance on both pratical and theoretical aspects of organic chemistry.

I am very grateful to Professor Mark Lautens for the opportunity to stay in his group at University of Toronto and for a warm welcome. Thanks to Harald Weinstabl, Juliane Keilitz and the rest of the research group for their helpfulness and especially their inspiring positive approach to organic chemistry.

A number of current and former employees at the Technical University of Denmark have helped me getting to where I am now — thank you! The Madsen group both present and former, for making the daily hours interesting and pleasant. Agnese Maggi, Amanda Birgitte Sølvhøj and Kennedy Taveras for greate discussions during lunch. Also thanks to the technical staff, especially Anne Hector, Janne Borg Rasmussen, Tina Gustafsson and Brian Ekman-Gregersen for help at desperate times. I would like to thank Casper Junker Engelin for computational calculations of my compounds. My lab partners Amanda and Clotilde for good conversations, music tolerance and of course for not blowing up the lab.

Financial support from The Danish Council for Strategic Research is gratefully acknowledged.

Last but not least, I would like to thank my friends and family for their support and understanding especially my husbond Karsten Stein Jennum. Thank you for making life so much brighter.

Camilla Arboe Jennum
Kongens Lyngby – 19th of August, 2013

Abstract

This thesis involves two distinct topics related to carbohydrate and organometallic chemistry.

The first chapter includes chemical synthesis of oligosaccharides present in human milk. Human milk oligosaccharides are a family of structurally diverse glycans, which are highly unique for human milk. These oligosaccharides participate in multiple mechanisms by which breast-fed infants become protected against infections and they are believed to serve as nutrients for the early brain development.

The synthesized oligosaccharides are based on the motif Galβ1–3/4GlcNAcβ1–3Galβ1–4Glc, which is the core of many human milk oligosaccharides. Three distinct human milk oligosaccharides were synthezised; Lacto-*N*-tetraose, Lacto-*N*-fucopentaose I and Lacto-*N*-neofucopentaose I. A one-pot strategy was developed for the synthesis of the tetrasaccharide backbone core based on the different reactivity of thioglycoside donors and acceptors. The tetrasaccharides were formed both by sequential and the developed one-pot method. Deprotection of the protecting group at the C-2-position on the galactose moiety liberated an acceptor for the fucosylation eventually creating the two linear pentasaccharides Lacto-*N*-fucopentaose I and Lacto-*N*-neofucopentaose I.

The scope of the developed one-pot method was further enhanced by performing selective 1-4 glucosylations utilizing a glucosamine building block containing two free hydroxy groups. Furthermore, this work was executed in the hope to synthesize the branched oligosaccharide Lacto-*N*-fucopentaose III where the fucose is also attached in the same pot.

In addition, *p*NP-neuraminic acid was synthesized for the purpose to perform activity studies on enzymes. The enzymes were designed to perform sialyl transfer reactions in the synthesis human milk oligosaccharides containing neuraminic acid.

Lacto-*N*-fucopentaose **Lacto-*N*-neofucopentaose**

The second chapter describes the regioselective ring opening of enantiopure oxabicycles primarily by the use of rhodium catalysts and phosphine ligands. The ring opened

products were similar to compounds, which had shown to be potential protein Bcl-X_L antagonists, a target for future drugs in cancer treatment.

The aim was to create a general asymmetric ring opening method of several enantiopure oxabicycles having different functional moieties attached to the ring. By employing a $[Rh(COD)Cl]_2$ catalyst with a Josiphos ligand, it was possible to perform the ring opening of oxabicycles with ester moieties in good yield end excellent regioselectivity.

Other functional groups showed to be more challenging, therefore several alterations of the functional groups were performed to form oxabicycles, which could be ring opened regioselectively by the $[Rh(COD)Cl]_2$ catalyst and Josiphos ligand.

Ph–B(O)O [Rh(COD)Cl]2 Josiphos
Ph OH + Ph OH

Resumé

Denne afhandling omfatter to adskilte emner, et vedrørende kulhydrat kemi og et omhandlende organometallisk kemi.

Det første kapitel omhandler kemisk syntese af oligosakkarider til stede i modermælk. Human mælk oligosakkarider er en familie af strukturelt forskellige glycaner, som er meget unikke for modermælk. Disse oligosakkarider tager del i adskillige mekanismer, der beskytter ammede børn mod infektioner, og desuden menes de at fungere som næringsstoffer for den tidlige udvikling af hjernen.

De syntetiserede forbindelser indeholder Galβ1–3/4GlcNAcβ1–3Galβ1–4Glc, som er en gennemgående central del i mange human mælk oligosakkarider. Tre forskellige human mælk olligosakkarider er blevet syntetiseret; Lacto-*N*-tetraose, Lacto-*N*-fucopentaose I og Lacto-*N*-neofucopentaose I. En "one-pot" syntese strategi blev udviklet, baseret på forskellen i reaktivitet mellem thioglycosid donor og acceptor, og anvendt til dannelse af tetrasakkarid kernen. Tetrasakkariderne blev syntetiseret ved brug af den udviklede "one-pot" mekanisme, samt ved sekventiel syntese. Ved afbeskyttelse af beskyttelsesgruppen i C-2 stillingen på galactose delen blev en ny acceptor dannet som ved efterfølgende fucosylering dannede de to linære pentasakkarider Lacto-*N*-fucopentaose I og Lacto-*N*-neofucopentaose I.

For at udvide anvendelses mulighederne for "one-pot" motoden, blev der udført selektive 1-4 glycosyleringer ved anvendelse af en glucosamin byggeblok med to frie hydroxygrupper. Endvidere blev dette arbejde udført i håb om at syntetisere den forgrenede oligosakkarid Lacto-*N*-fucopentaose III, hvor alle byggeblokke blev samlet i samme kolbe.

Derudover blev *p*NP-neuraminsyre syntetiseret for at foretage enzymaktivitets målinger. Enzymerne som skulle undersøges havde til formål at overføre dislyl grupper i enzymatisk syntese af human mælk oligosakkarider, som indeholder neuraminsyre.

Lacto-*N*-fucopentaose **Lacto-*N*-neofucopentaose**

Det andet kapitel beskriver den regioselektive ringåbning af enantiorene oxabicykler, primært ved anvendelse af rhodium-katalysatorer og phosphinligander. De ringåbnede produkter var analoger til forbindelser, der har vist sig at være potentielle protein

Bcl-x_L antagonister, et protein der er fokus på til udvikling af fremtidige lægemidler i kræftbehandlingen.

Der ønskedes at udvikle en generel metode for asymmetrisk ring åbning af enantiorene oxabicykler med varierende funktionelle grupper på ringen. Ved anvendelse af katalysatoren $[Rh(COD)Cl]_2$ og en josiphos ligand var det muligt at foretage regioselektive ringåbninger af oxabicykler i højt udbytte og god regioselektivitet, når en af de funktionelle grupper var en ester gruppe.

Det viste sig at være mere udfordrende at foretage ringåbninger, ved tilstedeværelse af andre funktionelle grupper end ester grupper. Derfor blev der foretaget flere ændringer af de funktionelle grupper, med det mål at disse kunne blive ringåbnet regioselektivt ved anvendelse af katalysatoren $[Rh(COD)Cl]_2$ og en josiphos ligand.

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CHAPTER 1

Human Milk Oligosaccharides

This chapter will cover the synthesis of tetra- and pentasaccharides present in human milk along with the synthesis of p*NP-Neu5Ac used for measuring the activity of enzymes for the synthesis of human milk oligosaccharides.*

1.1 Human Milk Oligosaccharides

Human milk oligosaccharides (HMOs) are a family of structurally diverse glycans that are highly abundant in and unique to human milk. The discovery of HMOs was driven by both scientists and physicians. Physicians and microbiologists were trying to understand the observed health benefits associated with breast-feeding, whereas chemists were trying to characterize the highly abundant carbohydrates. At the end of the 19th century it was observed that breast-fed infants had lower incidences of diarrhea and other diseases than bottle-fed infants, hence had a greater chance of survival.[1]

In 1900 Tissier and Moro reported that there was a difference in the bacterial composition in the feces of breast-fed compared with formula-fed infants.[2,3] Around the same time it was observed, that human milk contained additional unknown carbohydrate fractions than bovine milk. Through the 1930th small progress in structural characterisation was made but at the end of the 1950th several HMOs were characterized in detail.[4–9] Around this time it was also confirmed, that the growth-promoting factor for *Bifidobacterium bifidus* consisted of oligosaccharides.[10–14] Some of the oligosaccharides showed activities of blood group determinants, which contributed to the structural characterization of H and Lewis blood group determinants. As the interest in blood group glycans increased, new methods and tools were developed leading to characterization of additional HMOs.[15–17] In the time following, even more HMOs were discovered and today more than 200 distinct complex oligosaccharides have been identified in human milk.[18]

Human milk containes a broad range of biomolecules including lipids (vitamins), enzymes, proteins and oligosaccharides. The third most abundant component in human milk is the oligosaccharides (5–15 g/L).[19] HMOs are composed of the five monosaccharide building blocks galactose (Gal), glucose (Glc), *N*-acetylglucosamine (GlcNAc), fucose (Fuc) and *N*-acetylneuraminic acid (Neu5Ac). With a few exceptions they are all composed of a lactose (Galβ1–4Glc) unit at the reducing end and can be elongated with up to 15 repeating units of the disaccharides (Galβ1–3/4GlcNAc). Lactose or the resulting linear core structure can be sialylated with α2–3 and/or α2–6 linkages and/or fucosylated with α1–2, α1–3, and/or α1–4 linkages. The different linkages that are observed in HMOs are shown in Figure 1.1.[20]

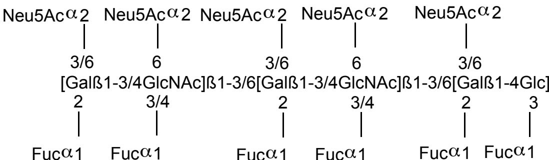


Figure 1.1: Core structure of HMOs with possible linkages of *N*-acetylneuraminic acid and fucose.

The oligosaccharide amount and composition vary between women, and the composition is very different compared with bovine milk. The oligosaccharides in bovine milk are less abundant and structurally less complex than oligosaccharides in human milk, and therefore formula-fed infants are not provided with the same amount of HMOs as breast-fed infants.[1] In Tabel 1.1 is shown the difference in oligosaccharide composition of human milk and bovine milk.

As can be seen the fucosylated oligosaccharides are the most abundant in human milk, but bovine milk only containes traces of fucosylated oligosaccharides. While sialylated oligosaccharides are common in both human and bovine milk, the latter also contains sialyloligosaccharides containing *N*-glycolylneuraminic acid (Neu5Gc), which is not present in human milk.[22] The postulated beneficial effects of some of the most abundant HMOs will be discussed in the following section.

1.1.1 Postulated beneficial effects of human milk oligosaccharides

HMOs were mainly known for their prebiotic effects, as the first discovery was the growth-promoting factor of HMOs for *Bifidobacterium bifidus.* However, since the early 1990s research have provided more and more evidence suggesting that HMOs are more than a substrate to promote the growth of desired bacteria in the infant's intestine.

Table 1.1: Oligosaccharide composition of human milk and bovine milk.

	Human milk [g/L]	**Bovine milk [g/L]**
Lactose[a]	55–70	40–50
Oligosaccharides[a]	5–15	0.05
Fucosylated[a]	50–80%	∼1%
Sialylated[a]	10–20%	∼70%
Neutral oligosaccharides[b]		
Galβ1–3GlcNAcβ1–3Galβ1–4Glc (LNT)	0.5–1.5	Traces
Fucα1–2Galβ1–3GlcNAcβ1–3Galβ1–4Glc (LNFP I)	1.2–1.7	—
Galβ1–3[Fucα1–4]GlcNAcβ1–3Galβ1–4Glc (LNFP II)	0.3–1.0	—
Galβ1–4[Fucα1–3]GlcNAcβ1–3Galβ1–4Glc (LNFP III)	0.01–0.2	—
Fucα1–2Galβ1–3[Fucα1–4]GlcNAcβ1–3Galβ1–4Glc (LNDFH I)	0.1–0.2	—
Acidic oligosaccharides[b]		
Neu5Acα2–3Galβ1–4Glc	0.1–0.3	0.03–0.06[c]
Neu5Acα2–6Galβ1–4Glc	0.3–0.5	0.03–0.06[c]
Neu5Acα2–3Galβ1–3GlcNAcβ1–3Galβ1–4Glc	0.03–0.2	Traces
Neu5Acα2–6Galβ1–4GlcNAcβ1–3Galβ1–4Glc	0.1–0.6	Traces
Neu5Acα2–3Galβ1–3[Neu5Acα2–6]GlcNAcβ1–3Galβ1–4Glc	0.2–0.6	Traces

[a] Data from Bode[1] [b] Data from Kunz *et al.*[21] [c] Combined amount

Prebiotic effect

The effect is defined as 'A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health'.[23] Three criteria need to be met for HMOs to be defined as prebiotics, firstly, they have to resist gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption, secondly, they need to be fermented by the intestinal microflora, and thirdly, they should selectively stimulate the growth and/or activity of intestinal bacteria associated with health and well being. HMOs meet all three criteria.

HMOs serve as prebiotics by increasing the number of beneficial *bifidobacteria* and *Lactobacillius* species. As a consequence, the competition between the beneficial bacteria and undesired pathogenic bacteria for binding sites on the intestinal epithelium and for nutrients is increased, thus inhibiting survival of the pathogenic strains (Figure 1.2). The fermentation of prebiotics by *Bifidobacterium* species produce short chain fatty acids, which acts as nutrients for the epithelial cells. Also the short chain fatty acids creates an acidic environment in the colon inhibiting the growth of some pathogenic strains of bacteria such as colibacteria.[24]

As about 90% of all HMOs are found intact and not metabolized in the infant's feces the HMOs are postulated to have additional effects to establish a certain microbiota composition.

Anti-adhesive antimicrobial effect

HMOs directly reduce microbial infections by serving as anti-adhesive antimicrobials. It is essential for many pathogens (e.g. *Campylobacter jejuni, Escherichia coli (E. Coli), Vibrio cholera*, and *Salmonella* species) to adhere to the mucosal surfaces for the spreading of these bacteria.[21] Bacterial adhesion is often initiated by lectin-glycan interactions, where lectins bind to oligosaccharides on the epithelial cell surface. Some HMOs resemble mucosal cell surface glycans and hereby are able to serve as soluble ligand analogs and block pathogen adhesion, thus protecting the infants against infections.[18] A good example of this, is *Campylobacter jejuni* that adhere to intestinal 2'-

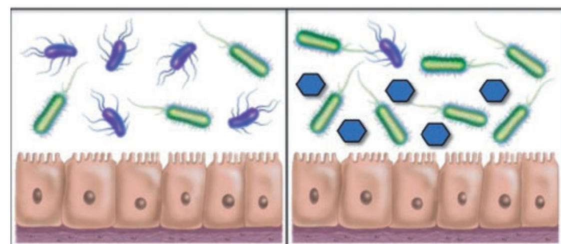


Figure 1.2: Simplified figure showing the intestine without HMOs (left) and with HMOs (right). In the absence of HMOs the undesired pathogenic bacteria (purple) and the desired bacteria (green) compete for the binding sites. When HMOs (blue) are present, beneficial bacteria (green) metabolize HMOs and thrive, while undesired bacteria (purple) can not metabolize HMOs. Hereby fewer undesired bacteria will bind to the intestinal epithelium.[18]

fucosyllactosamine (Fucα1–2Galβ1–3GlcNAc). The incidences of *Campylobacter* diarrhea in breast-fed infants were less for breast-fed infants receiving milk with high concentration of 2'-fucosyllactose than those with low concentration of 2'-fucosyllactose.[25] Also influenza A, B and C viruses have a specificity for Neu5Acα2–3Galβ1–4Glc and Neu5Acα2–6Galβ1–4Glc, which both are of high concentration in human milk.[21]

Scientific studies have shown that HMOs might protect infants against postnatal human immunodeficiency virus (HIV) transmission. HIV entry across the infant's mucosal barrier is partially mediated through binding of the viral envelope glycoprotein gp120 to DC-SIGN (dentritic cell-specific ICAM3-grabbing non-integrin) on human dendritic cells. This initial gp120/DC-SIGN interaction is important for HIV transmission from mother to child via breast-feeding. DC-SIGN recognizes mannose containing glycoconjugates, such as HIV-gp120, but has even higher binding affinities for Lewis blood group antigens.[26] The Lewis antigens contain fucosylated Galβ1–3/4GlcNAc residues. As HMOs contain these Lewis antigens they can compete with gp120 for binding to DC-SIGN *in vitro*. This may explain the low HIV transmission through breast-feeding as 80–90% of infants are not infected, despite continuous exposure to the virus in milk over many months.[1]

It has also been suggested that HMOs reduce upper respirartory and urinary tract infections.

In summary, the antiadhesive antimicrobial effects of HMOs may contribute to a lowering of intestinal, upper respiratory and urinary tract infections in breast-fed compared with formula-fed infants.

Other postulated benefits

Many other benefits of HMOs have been postulated, among these is their ability to modulate the expression of intestinal epithelial cell surface glycans. When cultured human intestinal epithelial cell lines are incubated with 3'-sialyllactose the gene expression levels of sialyltransferases is reduced, thus diminishing α2-3- and α2-6-sialylation on cell surface glycans. Consequently, binding of enteropathogenic *E. coli* is reduced as it uses sialylated cell surface glycans to attach to the host's intestinal epithelial cell.[27]

It is also postulated that certain sialylated HMOs may contribute to prevent allergy and autoimmune diseases.[29] Breast-feeding promotes an anti-inflammatory cytokine milieu, which is maintained throughout infancy. Higher serum concentrations of the potentially pro-inflammatory cytokines were found in formula-fed infants than in those who were breast-fed.[30]

Necrotizing enterocolitis (NEC) is a disorder primarily seen in premature infants, and is often fatal. Breast-fed infants have a 6–10 fold lower risk of developing NEC than formula-fed infants.[28] Studies on rats have shown that some HMOs protect againts NEC.[31] Neu5Acα2–3Galβ1–3(Neu5Acα2–6)GlcNAcβ1–3Galβ1–4Glc (DSLNT) was found to protect rats against NEC disorder, and for that reason it could be interesting to investigate whether these effects translate to infants.

They are believed to serve as nutrients for the early brain development. Many other beneficial effects of HMOs have been suggested, and they are currently being investigated.

Unfortunately, large quantities of these oligosaccharides are not easily available. This calls for other ways to provide the quantity needed, if formula-fed infants are to

be provided with some of the same benefits as breast-fed infants. As a consequense enzymatic, chemoenzymatic and chemical syntheses of HMOs have become the aim for many research groups. In the present project focus was devoted to chemical synthesis some of the more abundant HMOs in human milk.

1.2 Chemical synthesis of human milk oligosaccharides

The oligosaccharide moieties of glycoconjugates are involved in a number of biological interactions within the human body. This have led to a growing interest in obtaining large amounts of natural and modified oligosaccharides for biological and pharmacological investigations. As a consequence much research have been performed in finding new methods and strategies for assembling these oligosaccharides. Such as enzymatic, chemoenzymatic or chemical syntheses. The first glycoside synthesis was reported in 1879 [32] and since then the field of chemical synthesis of glycosides has been widely explored and expanded. [33–37] In this thesis, however, a comprehensive overview of oligosaccharide synthesis will not be provided. Instead the strategies employed in this work will be discussed together with an overview of the reported chemical synthesis of HMOs.

1.2.1 The glycosylation reaction

In a glycosylation reaction a glycosidic linkage between a glycosyl donor and a glycosyl acceptor is being formed. It is most common to form *O*-glycosides, but *N-*, *S-*, and *C*-glycosides are also possible to form, but is not relevant for HMO synthesis. The challenge with forming glycosidic linkages is to generate them stereo- and regioselectively. To achieve high stereo- and regioselectivity protecting group manipulation is applied. The most common way is to use a fully protected glycosyl donor, which bears a leaving group at its anomeric center, together with a suitably protected glycosyl acceptor that often contains only one free hydroxy group (this specifies the regioselectivity) (Scheme 1.1). [33]

It is believed that a typical glycosylation follows a unimolecular mechanism (S_N1). The mechanism of a given glycosylation reaction possesses partial or complete S_N1 type character, but it has not yet been possible to achieve a clean S_N2 type character. The rate limiting step often involves the development of positively charged oxocarbenium ion. Consequently, the electronic effects of the substituents of the glycosyl donor can affect the reactivity remarkably. Thus, the rate limiting transition state will be stabilized by electron donating substituents. [35]

As a result of the S_N1 type character of the glycosylation reaction, controling the stereochemistry is the most challenging aspect of oligosaccharide synthesis. When considering only the *D*-sugars (in pyranose form), the nature of the protecting group at C-2 of the glycosyl donor determines the selectivity. When a participating neighboring

PGO LG → PGO O+ —HOR→ PGO OR

Glycosyl donor oxocarbenium ion glycoside

Scheme 1.1: Genereal glycosylation mechanism.

a)

1,2-*trans*

b)

1,2-*trans*

1,2-*cis*

Scheme 1.2: Glycoside synthesis with a) neighboring group participation b) no neighboring group participation.

group is employed, the glycosidic linkage will be a 1,2-trans glycosidic linkage (β) and the reaction is stereospecific. Whilst glucosylation with a non-participating group at C-2 will lead to a mixture of the 1,2-trans and the 1,2-cis product. In this case the reaction conditions will determine the stereoselectivity (Scheme 1.2).[38]

A wide range of glycosyl donors are used in glycoside synthesis and the number is still increasing. By selecting the appropriate reaction conditions, high yields and good α:β ratios can be obtained. As Hans Paulsen so beautifully put it "...each oligosaccharide synthesis remains an independent problem, whose resolution requires considerable systematic research and a good deal of know-how. There are no universal reaction conditions for oligosaccharide synthesis".[39]

1.2.2 Glycosyl donors

Today the selection of glycosyl donors is huge, they all have advantages and disadvantages. When performing an unknown glycosidic linkage it can be an advantage to consider some of them. A selection of the wide array of glycosyl donors to choose from spands from anomeric halides,[40–42] glycals,[43] selenoglycosides,[44] thioglycosides,[45] sulfoxides,[46] vinyl glycosides,[47] xanthates,[48] through pentenyl glycosides,[49,50] phosphorus containing compounds,[51,52] orthoesters,[53] imidates,[54,55] and even more (Figure 1.3). Each of these glycosyl donors have a certain set of promotors which establishes the reaction conditions. The methods employed in the present work uses glycosyl halides, pentenyl glycosides, *N*-phenyl trifluoroacetimidates, trichloroacetimidates and thioglycosides, hence a more thorough discussion of these will be given.

Glycosyl bromides and chlorides

One of the oldest methods, and traditionally the most widely used method for the synthesis of complex glycosides, is the Koenigs-Knorr method.[40,56] The classical glycosyl donors are glycosyl bromides and chlorides and the promoters used are heavy metal salts. Over the years a wide variety of promotors have been employed but the most widely used till date are silver salts such as Ag_2CO_3,[40,56] Ag_2O,[56] silver perchlorate ($AgClO_4$)[57] and silver triflate (AgOTf).[58] This method aquire at least stoichiometric amounts of the promoters. Water is beeing formed during this reaction with the

sulfoxides glycals selenoglycosides thioglycosides pentenylglycosides

X = F, Cl, Br, I halids vinyl glycoside phosphates/phosphites orthoesters

N-phenyl trifluoroacetimidate trichloroacetimidate xanthates

Figure 1.3: Glycosyl donors.

first two salts, and as a consequence of this they are often low yielding if a no drying agent is used. The silver carbonate and oxide also act as acid scavengers for the acid released, but organic bases, such as collidine, lutidine, tetramethylurea (TMU) and 2,4,6-tri-^{t}butylpyrimidine (TTBP) may also be used for the same purpose.[37]

The disadvantage of the Koenigs-Knorr method is the thermal instability of the glycosyl bromides and chlorides, especially when they bear electron-donating protecting groups. Often the halide is introduced immediately before the glycosylation step, and purification of the halides are often problematic.

Glycosyl trichloroacetimidates and *N*-phenyl trifluoroacetimidates

The first attempts to find alternatives to the glycosyl halide methodologies were the employment of imidate and orthoester procedures. Trichloroacetimidates were introduced by Schmidt and co-workers.[54] They can be prepared by base catalyzed addition of trichloroacetonitrile to the free hydroxyl of lactols. The strength of the base employed determines the relative stereochemistry.[59] They are sufficiently stable for purification, and can be stored at low temperatures for months. Trichloroacetimidates are activated by catalytic amounts of Brønsted or Lewis acid, with the currently most used Lewis acid being trimethylsilyl triflate (TMSOTf).[60] The stereoselectivity of the glycosylation is determined by the nature of the donor (α or β), the promotor and the solvent. Both 1,2-cis and 1,2-trans products can be favored. In many ways trichloroacetimidates are prefered over glycosyl halides, as they are more stable, only catalytic amounts of promotor is needed, and there are various possibilities for stereocontrol. The high reactivity of trichloroacetimidates can lead to side reactions or even decomposition of the donor. Also when the acceptor is unreactive, rearrangement of the trichloroacetimidate donor into the corresponding glycosyl trichloroacetamide has been observed under Lewis catalysis (Scheme 1.3). The glycosyl acetamide is not reactive towards glycosyla-

Lewis acid

glycosyl acetimidate glycosyl acetamide

Scheme 1.3: Rearrangment of trichloroacetimidate into the corresponding trichloroacetamide under Lewis acid catalysis.

tion.[61] To circomvent this problem *N*-phenyl trifluoroacetimidates can be employed, as these are less reactive and the rearrangment is not possible. They are synthesized from *N*-substituted trifluoroacetimidoyl halides and are also activated by Lewis acids.[55]

n-Pentenyl glycosides

Another group of stable glycosyl donors are the *n*-pentenyl glycosides, which was introduced by Fraser-Reid.[49] They can be stored at room temperature for several months some even years. *n*-Pentenyl glycosides are activated by halogenation of the double bond, leading to the formation of a cyclic halonium ion intermediate, followed by subsequent rearrangement into a cyclic intermediate containing the leaving group. Elimination of the leaving group provides the active glycosylation species (Scheme 1.4).

X^+ ROH OR X halonium ion intermediate cyclic intermediate

Scheme 1.4: Mechanism for the activation of *n*-Pentenyl glycosides.

The promotors employed are *N*-bromosuccinimide (NBS), *N*-iodosuccinimide (NIS), iodonium di-*sym*-collidine perchlorate (IDCP) or iodonium dicollidine triflate (IDCT). Equimolar amounts of the promoter are required. When NBS or NIS is used with a protic acid such as triflic acid (TfOH) or a Lewis acid such as triethylsilyl trifluoromethanesulfonate (TESOTf) a more active catalyst is formed.[62] It is possible to form the 1,2-trans glycosides with participating neighboring groups, but without it leads to a mixture of α and β in favor of the α-glycoside. As the reactivity of *n*-pentenyl glycosides vary with the protecting groups employed, they can be used together in the armed-disarmed concept, which will be discussed more thoroughly in Section 1.2.3.

Thioglycosides

Thioglycosides are among the most widely used choices of glycosyl donors and were introduced by Ferrier and co-workers.[63] This popularity is partly due to their ready synthesis and partly to their easy conversion into sulfoxides and other glycosyl donors, offering an alternative glycosyl donor source. Thioglycosides can easily be prepared from acetylated sugars (often 1,2-trans acetates) with thiols in the presence of a Lewis acid such as TESOTf, $SnCl_4$ and $BF_3{\cdot}OEt_2$. But they can also be obtained from other common glycosyl donors like glycosyl halides. The advantage of thioglycosides are their remarkable stability. They have long shelf lives, but more importantly, they posses an increased stability against protecting group manipulation and other chemical transformations. Activation of thioglycosides can be achieved by a wide variety of electrophilic promotors. The most commonly employed activators are NIS/TfOH, IDCP, methyl trifluoromethanesulfonate (MeOTf), phenylselenyl trifluoromethanesulfonate (PhSeOTf), dimethylthiomethylsulfonium trifluoromethanesulfonate (DMTST), 1-benzenesulfinyl piperidine-triflic anhydride (BSP-Tf_2O), and diphenyl sulfoxide-Tf_2O (Ph_2SO-Tf_2O) (Figure 1.5).[45] All promotors are required in at least stoichiometric amounts.

Scheme 1.5: The most commonly employed activators for thioglycosides

The configuration of the glycosidic linkage follows the same pattern as for other glycosyl donors bearing a participating neighboring group leading to the 1,2-trans product. Wheras the use of non participating neighboring group lead to a mixture of α and β. The use of diethylether will favor the α-glycoside, while the use of acetonitrile will favor the β-glycoside.[64] Thioglycosides can be applied in the armed-disarmed concept, both with other thioglycosides and/or other glycoside donors and acceptors such as pentenyl glycosides.

1.2.3 Synthetic strategies for oligosaccharide formation

In the synthesis of natural products, one aim is to apply as few steps as possible, especially as few linear steps as possible. This is also a goal in oligosaccharide synthesis. Multiple ways of acheiving this have been employed. A short overview of some of the different strategies will be given hereafter.

Two major strategies can be considered for oligosaccharide synthesis, one is the linear approach using stepwise synthesis and the other is block synthesis. In the stepwise (linear) synthesis a donor and an acceptor is first coupled to form a disaccharide intermediate (Scheme 1.6). The disaccharide can either be deprotected to form a glycosyl acceptor, or the R-group at the anomeric center of the reducing end can be converted into a leaving group (LG), hence become a glycosyl donor. The formed disaccharide acceptor can be reacted with a monosaccharide donor to furnish a trisaccharide. The disaccharide doner would by reaction with an acceptor create the same (or a similar) trisaccharide. The trisaccharide can be further elongated by deprotection/introduction of a leaving group followed by another glycosylation.

In the block synthesis, a disaccharide building block is first assembled and then transformed into a glycosyl donor and a glycosyl acceptor. The two disaccharides are then coupled yielding a tetrasaccharide. The tetrasaccharide can be further elongated by deprotection/introduction of a leaving group followed by another glycosylation (Scheme 1.7).[36]

Scheme 1.6: Stepwise (linear) synthesis

When higher oligosaccharides are wanted, the block strategy is to be prefered especially when two or more sequential rrepeating units are employed. Block synthesis also have the advantage that critical steps can be performed at an early stage on smaller compounds, hence facilitating chromatographic separation and hopefully avoid complicated separation steps of diastereoisomers.

As the selection of glycosyl donors and promoter systems have multiplied over the years, the methods for assembling oligosaccharides have been similarly expanded. In the following, a short overview of selected concepts in glycoside formation will be summarized.

Selective activation involves a glycosyl donor, bearing a reactive leaving group (LGa), which is coupled with a glycosyl acceptor, bearing a relative stable leaving group (LGb) at the anomeric center. The requirements for the promoter is to selectively activate LGa over LGb, and LGb should not be altered under the applied conditions. One of the first reported examples involved a bromide as LGa and a thioethyl as LGb.[65] Demchenko and co-workers have developed many glycosylations by this strategy.[66,67]

Scheme 1.7: Convergent block synthesis

The armed-disarmed concept was developed by Fraser-Reid and co-workers.[68] The concept rely on the observation, that electron donating substituents such as ethers tend to stabilize the rate limiting transition state of the glycosylation reaction. Thus the reactivity is increased and these donors are termed 'armed'. On the contrary, electron withdrawing groups such as esters lowers the glycosylating capability and these glycosides are termed 'disarmed'. The armed saccharide will be chemoselectively activated over the disarmed, hence acting as the donor by the use of a mild promoter. The newly formed dissacharide can be coupled with other saccharides using a more powerful promoter.

As the chemoselective activation principles have expanded since, a database of relative reactivity values (RRVs) has been established.[71] Several observations were done and some are listed below.

1. the trend of reactivity of aminosugars is as follows for the *N*-protecting groups NHCbz > NHTroc > NPhth > N_3 > NHAc. Hence an aminosugar with a NHCbz group is more reactive than one with a NHAc group.[72]

2. the general trend for reactivity enhancement of glycosides by protective groups is (H) > OSilyl > OH » OBn > NHTroc > OAc > OBz > OClAc.[72]

3. pyranosides show reactivities, which differ as a function of sugar. The reactivity decreases in the order fucose > galactose > mannose > glucose > neuraminic acid. It should be noted that the differences in reactivity are not significant.[72]

4. the position that affects pyranoside reactivity most is not the same for each sugar.[72]

5. the anomeric deactivation can also be achieved by the torsional effect of cyclic acetals, bulky protecting groups and/or dispiroketal protecting groups.[69,70,73]

6. bulky leaving groups reduce the reactivity.[74]

7. axial substituents increase reactivity.[75]

The armed disarmed concept has been expanded by additional concepts such as the active-latent strategy,[76] orthogonal strategies[77] and one-pot multistep strategies.[45]

The aim of this PhD project was to develop a general one-pot method, which could be applied for the synthesis of several HMOs. The one-pot multicomponent strategy is, as the name implies, the synthesis of oligosaccharides by a controlled cascade of glycosylations in the same reaction flask. The one-pot reactions are controlled by tuning the reactivity of the glycosides. The advantage of one-pot glycosylations compared to the sequential glycosylations is that tedious purification steps are avoided. Also the yield of one-pot reactions tend to be higher due to the fewer purification steps.

Table 1.2: Reported chemical syntheses of HMOs.

Oligosaccharide	Reference[a]	Reference[b]
Linear oligosaccharides		
Galβ1–3GlcNAcβ1–3Galβ1–4Glc (LNT)	Noro *et al.*,[81] Takamura *et al.*,[82] Aly *et al.*,[84] Knuhr *et al.*,[87] Malleron *et al.*,[88]	Noro *et al.*,[81] Takamura *et al.*,[82] Aly *et al.*[84]
Galβ1–4GlcNAcβ1–3Galβ1–4Glc (LNnT)	Takamura *et al.*,[83] Aly *et al.*,[84] Maranduba *et al.*[89]	Takamura *et al.*,[83] Aly *et al.*[84]
Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc	Shimizu *et al.*, Aly *et al.*[86]	Shimizu *et al.*, Aly *et al.*[86]
Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc	Aly *et al.*[86]	Aly *et al.*[86]
Fucα1–2Galβ1–3GlcNAcβ1–3Galβ1–4Glc (LNFP I)	Hsu *et al.*[90]	—
Fucα1–2Galβ1–4GlcNAcβ1–3Galβ1–4Glc (LNnFP I)	Love *et al.*,[91,92] Bröder *et al.*[93]	—
Neu5Acα2–3Galβ1–3GlcNAcβ1–3Galβ1–4Glc	Sherman *et al.*,[94] Schmidt *et al.*[95c]	Schmidt *et al.*[95,c]
Neu5Acα2–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc	Sherman *et al.*,[94] Mandal *et al.*,[96] Hsu *et al.*[97]	—
Branched oligosaccharides		
Galβ1–4GlcNAcβ1–3[Galβ1–4GlcNAcβ1-6]Galβ1–4Glc	Maranduba *et al.*[89] Roussel *et al.*[98]	—
Galβ1–3GlcNAcβ1–3[Galβ1–3GlcNAcβ1–3Galβ1–4GlcNAcβ1-6]Galβ1–4Glc	Knerr *et al.*[100] Knuhr *et al.*[87]	Knuhr *et al.*[87]
Galβ1–4[Fucα1–3]GlcNAcβ1–3Galβ1–4Glc (LNFP III)	Lay *et al.*,[99] Manzoni *et al.*,[101] Lubineau *et al.*,[102] Love *et al.*,[92] Zhang *et al.*[103]	Lay *et al.*,[99] Manzoni *et al.*[101]
Galβ1–3[Fucα1–4]GlcNAcβ1–3Galβ1–4Glc (LNFP II)	Manzoni *et al.*,[101] Malleron *et al.*[88]	Manzoni *et al.*[101]
Fucα1–2Galβ1–4[Fucα1–3]GlcNAcβ1–3Galβ1–4Glc	Love *et al.*[92]	—
Fucα1–2Galβ1–3[Fucα1–4]GlcNAcβ1–3Galβ1–4Glc	Chernyak *et al.*[104]	—
Galβ1–4[Fucα1–3]GlcNAcβ1–3Galβ1–4[Fucα1–3]GlcNAcβ1–3Galβ1–4Glc	Toepfer *et al.*[105]	Toepfer *et al.*[105]
Galβ1–3[Fucα1–4]GlcNAcβ1–3[Galβ1–4[Fucα1–3]GlcNAcβ1–6]Galβ1–4Glc	Kim *et al.*[106]	Kim *et al.*[106]
Galβ1–4[Fucα1–3]GlcNAcβ1–3[Galβ1–4[Fucα1–3]GlcNAcβ1–6]Galβ1–4Glc	Kim *et al.*[106]	Kim *et al.*[106]

[a]Protected saccharide [b]Deprotected saccharide [c]Chemoenzymatic synthesis

1.2.4 Reported synthesis of human milk tetrasaccharides and higher oligosaccharides

Chemical synthesis of more than 15 different structures ranging from tetrasaccharides to octasaccharides has been prepared so far. The main part of these were presented as the parent oligosaccharides, but some were synthesized with a chemical deviant group at the reducing end. This includes alkyl azides [90,97] or potential donor aglycons such as pent-4-enyls. [91,92] Much focus has been on the synthesis of the backbone core [Galβ1–3/4GlcNAcβ1–3/6]$_n$Galβ1–4Glc, and various approaches have been employed for the assambly of this, including solid phase methods. Numerous groups have synthesized Lewis antigens, which consists of Galβ1–3[Fucα1–4]GlcNAc—R (Le^a), Galβ1–4[Fucα1–3]GlcNAc—R (Le^x), Fucα1–2Galβ1–3[Fucα1–4]GlcNAc—R (Le^b) and Fucα1–2Galβ1–4[Fucα1–3]GlcNAc—R (Le^b). When the R-group is lactose, all of these compounds are present in human milk. For further information of Lewis antigens synthesis see ref. [78–80]

An overview of the reported synthesis of HMOs is summerized in Table 1.2. A selection of these will be reviewed more thoroughly.

The first chemical synthesis of the backbone core lacto-*N*-tetraose (LNT) was repoted by Noro and co-workers [81] in 1979 and one year later by another group who utilized the exact same experimental procedure. [82] They reacted donor **1** with acceptor **2** in a toluene-nitromethane mixture with *p*-toluenesulfonic acid as catalyst for 48 h at 60 °C. This yields the protected LNT **3** in 77-79% that upon deprotection provides the desired product **4** (Scheme 1.8). In 1981 lacto-*N*-neotetraose (LNnT) was prepared by a similar strategy. [83]

The pioneer within synthesis of HMOs is Richard R. Schmidt. [84,86,87,99,101,105] Quite a few research groups have focused on the synthesis of HMO analogs containing lactose at the reducing end, and with a group attached (e.g. glycosyl ceramides) to the anomeric center of glucose. [94,107–109] Schmidt and co-workers [84] have synthesized both of the backbone cores LNT and LNnT. A TMSOTf promoted coupling of the trichloroacetimidate donor **5** and glucosamine acceptor **6** yielded **7** in 89%. [110] The *tert*-butyldimethylsilyl (TBDMS) group was removed and the newly formed disaccharide

Scheme 1.8: The first reported synthesis of LNT. [81,82]

Scheme 1.9: Synthesis of LNT by Schmidt and co-workers.[84,110]

was converted into the trichloroacetimidate donor **8**. TMSOTf promoted glycosylation of trichloroacetimidate donor **8** and lactose acceptor **9** to created the fully protected tetrasaccharide **10** in 76% yield, which upon deprotection and *N*-acetylation yielded LNT (**4**) (Scheme 1.9).

LNnT was prepared using the same reaction conditions as for the synthesis of LNT, employing glucosamine acceptor **11** to get the desired 1–4 correlation (Scheme 1.10). The TMSOTf promoted coupling of **5** and **11** yielded **12** in 84%, which after conversion into trichloroacetimidate **13** was coupled with **9** forming **14** in high yield. LNnT was isolated after deprotection and *N*-acetylation.

Schmidt and co-workers further extended the work by synthesizing the linear hexasaccharide lacto-*N*-neohexaose **15**[86] and octasaccharide lacto-*N*-neooctaose **16**[86] along with the branched octasaccharide **17** (Figure 1.4).[87] They used the block strategy with trichloroacetimidate donors and acceptors with their anomeric center being TBDMS protected.

Schmidt and co-workers also synthesized Lacto-*N*-fucopentaose II and III (LNFP II and III, see Table 1.2).[99,101] Once again trichloroacetimidate donors were utillized and a temporary protecting group was used at the anomeric center of the acceptor.

Scheme 1.10: Synthesis of LNnT by Schmidt and co-workers.[84,110]

15 : n = 1
16 : n = 2

17

Figure 1.4: Structures of hexasaccharide **15** and octasaccharides **16** and **17**.

Two different approches were used; in one of the cases only a single hydroxy group was left free at C-3,[101] and in the other case both C-3 and C-4 have free hydroxy groups.[99] The latter case is particularly interesting as this provides an insight into the reactivity of the different hydroxy groups on glucosamine and prefered attachments for different glycosyl donors. In the case of only one free hydroxy group, either the galactose or fucose building block is coupled with glucosamine **18** (Figure 1.5) followed by regioselective opening of the benzylidene acetal, leaving a hydroxy group at C-4 open for glycosylation with either a fucose or galactose. All of the glycosylations in this method proceeded in high yields. In the second method with two free hydroxy groups, it was attempted to prepare trisaccharide **22** in two different ways (Scheme 1.11); method *a* by first connecting the galactose donor **5** with the glucosamine acceptor **19**, followed by a glucosylation with fucose donor **21** or method *b* by first connecting the fucose donor **21** with the glucosamine acceptor **19**, followed by a glycosylation with the galactose donor **5**. As seen from this scheme, pathway *a* leads to disaccharide **20** as the only product, whilst pathway *b* leads to the two disaccharides **23** and **24** in a 2:1 ratio. This shows that the galactose has a preference for the 4-position while the fucose has a preference for the 3-position. The formed trisaccharides were transformed into trichloroacetimidate donors and coupled with a protected lactose acceptor yielding the fully protected pentasaccharides, which were converted into LNFP II and LNFP III (Table 1.2).

All methods reviewed until this point involved a temporary protecting group at the anomeric center of the acceptor, necessitating additional steps for introduction of an appropriate leaving group for the following glycosylations. The first reactivity-based synthesis of HMOs was only developed recently.[97] Disaccharide donor **26** and glucosamine **27** were combined and then coupled with lactose acceptor **28** in a one-pot synthesis giving the pentasaccharide product **29** in moderate yield (Scheme 1.12). Moreover, it was resently shown that a one-pot synthesis of fully protected LNT could

18

Figure 1.5: Glucosamine building block **18** used for the synthesis of LNFP II and LNFP III.

Scheme 1.11: Synthesis of the protected trisaccharides **22** and **25**.

be carried out by reactivity-based synthesis. The yield depended on the RRVs of the super-armed donors, giving higher yields with higher RRVs.[90]

The main part of the synthesized HMOs were formed using trichloroacetimidate donors,[88,89,91,94,99] but other employed glycosyl donors are thioglycosides,[90,94,95,97] and phosphates.[91,92,97]

Scheme 1.12: Synthesis of the protected trisaccharide **21** for synthesis of LNFP III.

1.3 Synthesis of Lacto-*N*-tetraose (LNT)

LNT (**4**) is one of the most abundant oligosaccharides in human milk and it is a backbone core for many of the HMOs identified (Table 1.1). LNT was chosen as target molecule for several reasons. First of all, LNT is the core of many HMOs, which makes this tetrasaccharide of high interest for enzymatic studies in synthesis of higher HMOs and biological activity measurements. Secondly, it would hopefully provide building blocks for the synthesis of penta- and hexasaccharides. Thirdly, the size and linear conformation made it a suitable target for development of a new one-pot strategy.

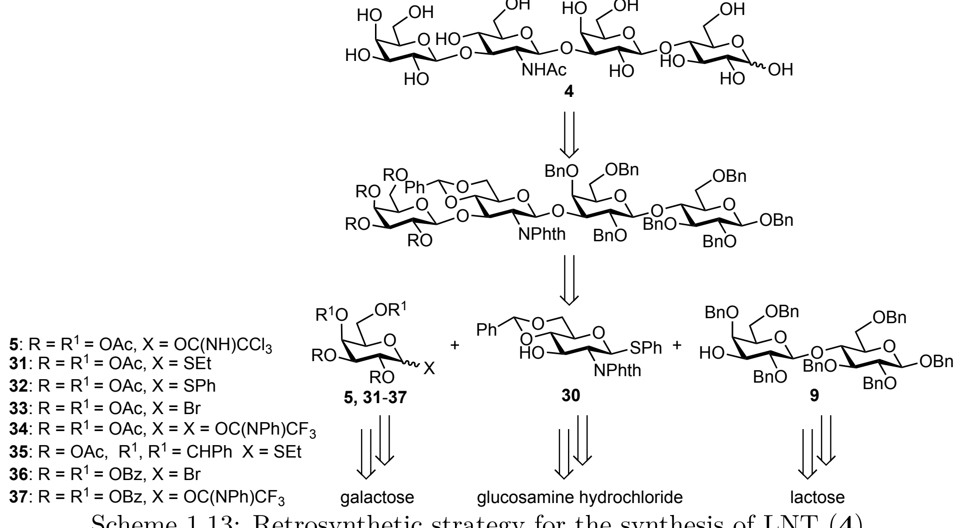
1.3.1 Retrosynthetic strategy

Well known building blocks were chosen for the retrosynthetic strategy. A variety of well known galactose building blocks **5, 31**–**37** were chosen as all could be synthesized in few steps from the same starting material. Furthermore, it was decided to settle for glucosamine building block **30** along with the lactose building block **9** (Scheme 1.13).

1.3.2 Building block synthesis

As the lactose building block **9** had been used successfully for the synthesis of LNT, it seamed to be the obvious choice. It renders only one free hydroxy group for the required β1-3 connection. The benzyl protecting groups improve the solubility in organic solvents and they can be cleaved off in one synthetic step. Building block **9** was synthesized from lactose in 6 steps with a low overall yield of 6%. Two steps were especially lowering the overall yield; the incorporation of a benzyl group at the anomeric center and the selective allylation of the 3'-position (Scheme 1.14).

Peracetylated lactose **38** was benzylated by an analogous procedure[114] yielding **39** in moderate 45%. An explanation for the low yield can be epimerization of the



Scheme 1.13: Retrosynthetic strategy for the synthesis of LNT (**4**).

Scheme 1.14: Synthesis of lactose building block **9**.

β-acetate **38** into the corresponding α-acetate which is less reactive. An alternative to the glycosylation of an acetal would be to convert the anomeric OAc group into a bromide and then perform the glycosylation with benzyl alcohol under lesser acidic and mare reactive Koenigs-Knorr conditions.

Removal of the acetyl grups with sodium methoxide yielded **40** in 88%. Herefter, dibutyltinoxide (Bu_2SnO) was employed for the selective attachment of the allyl group at the 3'-position. It is well known that the stannylene activation af hydroxy groups increases the nucleophilicity of the oxygen atom to react with various electrophiles. Tin can coordinate either to a primary hydroxy group or a vicinal *cis*-diol, hence for lactose **4o** three positions are able to coordinate with tin; the two primary alcohols and the 3' and 4' can coordinate forming a five membered ring with tin. The regioselectivity is determined by the electrophile. Reactive electrophiles such as acyl or alkyl electrophiles tend to react with the thermodynamically stable 3',4'-*O*-stannylated intermediate to afford 3'-*O*-acylated/alkylated lactose. On the contrary, 6/6'-*O*-glycosylation is afforded when relatively unreactive and bulky electrophiles are employed as they react with the faster accessible 6-*O*-stannylated intermediate.[37,112] The prefered conformation of the 3',4'-*O*-stannylated intermediate is a trigonal bipyramid (Figure 1.6). In general, the most electronegative ligands are located on the epical sites,[113] hence the hydroxy group at the 3'-position is more electronegative and the electrophile (allylbromide) will be attacked from this position. Addition of tetrabutylammonium iodide (TBAI) or tetrabutylammonium bromide (TBABr) will weaken the strength of the Sn-O bond of the most nucleophilic oxygen, and therefore catalyze the reaction.

The allylation of the 3'-position of a partly unprotected lactose (except for the reducing end aglycon) has been reported by several groups. Three different solvent systems have been employed; methanol/toluene (64% yield),[111] acetonitrile (47% yield)[114] and benzene (70% yield).[115] In this work the procedure by Alais *et al.*[115] was followed

Figure 1.6: Trigonal bipyramidal structure of the 3,4-*O*-stannylated intermediate.

changing the solvent to toluene instead of benzene. By running the reaction twice, they were able to drive the reaction nearly to completion with limited formation of further substituted compounds. In our case, **41** was furnished in 49% yield and small amounts of starting material was observed, however no additional products could be identified.

The subsequent benzylation generated **42** in 77% yield, which could be converted into **9** by the use of Wilkinsons catalyst in 69% yield. The most frequently used method for removing *O*- allyl ether is by transition metal catalyzed isomerization to the labile prop-1-enyl ether, which can be cleaved under mild acidic conditions. Wilkinsons catalyst ($ClRh(PPh_3)_3$) together with *n*-BuLi was used for the isomerisation of the double bond as the activity of the catalyst is significantly enhanced by *n*-BuLi. As a consequence, the catalytic loading can be lowered significantly. The isomerisation was followed by ^{1}H-NMR.

The glucosamine building block **30** (synthesis shown in Scheme 1.15) was designed to fulfill several criteria.

1. the *N*-protecting group should be a participating neighboring group to form the desired 1,2-trans product. In addition, the protecting group should endure the glycosylation conditions and the conditions employed for protecting group manipulations, such as strong base/acid.

2. only the hydroxy group at C3 is unprotected.

3. as few steps as possible is desired.

4. the leaving group (anomer protecting group) should withstand the glycosylation conditions employed for the first glycosylation and the protecting group manipulations.

5. if the protecting groups at C4 and C6 can be selectively removed one over the other it will be an advantage, but it is not a requirement. This will enable possible synthesis of branched penta- and hexasaccharides.

It would be optimal, if no *N*-protecting group was needed, as the 2-acetamido group can participate as a neighboring group, however a stable oxazoline is often formed instead of the desired glycoside. A phthalimide was chosen as the *N*-protecting group, since the stable oxazoline is not formed in this case and it can endure the basic conditions employed for acetyl removal, whereas many other *N*-protecting groups such as tetrachlorophthalimides and dimethylmaleimides are labile to these conditions.[116] Thiophenyl was chosen as the leaving group, since it is stable during the protecting group manipulations. A benzylidine protecting group was chosen, as this renders the possibility of selective opening of the benzylidine leaving a free hydroxy group at either the 4- or the 6-position. If two different protecting groups had been employed, it would result in additional synthetic steps. In addition, compound **30** has been involved in several glycosylations.[117–119]

The glucosamine building block **30** was synthesized from glucosamine hydrochloride in 4 steps with an overall yield of 37% by following literature procedures (Scheme 1.15). The bottleneck for this synthesis is the first protection of glucosamine hydrochloride as many parameters can affect the yield of **44**. Firstly, if to much heat is evolved when the amine is generated by addition of NaOMe the yield will be lowered significantly.

Scheme 1.15: Synthesis of glucosamine building block **30**.

Secondly, any presence of phthalic acid will lower the yield, as the reaction is quenched by protonation of the amine, hence lowering the nucleophilicity. Triethylamine is added to neutralize the formed acid on compound **43**. Thirdly, it is important not to stirr the glucosamine **43**, acetic anhydride and pyridine for too long, as this will generate more of the thermodynamically stable α product, which is less reactive in the following step. The thioglycoside **45** was formed in 78% yield when only the β anomer of **44** was employed. For the deprotection of **45**, the reaction was closely monitored by TLC, as the phthalimido group can be cleaved if the reaction is left for to long. Gratifyingly, this afforded **46** in 99% yield. The acid catalyzed attachment of the benzylidene group furnished **30** in 88% yield.

Several galactose building blocks were synthesized from peracetylated galactose **47** or perbenzoylated galactose **48** (Scheme 1.16), giving rise to the needed participating neighboring group. All galactose donors are known, and they were synthesized following known analog procedures. The detailes with regard to the synthesis of these donors will not be further described here, but all reactions are shown in Scheme 1.16 and the syntheses are described in the experimental section.

1.3.3 Glycosylations

Thiophenyl glycoside**32** was the first to be synthesized and coupling with **30** was attempted. None of the desired disaccharide could be isolated when the reaction mixture was subjected to NIS/TESOTf at -20 – +25 °C. All of **32** and most of **30** were isolated. The RRVs for a similar donor and acceptor (STol instead of SPh) show that **30** is a better donor than **32**, which is an undesired reactivity relation ship between the two compounds.[120] A variety of galactose donors were synthesized assuring the galactose donor could be selectively activited over glucosamine **30**.

In Table 1.3 is shown a selection of the glycosylations tested. The workload was distributed equally with post doc Thomas Hauch Fenger.

Thioglycosides **31** and **35** (Scheme 1.16) were synthesized as they supposedly are more reactive than **30**. Thomas Hauch Fenger performed the glycosylation studies of **31** (entry 1–3). The most promising promoter was DMTST, although this gained less than 10% of the disaccharide and **31** was therefore abandoned as donor. Galactose donor **35** was employed for two reasons, firstly the benzylidine enhances the reactivity compared to the fully acetylated thiophenyl donor **32**,[120] secondly, the benzylidene

Scheme 1.16: Synthesis of galactose donors **5, 31**–**37**.

can be opened selectively leaving a free hydroxy group at the 4- or 6-position, enabling further expantion of the tetrasaccharide to form larger oligosaccharides. Three different promoter systems were tested (entry 4–6) and the most promissing promoter was NIS/TESOTf yielding the desired product in 26% yield. From this reaction some of the donor could be reisolated (entry 4). Instead of using the Lewis acid TESOTf, the stronger Brønsted acid TfOH was employed, which disappointingly resulted in even more decomposition of both donor and acceptor. Both the BSP-Tf_2O (entry 5) and the NBS/TESOTf (entry 6) promoters resulted in decomposition of both donor and acceptor.

Table 1.3: Glycosylation between various galactose donors and glucosamine acceptor **30**, CH_2Cl_2 was used as solvent for all reactions.

#	Donor	Acceptor (30)	Temp. [°C]	Promotor	Yield [%]
1[a]	31		-50	MeOTf	—
2[a]	31		rt	DMTST	< 10
3[a]	31		-50	NIS/TfOH	—
4	35		-30	NIS/TESOTf	26
5	35		-30 – rt	BSP-Tf_2O	—
6	35		-40 – -20	NBS/TESOTf	< 5
7	33		-30 – rt	AgOTf	—
8	36		-30	AgOTf	—
9	36		-60 – rt	Ag_2CO_3	—
10	36		-30 – rt	Ag_2O	—
11[a]	5		-40	TESOTf	75
12	34		-35	TESOTf	28
13	37		-35	TESOTf	68

[a] performed by Thomas Hauch Fenger

Scheme 1.17: Synthesis of **50** by sequential and one-pot fashion leading to LNT after deprotection.

The thioglycoside donors were then abandoned and the Koenigs-Knorr glycosylation method came into use (entry 7–10). Two different donors were employed, the acetylated **33** and the benzoylated **36**. The benzoylated donor **36** was employed as it is more stable than **33** and can be stored in the fridge for several months. First it was attempted to generate **33** *in situ*, but the conversion was not quantitative, which required workup prior to the glycosylation. AgOTf was utilized as the promoter and collidine as acid scavenger. Unfortunately, only decomposition of both donor and acceptor took place (entry 7). For the benzoylated donor **36** three different silver salts were employed as promoters (entry 8–10). The AgOTf promoted reaction was tried both with and without an acid scavenger (collidine or TMU), which all resulted in decomposition of the donor. Nevertheless, the reaction allowed the acceptor to be reisolated. Neither Ag_2O nor Ag_2CO_3 were capable of activating the donor, which resulted in reisolation of both donor and acceptor.

The most successful galactose donors turned out to be the imidate donors **5**, **34** and **37**. Thomas Hauch Fenger performed the glycosylation between the trichloroacetimidate donor **5** and **30** yielding the desired disaccharide in 75% (entry 11) yield. *N*-Phenyl trifluoroacetimidate donor **34** and **37** were synthesized in the hope of getting a better yield by diminishing the possibility of rearrangment of the trichloroacetimidate into the unreactive amide (Scheme 1.3). Donor **34** only afforded the desired disaccharide in 28% yield (entry 12), which was rather low compared to the more successful glycosylation of donor **37** that yielded the desired disaccharide in 68% yield(entry 13).

The disaccharide **49** formed by the reaction shown in entry 11 was subjected to NIS/TfOH and the glycoside acceptor **9** yielding the protected tetrasaccharide **50** in 50% (Scheme 1.17). The tetrasaccharide **50** was formed using the same reaction conditions in a one-pot fashion providing 8% yield (Scheme 1.17). The fully protected tetrasaccharide **50** was deprotected over four steps yielding LNT in 42% (This work was performed by Thomas Hauch Fenger).

Attempts were also made to couple galactose donor **37** with glucosamine **30** and lactose **9** in a one-pot fashion with the hope that this would lead to a higher yield than for the one-pot synthesis of **50**. Unfortunately, only the dimer **51** could be isolated, and **52** was not isolated or detected (Scheme 1.18).

A pentenyl glucosamine acceptor (**53**) was synthesized in the aim to activate a glycoside donor prior to the pentenyl glycoside **53**. As seen in Table 1.4, none of the

Scheme 1.18: Attempted one-pot reaction for the synthesis of **52**

executed trials was fruitful.

The glucosamine acceptor **53** decomposed when subjected to a NIS/TfOH promoter. Some of the galactose donor could be reisolated indicating that **53** is more labile than **32**. No disaccharide could be identified (entry 1, Tabel 1.4). To avoid decomposition of the glucosamine acceptor, the promoter Me_2S_2-Tf_2O was chosen. The promoter contains no halogen and is therefore incapable of activating the pentenyl. Alternatively, DMTST or BSP-Tf_2O can be used as possible promoters. Neither of the thioglycoside donors **31** and **32** resulted in the desired disaccharide (entry 2 and 3). Surprisingly, a disaccharide product could be identified as a Gal-Glc structure, but the pentenyl double bond was reduced (entry 2). Using the Koenigs-Knorr conditions led to decomposition of the donor, and the acceptor could be reisolated (entry 4). As a result, the pentenyl strategy was abandoned at this point.

Table 1.4: Glycosylation between various galactose donors and acceptor **53**.

#	Donor	Acceptor (53)	Temp. [°C]	Promotor	Comment
1	32		-30	NIS/TfOH	Acceptor decomp.
2	32		-20	Me_2S_2-Tf_2O	Decomp.
3	31		-30	Me_2S_2-Tf_2O	Decomp.
4	33		-30 – rt	AgOTf	Donor decomp.

The tetrasaccharide Lacto-*N*-tetraose (**4**) was successfully synthesized by a sequential method. Unfortunately, our aim of develloping a one-pot strategy was not accommodated yet. The amount of LNT synthesized was suficient for our collaborators to perform initial biological activity and enzymatic studies. The next objective was to find a new galactose donor suitable for a one-pot method for the formation of a tetrasaccharide, which in a few steps could be altered into a glycosyl acceptor enabling enlargement to penta- and/or hexasaccharides.

1.4 Synthesis of the linear pentasaccharides Lacto-*N*-fucopentaose I and Lacto-*N*-neofucopentaose I

The two linear pentasaccharides Fucα1–2Galβ1–3GlcNAcβ1–3Galβ1–4Glc (LNFP I) **54** and Fucα1–2Galβ1–4GlcNAcβ1–3Galβ1–4Glc (LNnFP I) **55** were the next HMO target molecules (Figure 1.7).

Figure 1.7: Structure of target pentasaccharides Lacto-*N*-fucopentaose I (**54**) and Lacto-*N*-neofucopentaose I (**55**).

1.4.1 Retrosynthetic strategy for Lacto-*N*-fucopentaose I (LNFP I)

The knowledge gained from the synthesis of Lacto-*N*-tetraose convinced us that, the benzylidene protecting group caused synthetic problems. Despite the fact that several groups have published work involving the exact same or similar glycosyl acceptors providing disaccharides in moderate to high yield.[110,121,122] It was suspected that the benzylidine protecting group was hydrolyzed under the acidic conditions giving rise to additional di- and trisaccharides, hence lowering the yield of the desired disaccharide. In general, a *trans*-fused benzylidine (eg. in Glc) is hydrolyzed faster than a *cis*-fused (eg. in Gal) benzylidene. It has been shown that when a week donor is employed the overall yield drops significantly.[128,129] *N*-protecting group manipulation was attempted by Zhu *et al.*[129] in order to increase the reactivity of the C-3 hydroxy group in the presence of the bulky phthalimido group at C-2 that dampens the reactivity. Changing to a NHTroc group instead of NPhth did not favor glycosylation. Ultimately, a new glucosamine building block **61** was designed (Scheme 1.19), by keeping the phthalimido group and then change the benzylidene acetate into two benzyl protecting groups leaving C-3 open for attachment.

A galactose donor bearing a temporary protection group at C-2 was needed, since it was of interest to introduce a fucose at the C-2 position. In this case, it was important that the donor was armed and thereby enabling selective activation. On these grounds, the super-armed galactose donor **60** was chosen. This building block contains a benzoyl protection group at C-2 to form the desired β glycosidic bond. In addition, the benzoyl group can selectively be removed leaving a free hydroxy group at C-2 available for fucosylation. A thioethyl group was chosen as the leaving group, as this can be selectively activated over thiophenyl glycosides (Scheme 1.19).

The benzylated lactose **9** was left untouched, as it fulfills all demands. In Scheme 1.19 is shown the retrosynthetic plan for the synthesis of Lacto-*N*-fucopentaose I. The compounds **9**, **60** and **61** are to be glycosylated in a one-pot fashion to form **58**, which in one step is converted into **57**. Tetrasaccharide **57** and the fucose building block **59**

Scheme 1.19: Retrosynthetic strategy for the synthesis of LNFP I **54**.

will furnish fully protected pentasaccharide **56** that finaly can be transformed into the target molecule **54**.

1.4.2 Retrosynthetic strategy Lacto-*N*-neofucopentaose I (LNnFP I)

For the synthesis of LNnFP I the same galactose donor **60** and lactose acceptor **9** was selected (Scheme 1.20). The glucosamine building block **65** is similar to the glucosamine building block **61**, but with the minor difference being that the free hydroxy group is positioned at C-4. In Scheme 1.20 is shown the retrosynthetic plan for the synthesis of Lacto-*N*-neofucopentaose I. This strategy follows the same pattern as for the synthesis of LNFP I; one-pot glycosylation of **9**, **60** and **65** will lead to tetrasaccharide **64**, which can be converted into **63** and coupled with **59** to form fully protected pentasaccharide **62**. Ultimately, compound **62** is transformed into the target molecule **55**.

1.4.3 Building block synthesis

The super-armed galactose donor **60** was synthesized by Thomas Hauch Fenger and the glucosamine building block **65** was synthesized by Linda Maria Bruun, under my supervision. The synthesis of the lactose building block **9** was previously described in Section 1.3.2.

Several different pathways were considered for the synthesis of the new glucosamine building block **61**. It was of interest to use as few steps as possible and if possible to avoid the use of NaH as the phthalimido group is labile to strong base especially

Scheme 1.20: Retrosynthetic strategy for the synthesis of LNnFP I **55**.

in the presense of water. The two main routes are illustrated in Scheme 1.21 and both arise from glucosamine **30**. For pathway *a*, the benzylidene is regioselectively opened forming the 4-*O*-benzylether **66** followed by regioselective benzylation of the primary hydroxy group. For pathway *b*, the hydroxy group is protected by a temporary group (**67**) followed by hydrolysis of the benzylidene (**68**). The formed hydroxy groups are then benzylated (**69**) and in the last step the C-3 hydroxy moiety is liberated by deprotection of the temporary protecting group (PG). Pathway *a* is prefered as it only involves two steps.

Both pathways involve the cleavage of the benzylidene moiety. A benzylidene acetal can be opened using different methods. It can be completely removed by mild acid hydrolysis, or by hydrogenolysis. The benzylidene group can regioselectively be opened under oxidative or reductive conditions. Oxidative conditions only give the

Scheme 1.21: Synthetic pathways for the synthesis of **61**.

Scheme 1.22: Selective protection of hydroxy group at 6-position.

4-*O*-benzoate and can be done by NBS in CCl_4 under basic conditions. Using reductive cleavage of the benzylidene can result in the 4- or 6-*O*-benzyl ethers depending on the conditions. Much research have been done since the introduction of regioselective reductive opening of benzylidene moiety, and today there is a broad array of methods to choose from. The most common methods for obtaining the 4-*O*-benzyl is by using BH_3·THF-$AlCl_3$ or $LiAlH_4$-$AlCl_3$. The most common methods for obtaining the 6-*O*-benzyl is by using BH_3·NMe_3-$AlCl_3$, $NaCNBH_3$-HCl or Et_3SiH-Lewis acid.[123]

For pathway *a*, BH_3·THF with $Cu(OTf)_2$ as Lewis acid in THF was used yielding the 4-*O*-benzyl protected glucosamine **66** in 63%. It was attempted to selectively glycosylate compound **66** 6-position following two different procedures. First by refluxing **66** with *N,N*-diisopropylethylamine (DIPEA), benzylbromide and TBAI. After refluxing for 5 h all starting material was consumed resulting in a mixture of products. The desired product could only be isolated in 14% yield, giving an overall yield of 9% for the two steps. The second method conducted, was catalyzed by tin ($(Bu_3Sn)_2O$) in refluxing toluene for 4 h, followed by addition of benzylbromide and Bu_4NBr, which was further refluxed for 16 h. After purification, 35% of compound **66** could be isolated along with a poor 11% of the desired product **61** (Scheme 1.22).

For pathway *b*, two different protecting groups at C-3 were employed; an allyl and a fluorenylmethyloxycarbonyl (Fmoc). Fmoc was chosen, since the protection and deprotection can be carried out under mild basic conditions (pyridine/DMAP and piperidine respectively). As depicted in Scheme 1.23 both the Fmoc-protection leading to **70** and the regioselective opening of the benzylidene acetal forming compound **71** proceeded smoothly in 78% and 80% yield respectively. Unfortunately it was not possible to benzylate **71** under neutral conditions and only decomposition of the starting material could be observed. Here, most of the starting material was reisolated. It seams that the Fmoc-group is too bulky for the benzyl-group to be attached.

As an alternative, the 3-position was *O*-allyl-protected, unfortunately this strategy involves several steps including NaH, which is the most efficient reagent for the protection. The most common way of forming ethers is by Williamsons ether synthesis[124] employing NaH or NaOH as base in a polar aprotic solvent such as DMF. Both acetates and esters can be cleaved under these conditions. To get to fully protected glucosamine

Scheme 1.23: Attempts at synthesizing **61** by the use of a temporary Fmoc protecting group.

Scheme 1.24: Allyl protection of **30** and benzyl protection of **73** under basic and neutral conditions.

74, both the common Williamson ether synthesis and an alternative neutral method were employed (Scheme 1.24). Allylation with NaH did not give reproducible results, but occured with a varying yield of 22–68%. A tiny amount of water lowered the yield significantly, and as DMF is difficult to acchive extremely dry, it is challeging to get anhydrous reaction conditions. A palladium(0) catalyst was employed for the conversion of the alcohol into the allylether under neutral conditions. $Pd_2(dba)_3$, 1,4-bis(diphenylphosphino)butane (dppb) as ligand and allyl ethyl carbonate were used to introduce the allyl group by *in situ* CO_2 extrusion (Scheme 1.24). This procedure involves an oxidative addition of the allyl ethyl carbonate to the Pd(0) species, followed by a decarboxylation to give a (π-allyl)-palladium ethylate complex. Upon proton exchange between the ethoxide and the carbohydrate a cationic alkoxide complex is formed and the allylation can take place.[162] By employing this method it was possible to obtain **70** in reproducible yields. The formed allylether **72** was subjected to acidic methanol to give **73** in 99% yield. The following benzylation was carried out using NaH in either DMF or THF both in very poor yield, 40% and 32% respectively. Whilst employing neutral conditions by the use of Ag_2O, KI and benzylbromide in DMF the yield of compound **74** was markedly higher. The reaction can be carried out without KI, but several experiments showed that the reaction rate was significantly lowered without KI and the reaction did not run to completion. The enhanced reactivity could be related to an *in situ* Finkelstein halogen exchange, forming KBr and benzyliodide. As iodide is a better leaving group and silver has a higher affinity for I than Br (HSAB principle), thus the benzylation is ought to proceed faster.

With the fully protected compound **74** at hand, the last step to reach the target

Scheme 1.25: Cleavage of allyl to form glucosamine building block **61**.

molecule **61** was deprotection of the allyl group by Wilkinsons catalyst (see the synthesis of **9** for more information Section 1.3.2). The isomerisation could be monitored by TLC, which is unusual, but convenient as the anaerobic conditions are retained until **74** is fully converted into the prop-1-enyl ether **75** (Scheme 1.25). Amberlite IR-120 H^+ in methanol was chosen for the cleavage of the prop-1-enyl ether prior to refluxing in 1 M HCl, although the amberlite cleavage takes several days for completion. The desired product **61** was isolated in 64% yield. The overall yield for the four synthetic steps from glucosamine **30** is 48%, which is significantly better than the 9% following path *a*.

The fucose building block **59** was synthesized from fucose over three steps, where the final benzylation was performed under the standard basic NaH conditions giving **59** in 61% yield.

1.4.4 Di- and tetrasaccharide formation and initial one-pot glycosylations

With all the building blocks in possession, it was possible to perform the glycosylation by following the armed-disarmed strategy (Scheme 1.26). Whilst I was in Canada for my external stay (Chapter 2), some preliminary studies were executed by Thomas Hauch Fenger. He managed to get the protected disaccharide **76** in an impressive 97% yield. By subsequent glycosylation with lactose building block **9**, compound **64** was furnished in 59%. Thomas optimized the conditions for the one-pot glycosylation giving the fully protected tetrasaccharide **64**. When I returned, I repeated the one-pot method, and got compound **64** in 72% yield (Scheme 1.26).

These results were very promissing, therefore the same conditions were utilized for the one-pot glycosylation of **60**, **61** and **9** into the fully protected LNT **58** (Scheme 1.27). The TLC plate of the coupling between **60** and **61** showed several spots, whereas the coupling between **60**, **65** was spot to spot conversion. As a concequence, the reaction was quenched at this stage yielding **77** in 44% (entry 1 in Table 1.5). It was evident that the C-3 OH was significantly less reactive than the C-4 OH and this is probably due to the bulky phthalimido protecting group at C-2.[125] A screening of promoters was executed in an attempt to increase the yield of the reaction (Table 1.5). All of the donor **60** was converted in the first trial, hence slow addition of the donor was attempted, regrettably, this resulted in a lowering of the yield (entry 2). Changing

NIS, AgOTf, CH_2Cl_2, -30 °C, 97%

9, NIS, TESOTf, CH_2Cl_2, -30 °C, 59%

NIS, AgOTf (cat.) then **9**, NIS, TESOTf, CH_2Cl_2, -30 °C, 72%

Scheme 1.26: Sequential and one-pot synthesis of **64**.

Table 1.5: Glycosylation between **60** and **61** employing different promoters.

#	Ratio 60:61	Promotor	Temp. [°C]	Time	Yield [%]
1	1.1:1	NIS/AgOTf	-40	1 h	44
2[a]	1.1:1	NIS/AgOTf	-40	1 h	22
3	1.1:1	NIS/TESOTf	-30	80 min	32
4	1.1:1	NIS/TfOH	-50 – -20	2 h	34
5	1.2:1	MeOTf	-10	7 h	78
6	1.2:1	MeOTf	-10 – rt	16 h	—
7	1.2:1	MeOTf, TTBP	-10	6 h	33
8	1.1:1	Me_2S_2-Tf_2O	-20 – rt	3 h	—

[a] slow addition of donor

the catalyst to TESOTf or TfOH gave a similar low yield of 32% and 34%, respectively (entry 3 and 4). Exposing **60** and **61** to MeOTf for 7 h gave **77** in 78% (entry 5). If the reaction was left to reach room temperature no product could be obtained (entry 6). In an attempt to increase the yield even more, the acid scavenger 2,4,6-tri-*tert*-butylpyrimidine (TTBP) was used (entry 7). Unfortunately, this resulted in a lower yield. A last attempt to improve the yield was to use Me_2S_2-Tf_2O, but the reaction was very messy and the desired product could not be observed by TLC. Although the yield obtained by using MeOTf was satisfactory, the long reaction time is not suitable when running a one-pot reaction.

A glycosylation between **77** and **9** promoted by the NIS/TESOTf system yielded **58** in 80% (Scheme 1.27). This led to an overall yield of 62% with MeOTf as the promoter in the first step and 35% when NIS/AgOTf was used as promoter. For the sequential coupling in the synthesis of **64** the overall yield reached 59% under similar conditions (Scheme 1.26).

For comparison, a one-pot reaction was carried out using NIS/AgOTf for the first coupling and NIS/TESOTf for the following glycosylation. The desired product **58** was obtained in 7% yield as depicted in Scheme 1.27 (for **64** the yield was 72%, Scheme

Scheme 1.27: Sequential and one-pot synthesis of **58**.

1.26).

Deprotection of benzoyl protecting groups

Methanolic sodium methanolate was intended to execute the chemoselective deprotection of the benzoyl group in the 2-position of **58** and **64**. The results are illustrated in Scheme 1.28 and as can be seen neither of the two are chemoselective. After stirring benzoate **64** for 1 day at room temperature, TLC revealed that virtually all starting material was still present. As a consequense, the temperature was raised to 50 °C and the mixture stirred for an additional 2 days. The reaction was quenched with Amberlite IR-120 H^+ although some starting material (**64**) was still visual on TLC. Only 21% of the desired product **63** was isolated along with 30% of the starting material. A third compound was detected and assumed to be **79**. Likewise, compound **58** was stirred at 30 °C for 8 days, the reaction was quenched although there was still starting material remaining. The crude mixture was examined by NMR, and the main product was determined as **78**.

Other research groups have reported partial cleavage of the phthalimido group, when a debenzoylation was attempted.[126] Instead of executing a deprotection of both the benzoyl and the phthalimide with hydrazine, it was decided to change the benzoyl into an acetyl protecting group for the galactose building block. This will be further explored in the following section.

1.4.5 Glycosylation reactions with super-armed acetyl-protected galactose donor

The acetyl-protected super-armed galactose donor **80** was synthesized from the super-armed benzoyl protected galactose donor **60** (Scheme 1.29). The debenzoylation under alkaline conditions required stirring at 50 °C for 48 h to reach completion. It is evident that the phthalimido group can not endure these conditions and therefore it is

Scheme 1.28: Attempts of chemoselective benzoyl (Bz) deprotection of **58** and **64**.

Scheme 1.29: Synthesis of galactose donor **80**.

understandable that some of the phthalimide was cleaved when the tetrasaccharides were exposed to such reaction conditions. The reaction was completed by addition of acetic anhydride, hence providing the galactose donor **80** in 97% (Scheme 1.29)

Glycosylations between the new donor **80** and either **61** or **65** were conducted to gain the results presented in Scheme 1.30 and Table 1.6. In this case the overall yield is not significantly better for the 1-4 coupled disaccharide (mix of **83** and **84**) than for the 1-3 coupled disacharides (mix of **81** and **82**). For the 1-3 coupled disaccharide **81**, the best yield was obtained whit NIS/AgOTf as promoter (entry 3). When employing MeOTf only disaccharide **81** was formed in a moderate yield of 52% (entry 1). The usage of NIS/TESOTf yielded a nearly 1:1 mixture of **81** and **82** in an unimpressive yield of 53%.

What is evident from these results is the occurence of aglycon transfer when NIS is utilized. This is not a crucial problem as all disaccharide products can be activated under similar conditions in the following glycosylation reaction with the lactose building block **9**. Aglycon transfer can be a problematic side reaction, if the donor becomes inactive because of the aglycon transfer. Aglycon transfer is common for thioglycosides and have been reported numerous times.[45,127,129,130] It is difficult to predict beforehand whether or not aglycon transfor wil occur as small changes in protecting groups and promoter systems can have significant effects. Ways to effectively prevent the aglycon transfer have been reported,[131] but it was of no interest to us to perform further modifications of our donor **80**. In previous reported cases of aglycon transfer only armed thioglycosides suffered from transfer, but this is not true in the present case. The carbon-sulfur bond of the thioglycoside can break and reform for both the donor and the acceptor and as the thioethyl group is a better nucleophile than the thiophenyl group, an attack can take place transforming the acceptor into a thioethyl glycoside instead of a thiophenyl glycoside. An equilibrium between SEt and SPh could potentially occur even when formation of the transfer product is not energetically favored. The reason for the absence of aglycon transfer occuring MeOTf is employed, is that the formed

Table 1.6: Glycosylation between **61** and **80** employing different promoters.

#	80:61	Promotor	Temp.	Time	81:82	Yield 81 (overall) [%][a]
1	1.3:1	MeOTf	-10 °C	6 h	100:0	52 (52)
2	1.2:1	NIS/TESOTf	-40 °C	1 h	55:45	29 (53)
3	1.2:1	NIS/AgOTf	-50 °C	1 h	81:19	62 (76)

[a] Estimated from isolated yields and isolated mixtures of **81** and **82** by NMR ratios between the two.

Scheme 1.30: Formation of **85** by sequential and one-pot glycosylation.

MeSEt is stable opposed to ISEt, which is formed when NIS is employed.

The formed disaccharides **81** and the mix of **83** and **84** were further glycosylated with **9** utilizing NIS/TESOTf as promoter in each separate reaction (Scheme 1.30 and 1.31). A one-pot reaction was also performed for the synthesis of **85** using the developed one-pot method. As depicted in Scheme 1.30 all glycosylations proceeded with excellent yields, especially the one-pot glycosylation furnishing tetrasaccharide **85**. For comparison, the sequential glycosylation forming **85** had an overall yield of ∼61% over the two steps, which is significantly lower than the one-pot reaction. Tetrasaccharide **86** was created in 80% after the glycosylation between **81** and **9** leading to an overall yield of ∼61% over two steps (Scheme 1.31).

It is evident, that the one-pot method employed is significantly better than the sequential method. The improved yield by the one-pot synthesis might be explained by an enhancement of the formed disaccharide donor by the continuing presence of SEt in the reaction mixture, leading to possible aglycon transfer. Also any loss during purification between the sequential glycosylation steps lowers the yield.

These results also prove that the 4-*O*-position is a better acceptor than 3-*O* for glucosamine when a phthalimide is employed as *N*-protecting group. Moreover, a one-pot glycosylation between **9**, **61** and **80** ought to be carried out to futher emphasize this assumption and expand the tested compounds for the newly developed one-pot method.

Deprotection of acetyl protection group

The two tetrasaccharides **85** and **86** were subjected to the standard deacetylation conditions to selectively remove the acetyl group over the phthalimide (Scheme 1.32). Yet again, the two acetyl protected tetrasaccharides **85** and **86** showed different reactivity behavoir, as was observed for the two benzoylated tetrasaccharides.

Scheme 1.31: Glycosylation between **81** and **9** forming tetrasaccharide **86**.

Scheme 1.32: Deacetylation of **85** and **86**.

After stirring **85** in methanolic sodium methanolate for three days at room temperature the deacetylated tetrasaccharide **63** could be isolated in 81% yield. Whereas none of the desired deacetylated product of **86** could be isolated after 7 days of stirring. Instead both the alcohol and amine had underwent a deprotection reaction yielding **78** in 68% (Scheme 1.32).

It was puzzling why the two tetrasaccharides were behaving so different under similar reaction conditions, with the only conformational difference being the correlation between the galactose and the glucosamine. Consequently, lowest energy calculations were performed to see if an explanation could be found on these grounds (Figure 1.8 and 1.9). Indeed, the two lowest energy conformations are markedly different. For compound **86** the acetyl and the phthalimide moieties are situated side by side, hence minimizing the possibility for the nucleophile to differentiate between the two groups (Figure 1.8). On the other hand, the acetyl group and the phthalimido group are situated far from each other for **85** and the phthalimide is shielded by benzyl protecting groups whereas there is free access for attack on the acetyl (Figure 1.9). The calcu-

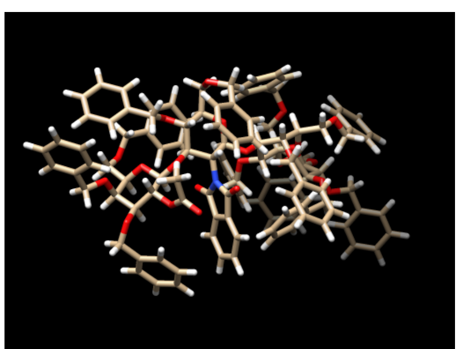


Figure 1.8: Calculated lowest energy conformation of **86**. N-atom is blue, O-atoms are red. The phthalimido group and the acetyl group are situated in the middle of the lower part. Calculations done by Casper Junker Engelin.

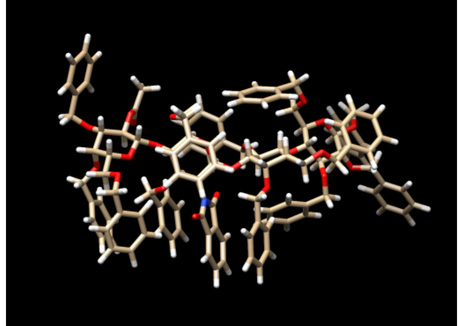


Figure 1.9: Calculated lowest energy conformation for **85**. N-atom is blue, O-atoms are red. The phthalimido group is situated in the middle of the lower part, and the acetyl group is situated in the top left. Calculations done by Casper Junker Engelin.

Scheme 1.33: Deprotection and selective *N*-acetylation of **86**.

lations are in agreement with the experimental results. Taking this into account, it seems obvious that **86** can not be selectively deprotected to form a free alcohol without affecting the phthalimido group. Hereof the new strategy was to deprotect both the acetate and the phthalimide with a following selective *N*-acetylation.

Ethylenediamine (EDA) was employed for the deprotection of the acetate and the phthalimide parts on **86**, as this lowers the reaction time compared to when sodium methoxide was utilized. The selective *N*-acetylation was carried out without purification of the formed amine **78**, as purification of amines by column chromatography can be very tedious. Treatment of "**78**" with acetic anhydride and triethylamine in ethanol furnished tetrasaccharide **87** in good yield (scheme 1.33). A primary amine is more nucleophillic than a secondary alcohol, and is therefore acetylated more easily. The conditions employed here are rather mild, but if acetyl chloride had been utilized instead of acetic anhydride, acetylation of the alcohol would possibly occur as well. Changing the triethyl amine to pyridine might also result in acetylation of the alcohol, as pyridine enhance the electrophilicity of acetic anhydride by the formation of an acyl pyridinium ion.

At this point the two tetrasaccharides **63** and **87** were ready for fucosylation. In the following section the final steps to form LNFP I and LNnFP I will be outlined separately.

1.4.6 Fucosylation and full deprotection

The fucosylation and deprotection of **63** proceeded smoothly. Treatment of **63** with fucose **59** and NIS/TESOTf in CH_2Cl_2 at -30 °C yielded **62** in excellent 89% yield (Scheme 1.34). At this point the overall yields for the sequential and the one-pot glycosylations to form **62** were 44% and 68% respectively.

Bröder and Kunz[93] synthesized the exact same compound from the lactoseamine fluoride donor **88** (formed in 17% yield from a galactose donor and a glucosamine acceptor[132]) instead of the thiophenyl glycoside employed in the current work together with the same lactose acceptor **9** (Scheme 1.35). They employed the same conditions for the deacetylation and fucosyl bromide **89** as donor for the final step. They achieved an overall yield of 45%, if this was to be compared with the synthesis described in this thesis, the first step for the formation of the mixture of **83** and **84** (Scheme 1.31) should not be considered in the overall yield. Thereby the overall yield is 52% for

Scheme 1.34: Fucosylation of **63** to form fully protected pentasaccharide **62**.

the same amount of steps by the sequential method, which is an improvement to their synthesis. Furthermore, the overall yield for the one-pot synthesis is an even greater improvement.

LNnFP I (**55**) was isolated after full deprotection of **62** (Scheme 1.36). First, the phthalimide was removed with EDA and the amine was acetylated with acetic anhydride and triethylamine, giving compound **90** in 74% yield. The following hydrogenolysis gave LNFP I **55** in 99% yield. A mixture of MeOH/THF was chosen as solvent to ensure solvation of both starting material and product throughout the reaction. TFA was used as catalyst for the reaction.

One of the two target human milk pentasaccharides was hereby synthesized by a sequential and a one-pot synthesis in good to excelent yields. Full NMR data are included in the appendix.

The fucosylation of **87** did not proceed as smoothless as for **63** (Scheme 1.37). A higher temperature was needed for the fucosylation to occur, since nothing happened below -20 °C. The change in reactivity can be due to the topological differences around the C-2 OH, as the galactose and glucosamine are coupled differently for the two molecules. Therefore, the lowest energy conformation might be significantly different

Scheme 1.35: Synthesis of **62** by Bröder and Kunz.[93]

Scheme 1.36: Deprotection and N-acetylation of **62**.

for the two compounds. Compound **91** was isolated in 68% yield after the glycosylation was carried out at -10 °C with NIS/TESOTf as the promoter (Scheme 1.37).

LNFP I (**54**) was isolated after hydrogenolysis in 98% yield. Again a mixture of MeOH and THF was employed to ensure solubility, and TFA was used as catalyst for the reaction (Scheme 1.37). Hereby the second human milk pentasaccharide was succesfully synthesized by a sequential and a one-pot process in moderate to good yields. Full NMR data for LNFP I (**54**) are included in the appendix.

Scheme 1.37: Fucosylation of **87** with fucose building block **59** and the following hydrogenolysis of **91** to furnish LNFP I (**54**).

1.5 Towards branched pentasaccharides using partly protected saccharide building blocks

As can be seen from Table 1.1, the branched penta- and hexasaccharides are also quite abundant in human milk. Therefore, it was also of great interest to synthesize some branched oligosaccharides containing either fucose or neuraminic acid attachments. Moreover, it was desired to apply the newly developed one-pot method for synthesis for the branched HMOs.

1.5.1 *N*-Acetyl neuraminic acid containing pentasaccharides

The use of *N*-acetyl neuraminic acid (Neu5Ac) as glycosyl donors is complicated for several reasons. For instance, there is no C-3 functionality present to direct the stereochemical outcome of the glycosylation. Furthermore, the electron withdrawing carboxylic acid at the anomeric center and the deoxy moiety at C-3 makes Neu5Ac prone to undergo elimination and thereby forming a glycal. Finally, glycosylation of Neu5Ac involves attack at a sterically hindered tertiary oxocarbenium ion intermediate. The commonly most applied glycosyl donors (e.g. thiols, imidates and halogens) are rarely employed for the attachment of Neu5Ac. On the contrary, phosphites[133] and xanthates[134] are some of the more successful leaving groups for sialyl donors.[135]

It was of interest to incorporate the Neu5Ac at an early stage of the synthetic route, primarily to avoid tedious α/β purifications of a pentasaccharide, since the glycosylation with derivatives of Neu5Ac lacks stereospecificity. It was chosen to persue the

Scheme 1.38: Retrosynthetic strategy for the synthesis of pentasaccharide **92**.

pentasaccharide Galβ1–4[Neu5Acα2–6]GlcNAcβ1–3Galβ1–4Glc (**92**), which retrosynthetic plan is shown in Scheme 1.38.

Once again both the super-armed galactose donor **60** and the lactose acceptor **9** were selected. They were to be combined with building block **94** containing a Neu5Ac residue at the 6-position of a glucosamine. Disaccharide **94** was envisioned to be synthesized from partly unprotected glucosamine **46** and fully protected neuraminic acid **95**. The primary hydroxy group for the glucosamine **46** is the most nucleophilic of the three hydroxy groups available. Research described in this thesis has proven that the C-3 OH is shielded by the phthalimido group, and other research groups have shown the C-4 OH typically is a poor glycosyl acceptor.[39]

Koenigs-Knorr conditions were attempted for the generation of **94**, by the use of two different silver salts (Table 1.7). Neither of the two silver salts were sufficiently powerful, since all starting material could be reisolated even after 2 days of stirring at room temperature (Entry 1 and 2). As an alternative, the thioglycoside donor **96** (synthesized by a former student) was tested. The thioglycoside donor **96** was attempted, since a similar glycosylation between **96** and a galactose acceptor formed the desired α adduct in moderate yield.[96] Regrettably, both donor and acceptor decomposed with the use of the NIS/TESOTf promoter system (entry 3). Altering the employed solvent might be a possibility to get dimer formation.

Table 1.7: Attempts of synthesizing disaccharide **94**.

#	**Donor**	**Acceptor (46)**	**Temp. [°C]**	**Promotor**
1	AcO, OAc, Cl, AcO, AcHN, O, CO_2Me, AcO **95**	OH, O, HO, HO, SPh, NPhth	-10 – rt	AgOTf
2	AcO, OAc, Cl, AcO, AcHN, O, CO_2Me, AcO **95**	OH, O, HO, HO, SPh, NPhth	-10 – rt	Ag_2O
3	AcO, OAc, CO_2Me, AcO, AcHN, O, SPh, AcO **96**	OH, O, HO, HO, SPh, NPhth	-10 – rt	NIS/TESOTf

Both of the utilized Neu5Ac donors (**95** and **96**) contained common leaving groups with regard to other glycosyl donors, however, a change to either a phosphite/phosphate or xanthate donor would be of interest, since they usually result in higher yields.

It was not of the highest priority to persue a solution to this problem and due to time limitations, the project was left at this stage.

1.5.2 Fucose containing pentasaccharides and hexasaccharides

To broaden the one-pot method employed for the synthesis of **54** and **55** (LNFP I and LNnFP I), pentasaccharide Galβ1–4[Fucα1–3]GlcNAcβ1–3Galβ1–4Glc (LNFP III) **97** was targeted (Figure 1.10).

Figure 1.10: Structure of pentasaccharide LNFP III (**LNnFP3**).

1.5.3 Retrosynthetic strategy and building block synthesis

The galactose donor part can be one of the two super-armed donors **60** or **80** (Scheme 1.39). The glucosamine **100** was designed to have two free hydroxy groups, one at C-4 and one at C-3 as our results have shown that the C-4 OH is more nucleophilic than the C-3 OH. Furthermore, studies by Schmidt and co-workers had shown that galactose donor **5** had a preference for the C-4 position (see Scheme 1.11).[99] The fucose donor **59** had proven to be a good donor for the attachment on the galactose, thus this was a good choice. Finally, the same lactose acceptor **9** used for all previous glycosylations was chosen. The overall idea was to create the backbone core first and then in the same pot add the fucose donor (last to be added) to form **98** or **99** (Scheme 1.39).

The glucosamine building block was easily synthesized by regioselective cleavage of the benzylidene by treating **30** with triethylsilane, TFAA and TFA yielding **100** in 69%. The first galactose donor to be tested was **60**, which was utilized in both a sequential and a one-pot glycosylation (Scheme 1.40). Treating **60** and **100** with NIS/AgOTf resulted in an inseparable 1:2 mixture of the thioethyl glycoside **102** and

Scheme 1.39: Retrosynthetic strategy for the synthesis of pentasaccharide **97**.

Scheme 1.40: Sequential and one-pot glycosylations in the formation of **103** and one-pot synthesis of **104**.

the thiophenyl glucoside **101** in approximately 61% yield. The mixture of **101** and **102** was subsequently subjected to the NIS/TESOTf promoter in the presence of the lactose acceptor **9**, forming ultimately tetrasaccharide **103** in approximately 43% yield, resulting in an overall yield of 27% for the two steps. The one-pot glycosylation yielded **103** in 57%, which is fairly good considering the fact that two hydroxy groups can act as a nucleophile. In addition, it is a significant improvement to the sequential glycosylation and none of the β1-3-coupled product was observed.

In a similar way galactose donor **80** and **100** were used in a one-pot glycosylation with lactose **9**, giving tetrasaccharide **104** in moderate 51% yield (Scheme 1.40). None of the β1-3-coupled product was observed.

The 1-4 correlation for **103** and **104** was determined by NMR spectroscopy. A correlation between C-1”’ and the hydrogen at C-4” was observed by HMBC. In addition, no correlation between C-3” and CH_2Ph could be observed in the HMBC spectra.

The two tetrasaccharides **103** and **104** were ready for fucosylation to form fully protected pentasaccharides (Scheme 1.41). For both tetrasaccharides NIS/TESOTf was employed as the promoter. The first glycosylation attempted was between fucose donor **59** and acceptor **103**. The promoter was added at -30 °C, but no reaction took

Scheme 1.41: Attempted fucosylation of **103** and **104**.

Scheme 1.42: Possible future work.

place, so the temperature was slowly raised to reach 0 °C. At the elevated temperature, it was observed that donor **59** was consumed after 1 h, unfortunately, none of the desired product could be identified. Treating fucose donor **59** and acceptor **104** with NIS/TESOTf at -10 °C and letting it reach room temperature resulted in full conversion of the donor, but yet again none of the desired product was isolated. It was a very disappointing result, as it would have been neat to extend the application of one-pot glycosylation by extending the amounts of glycosylations performed in the same pot. A change of fucose donor **59** to imidate could be a possibility, as **21** (Scheme 1.11) could successfully be attached at the C-3 position of a similar glucosamine acceptor.

Regrettably, no more work could be performed on this project, as my PhD enrolment had come to an end. If more work could be put into this project several ideas could be carried out. Two main ideas for further work on this would be to perform a deprotection of both tetrasaccharides (**103** and **104**) followed by selective *N*-acetylation to hopefully minimize the shielding at the C-3 position, hence making it possible for a glycosylation to occur (Scheme 1.42). Stereoselective deprotection of **103** should leave the benzoyl protecting group untouched so that a following *N*-acetylation could result in tetrasaccharide **105**, having only a single free hydroxy group at the 3-position of glucosamine. Subsequent, glycosylation with **59** would result in pentasaccharide **106**, which upon deprotection would give LNFP III. On the other hand, deprotection and selective *N*-acetylation of **104** would render a tetrasaccharide with two free hydroxy groups (**107**), one at C-2 at the galactose and one at the C-3 at the glucosamine. A following fucosylation could possibly lead to the branched hexasaccharide **108**.

1.6 Enzymatic synthesis of human milk oligosaccharides

An alternative to chemical synthesis of oligosaccharides is to involve enzymes, which couple monosaccharides with exquisite regio- and stereospecificity. One advantage with enzymatic glycosylation compared with chemical synthesis is the fact that protecting groups or elaborate precursors are unnecessary. On the other hand, preparation of non-natural oligosaccharide analogs is restricted as no suitable enzymes are available. Other concerns with employing enzymes is the restricted access to expensive or cloned enzymes and the requirement for co-factors. Many successful applications of enzymes in oligosaccharide production have employed chemoenzymatic synthesis, relying on both chemical and enzymatic steps, which typically begins with chemical synthesis and ends with an enzymatic extension.[136]

Two classes of enzymes can be utilized in enzymatic oligosaccharide synthesis, where the first class is glycosyltransferases that forms a glycosidic bond, while the second class is glycosidases whose natural function is the cleavage of glycosidic bonds by hydrolysis. The availability and stability of glycosidases are much greater than glycosyltransferases, and they are more tolerant to variations in substrate structure as well.[136]

Glycosidases can be used for glycoside synthesis by shifting the equilibrium of the glycoside cleavage in the opposite direction. It is possible to shift the equilibrium by exposing the enzyme to a large excess of the reaction products, hereby force a glycosidase to run in reverse. However, as the reverse reaction is endothermic, the equilibrium will always favor the cleavage products. Consequently, the yields obtained are typically low.[38]

Some advantages of the glycosyltransferases are that they produce the desired glycosides in higher yields and are highly specific towards donor as well as acceptor substrates. Unfortunately, the availability of glycosyltransferases is rather limited. The majority of glycosyltransferases used in glycan synthesis catalyze the transfer of a glycosyl donor to a sugar or amino acid acceptor. These transferases generally require nucleotide mono- or diphosphate saccharides as donor substrates (e.g., CMP-Neu5Ac or UDP-Gal), which are expensive and difficult to synthesize.[38]

Glycosyltransferases have been used to synthesize HMOs. For the more simple tetrasaccharides 2'-fucosyllactose, 3'-sialyllactose and 6'-sialyllactose the focus has been to find enzymes capable of performing regio- and stereospecific reactions with a possibility of a reaction scale-up.[137–139] On the contrary other more complex HMOs mainly have been subjected to glycosyltransferases for the purpose of finding excellent enzymes for specific transferase and not to run large scale synthesis of HMOs.[140–143]

When attachment of sialic acid is wanted, enzymatic synthesis is often to be prefered

Scheme 1.43: Mechanism for *Trans*-sialidase.

prior to chemical synthesis, since the purification step for the separation of alpha and beta anomers is very tedious in a non stereospecific reaction. And as the sialidase enzymes are programmed only to produce the desired alpha anomer they are naturally fabricated to perform the desired glycosylation.

Trans-sialidase catalyzes transfer of preferably α2,3-linked sialic acid directly to the terminal β-galactose or β-N-acetylgalactosamine-containing acceptors forming a new α2,3-linkage. In the absence of an appropriate acceptor, the enzyme acts as a hydrolase releasing glycosidically linked sialic acid. Cleavage is less efficient than transfer so in the presence of an appropriate acceptor transfer is dominant (Scheme 1.43).[144]

The activity of *trans*-sialidase can be monitored with a radioactive assay or a non-radioactive assay. For radioactive assays the most commonly used method is to apply sialyl-α2,3-lactose as donor, and a radioactively labeled lactose or N-acetyllactosamine as acceptor. The reaction can be followed as the radioactive and negatively charged sialylated products can be separated from labeled uncharged acceptor molecules by anion exchange chromatography, hence quantified to give a measure of enzyme activity. The nonradioactive *trans*-sialidase assays are based on a spectrophotometric technique, where either the donor or acceptor contain a UV-active group. The most commonly used UV-active moieties are *p*-nitrophenyl (*p*NP) or 4-methylumbelliferyl (MU). If the *p*NP or MU group is attached to the donor the activity is meassured by the release of phenol or 4-methylumbelliferone from the donor substrate. Whereas having the *p*NP or MU group on the acceptor acquires a workup where unreacted acceptor is washed away followed by hydrolysis of the *p*NP or MU group.[147]

Our collaborators were interested in finding a new enzyme with the ability to perform a *trans*-sialidations to produce 3'-sialyllactose on a large scale. For this purpose they needed a large amount of *p*NP-Neu5Ac for screening of their enzymes. I was asked to aid them by synthesizing *p*NP-Neu5Ac chemically.

1.6.1 Synthesis of *p*NP-Neu5Ac

Several research groups have synthesized *p*NP-Neu5Ac (**109**),[145,146] providing a well established synthetic pathway for the synthesis of *p*NP-Neu5Ac. The retrosynthetic strategy is shown in scheme 1.44.

Scheme 1.44: Retrosynthetic strategy for the synthesis of *p*NP-Neu5Ac.

Scheme 1.45: Synthetic pathway for the synthesis of the ester **115**.

The Fischer esterification of *N*-acetylneuraminic acid proceeded smoothly yielding **112** in 86% (Scheme 1.45). The first attempt to synthesize **95** was unsuccessful. The only product isolated after **112** had been subjected to acetylchloride for 36 h was **113**. It was apparent that the formed HCl gas was libarated from the reaction flask before an attack on C-2 occured. To circumvent this problem two alterations were performed, firstly, the reaction was carried out in a sealed flask with a screw cap capable of enduring high pressure, secondly, MeOH was added after 16 h, at which time **113** was formed, to produce additional HCl gas. With these alterations **95** was isolated in an excelent 100% yield (Scheme 1.45).

To synthesize **110** a two-phase system with benzyltriethylammonium chloride as phase transfer catalyst was employed. This is an alternative to the Koenigs-Knorr method and an improvement due to the fact that no expensive silver salt is necessary. Other advantages with this method is the short reaction time and the conditions are relatively mild.[145] Compound **110** was isolated in a modest 54% yield. The hydrolysis of the acetyl protecting groups with sodium methanolate yielded **115** in high yield (Scheme 1.45). Unfortunatly, the ester hydrolysis of **115** showed to be more tedious than expected.

The first attempt to synthesize **109** was executed following the procedure by Eschenfelder and co-workers.[146] This resulted in a crude mixture of starting material, the desired product and *N*-acetylneuraminic acid. It was attempted to purify the crude product by column chromatography, both flash column and dry column chromatography were employed here. Unfortunately, *p*NP-Neu5Ac is very unstable in both methanol and water. The purification resulted in a huge loss of product as *p*-nitrophenol was hydrolysed off forming the undesired Neu5Ac. In order to resolve this, a screening of base and solvents were performed to hopefully find a system only forming the desired *p*NP-Neu5Ac. In Table 1.8 can be seen all the hydrolytic systems tested. The same conditions were applied for entry 1 and 2, but not performed the same day. As of the ratios between the three compounds differed significantly it can be concluded that the procedure was not reproducible, and an unnoticed change in base concentration or reaction time have a significant influence on the outcome. Changing the base from NaOH to K_2CO_3 showed promissing results (entry 5–8), the best result being with 0.1

Table 1.8: Ester hydrolysis of **115**.

#	Solvent	Base	Conc. [M]	Time	Ratio; 115:109:111[a]
1	H_2O	NaOH	0.10	0.5 h	~3:5:4
2	H_2O	NaOH	0.10	0.5 h	~0:20:1
3	H_2O	NaOH	0.01	0.5 h	25:5:6
4	H_2O	LiOH	0.01	0.5 h	5:15:2
5	H_2O	K_2CO_3	0.01	0.5 h	3:1:~0
6	H_2O	K_2CO_3	0.10	1.5 h	1:10:2
7	MeOH/H_2O 95:5	K_2CO_3	0.10	2.0 h	0:3:2
8	THF/H_2O 90:10	K_2CO_3	0.10	3 h	2:~0:~0
9	THF	LiOH	0.10	0.5 h	1:~0:~0

[a] measured by ^{1}H-NMR

M of K_2CO_3 in water. Unfortunately, this still resulted in some hydrolysis (entry 6). A change of solvent was performed in an attempt to lower the amount of hydrolysis, but unfortunatly this resulted in a two-phase system with no significant conversion of starting material (entry 7 and 8). None of the attempted alterations showed significant improvement, and therefore the the initial method was employed with acceptance of the small amounts of impurities. The impurities were not crucial for the activity measurements, as they are also formed in the assays for *trans*-sialidase activity.

The *p*NP-Neu5Ac synthesized was used to test *trans*-sialidase activity of a recombinant *Pasteurella multocida* sialyltransferase exhibiting dual trans-sialidase activities along with a *Trypanosoma rangeli* mutant (papers in preparation).

1.7 Summary and Outlook of Chapter 1

In summary, the backbone core Lacto-*N*-tetraose (**4**) was synthesized by the use of a trichloroacetimidate galactose donor. It was possible to perform a one-pot synthesis, but the yield was very poor. Several galactose donors were tried, for the first coupling between galactose and glucosamine. Thioglycosidic donors showed not to be efficient for the glycosylation, and only imidate donors were sufficient for the coupling. The glucosamine building block (**30**) containing a 4,6-*O*-benzylidene protecting group showed to be the problem, as the benzylidene acetal can be cleaved under the employed conditions. In addition, the bulky phthalimido group is lowering the reactivity at the C-3 position. The knowledge gained from this study was utilized in the strategy for the formation of the larger oligosaccharides.

The linear pentasaccharides Lacto-*N*-fucopentaose I (**54**) and Lacto-*N*-neofucopentaose I (**55**) were synthesized by sequential and a newly developed one-pot strategy invented in our research group. The synthetic strategy involved the armed-disarmed concept using a super-armed galactose donor, with an acetyl or benzoyl group in the C-2 position. Two glucosamine acceptors were synthesized to get the desired 1-3 and

1-4 correlation between the galactose and glucosamine. Different strategies were performed for the synthesis of the glucosamine **61** with a free hydroxy group at the C-3 position. A recurring issue regarding the synthesis of the glucosamine building blocks were the lability of the phthalimido group under strong alkaline conditions. Neutral conditions were employed whenever possible, as this increased the yields significantly.

LNFP I showed to be far more difficult to synthesize than LNnFP I for several reasons. Firstly, the 4-OH proved to be more reactive than the 3-OH, hence giving significantly lower yields for both the sequential and one-pot glycosylation in the synthesis of tetrasaccharides **58** and **64**. The difference between the two glycosylations (1-3 vs. 1-4) were not as significant for the galactose donor with an acetyl protecting group (resulting in tetrasaccharides **85** and **86**). The one-pot glycosylations to form **86** yielded the desired product in nearly quantitative yield (1-4 coupled). It would be of interest to perform the one-pot glucosylation to form the 1-3 coupled tetrasaccharide **85**. Secondly, the regioselective deprotection of **58**, **64**, **85** and **86** proved only possible for the acetyl-protected 1-4 coupled tetrasaccharide **85**. Deprotection of the 1-3 coupled tetrasaccharides **58** and **86** also resulted in cleavage of the phthalimido group, hence selective acetylation was conducted prior to fucosylation. Lastly, the fucosylation of the phthalimido protected 1-4 coupled pentasaccharide **62** ran in high yield, whereas the fucosylation of the acetamido protected 1-3 coupled pentasaccharide **91** gave a slightly lower yield.

The developed one-pot glycosylation method was used in an attempt to create branched pentasaccharides. A glucosamine with both a free hydroxy group at C-3 and C-4 was utilized giving only 1-4 coupled tetrasaccharides, with a free hydroxy at C-3. Unfortunately, it was not possible to fucosylate the C-3 position, hence forming the branched pentasaccharide LNFP III (**97**). It would be of interest to perform alterations of the fucose building block, to form LNFP III, all in the same pot.

Few trials in the synthesis of a neuraminic acid containing disaccharides were conducted, but none were successful. The disaccharide was thought as a possible building block for expanding the scope of the developed one-pot strategy.

*p*NP-Neu5Ac (**109**) was successfully synthesized with the purpose to perform enzyme activity studies. Due to the fact that *p*NP-Neu5Ac is readily hydrolyzed in the presence of water, the ester hydrolysis proved to be tedious, forming a mixture of *N*-acetyl neuraminic acid, uncleaved starting material and the desired product. Different bases and solvents were tried to see whether the hydrolysis could be performed selectively. The initial conditions showed to be the best conditions.

1.8 Experimental

General Experimental Conditions. Unless otherwise noted, reactions were carried out under an inert atmosphere (Ar) in round bottom flasks with magnetic stirring. However, reaction work-up was performed in air. Air- or water-sensitive liquids and solutions were transferred via syringe. Organic solutions were concentrated by rotary evaporation at 23–30 °C under 40 Torr. Purification of products was carried out by flash chromatography on silica gel (Merck 40–63 micron) or dry column chromatography on silica gel (Merck 15–40 micron). Reverse phase chromatograhy was carried out on silica gel (YMC - C18, 120 Å, 5-10-20 μM). Thin-layer chromatography (TLC) was carried out using aluminum sheets pre-coated with silica gel 60F (Merck 5554). The plates were inspected under UV light or developed using a cerium ammoniumsulfate solution (1% cerium(IV)sulphate and 2.5% hexaammonium molybdate in a 10% sulfuric acid solution).
Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded with a Bruker 500 (500 MHz/125 MHz) NMR spectrometer equipped with a cryoprobe or a Varian Mercury 300 (300 MHz/75 MHz) or a Varian Unity Inova 500 (500 MHz/125 MHz) or a Bruker Ascend 400 (400 MHz/100 MHz) equipped with a cryoprobe using the residual solvent as the internal standard. Recorded chemical shifts are reported in parts per million (δ = scale) downfield from tetramethylsilane, and all coupling constants (J) are expressed in Hz. In the APT spectra measured on the Bruker 500 MHz instrument, CH and CH_3 correspond to negative signals and C and CH_2 correspond to positive signals In APT spectras measured on the Bruker ascend 400 MHz instrument the relation is reversed. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Melting points were recorded on a Stuart SMP30 apparatus and are uncorrected.
Reagents. Chemicals were purchased from Aldrich, Merck, and Carbosynth Limited and were used as received. Dry solvents (DMF, THF, toluene and acetonitrile) were obtained from a Solvent Purification System, Innovative Technology Inc. Dichloromethane was dried over sieves (4 Å). Methanol was dried over sieves (3 Å) 2-3 days prior to use. Acetylchloride was distilled from PCl_5 prior to use. Wilkinsons catalyst was synthesized from $RhCl_3 \cdot (H_2O)$ according to the procedure described in ref.[148] Me_2S_2-Tf_2O reagent was synthesized according to the procedure described in ref.[149] Compound **38** and **47** were synthesized by following the procedure described in ref.[114] Compound **38** was synthesized by following the procedure described in ref.[178] Compound **53** was synthesized from **44** following the procedure described in ref.[169] Some compounds synthesized have been shown in Scheme 1.16, but not numbered. In this section they will be refered to by Roman Nomenclature (i-iv) Compounds with clubs (♣) are new, they have all been submitted for HRMS and the results are pending.

General procedure for one-pot glycosylation reactions

Super-armed galactose donor (1.1 eq.) and the glucosamine acceptor (1 eq.) were dissolved in CH_2Cl_2 (1 mL / 100 mg reactants) and stirred under argon with 4 Å MS for 1 h. The mixture was cooled to -30 °C, NIS (1.15 eq.) and AgOTf (cat.) were added and the mixture was stirred for 15 min, where TLC revealed full conversion of the acceptor (toluene/acetone 9:1). Then a solution of the lactose acceptor (1 eq. in CH_2Cl_2 (0.5

mL / 0.1 mmol)) was added to the mixture together with NIS (1.15 eq.) and TESOTf (0.1 eq.). The mixture was stirred for 40 min where TLC showed full conversion of the acceptor. The reaction was quenched with Et_3N and the mixture stirred for 15 min, then filtered through Celite and evaporated. Purification by chromatography.

General procedure for sequential glycosylation reactions

Donor (1.1 eq.) and the lactose acceptor **9** (1 eq.) were dissolved in CH_2Cl_2 (1 mL / 100 mg reactants) and stirred under argon with 4 Å MS for 1 h. The mixture was cooled to -30 °C, NIS (1.15 eq.) and TESOTf (0.1 eq.) were added to the mixture, which was allowed to stirr for 40 min where TLC showed full conversion of the acceptor, the reaction was quenched with Et_3N and the mixture stirred for 15 min, then filtered through Celite and evaporated. Purification by chromatography.

Benzyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-*D*-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-*β*-*D*-glucopyranoside[114] (39)

38 → 39 (BnOH, $BF_3\cdot OEt_2$, CH_2Cl_2, 45%)

To a mixture of **38** (40.7 g, 60 mmol), benzylalcohol (21.6 mL, 139 mmol) and 4 Å MS in dry CH_2Cl_2 (280 mL), was $BF_3\cdot OEt_2$ (21.6 mL, 175 mmol) slowly added at 0 °C with an argon atmosphere. After stirring for 24 h at rt the reaction mixture was washed with a saturated solution of $NaHCO_3$ (250 mL), H_2O (2×200 mL), and the organic phase was dried over $MgSO_4$ and evaporated under reduced pressure giving an yellow oil. Crystallization of the residue from diethyl ether gave a white crystalline product (19.7 g, 45%). M.p. 150–151 °C. M.p. lit. 145–146 °C.[151] ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.36–7.14 (m, 5H, Ar), 5.27 (dd, J = 3.4 Hz, 1.0 Hz, 1H), 5.15–4.96 (m, 2H), 4.96–4.84 (m, 2H), 4.79 (d, J = 12.3 Hz, 1H, OCH_2Ph), 4.53 (d, J = 12.3 Hz, 1H, OCH_2Ph), 4.48–4.34 (m, 3H), 4.12–3.93 (m, 3H), 3.86–3.66 (m, 2H), 3.51 (ddd, J = 9.9, 4.9, 2.0 Hz, 1H), 2.11–2.03 (m, 6H), 2.02–1.94 (m, 9H), 1.94 (s, 3H), 1.89 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.5, 170.5, 170.3, 170.2, 169.9, 169.7, 169.2, 136.7, 128.6, 128.2, 127.9, 101.2, 99.1, 76.4, 72.9, 72.7, 71.7, 71.1, 70.8, 70.8, 69.2, 66.7, 62.1, 60.9, 21.0, 20.9, 20.8, 20.8, 20.7. NMR data are in accordance with literature values.[152]

Benzyl 4-*O*-*β*-*D*-galactopyranosyl- *β*-*D*-glucopyranoside[114] (40)

39 → 40 (NaOMe, MeOH, 88%)

Compound **39** (9.5 g, 13 mmol) was taken op in a solution of NaOMe (0.220 g Na in 170 mL MeOH) and stirred at rt for 3 h whereupon amberlite IR-120 H^+ was added and the mixture stirred for additional 45 min followed by filtration and addition of a few drops of aqueous ammonia (24%). Upon evaporation under reduced pressure a white solid precipitated. Recrystallization from methanol resulted in a white crystalline product (5.0 g, 88%). M.p. 173–174 °C. M.p. lit. 180 °C.[153] ^{1}H-NMR (300 MHz, D_2O): δ = 7.55–7.33 (m, 5H), 4.92 (d, J = 11.6 Hz, 1H), 4.74 (d, J = 11.6 Hz, 1H), 4.53 (d, J =

8.0 Hz, 1H), 4.42 (d, $J = 7.7$ Hz, 1H), 3.97 (dd, $J = 12.3$, 1.9 Hz, 1H), 3.90 (d, $J = 3.2$ Hz, 1H), 3.84–3.47 (m, 9H), 3.38–3.29 (m, 1H). ^{13}C-NMR (75 MHz, D_2O): $\delta = 136.7$, 128.9, 129.0, 128.6, 103.0, 101.2, 78.5, 75.5, 74.9, 74.6, 73.0, 72.6, 71.6, 71.1, 68.7, 61.2, 60.2. NMR data are in accordance with literature values.[154]

Benzyl 4-*O*-(3-*O*-allyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside[115] (41)

A suspension of **40** (3.03 g, 7.00 mmol) in toluene (75 mL) was heated until the temperature reached 80 °C whereupon Bu_2SnO (2.09 g, 8.40 mmol) was added and the mixture refluxed (125 °C) overnight in a flask equipped with a Dean-Stark separator. The temperature was lowered to 80 °C followed by addition of allylbromide (10 mL, 0.12 mol) and Bu_4NBr (1.0 g, 3.1 mmol). This mixture was heated to reflux (130 °C) with a Dean-Stark separator for 4 h, cooled to rt and evaporated under reduced pressure. The resulting solid was dissolved in water (100 mL) and washed with EtOAc (2×100 mL). The aqueous phase was concentrated and toluene (75 mL) was added followed by Bu_2SnO (1.92 g, 7.71 mmol) this mixture refluxed (130 °C) overnight with a Dean-Stark separator. The temperature was lowered to 80 °C followed by addition of allylbromide (8.0 mL, 0.092 mol) and Bu_4NBr (0.75 g, 2.3 mmol). This mixture was heated to reflux (130 °C) with a Dean-Stark separator for 1 h. Cooled to rt, concentrated under reduced pressure. Methanol (50 mL) was added to the residue, filtered and washed with methanol. The filtrate was concentrated under reduced pressure and purified by flash column chromatography (SiO_2, MeOH/CH_2Cl_2 1:9) resulting in a white solid (1.61 g, 49%). ^{1}H-NMR (300 MHz, CD_3OD): $\delta = 7.42$–7.33 (m, 2H), 7.33–7.18 (m, 3H), 5.96 (ddt, $J = 17.3$, 10.4, 5.7 Hz, 1H, CH=CH_2), 5.30 (dq, $J = 17.3$, 1.6 Hz, 1H), 5.13 (ddt, $J = 10.4$, 1.9, 1.2 Hz, 1H), 4.88 (d, $J = 11.8$ Hz, 1H), 4.63 (d, $J = 11.8$ Hz, 1H), 4.35 (2×d, $J = 7.8$ Hz, 2H), 4.19 (ddt, $J = 12.7$, 5.7, 1.4 Hz, 1H), 4.09 (ddt, $J = 12.7$, 5.7, 1.4 Hz, 1H), 3.96 (d, $J = 2.6$ Hz, 1H), 3.90 (dd, $J = 12.1$, 2.6 Hz, 1H), 3.82 (dd, $J = 12.1$, 4.2 Hz, 1H), 3.75 (dd, $J = 11.4$, 7.5 Hz, 1H), 3.70–3.45 (m, 5H), 3.41–3.33 (m, 1H), 3.30–3.25 (m, 2H). ^{13}C-NMR (75 MHz, CD_3OD): $\delta = 139.0$, 136.4, 129.3, 129.2, 128.7, 117.5, 105.0, 103.2, 82.0, 80.7, 76.9, 76.5, 76.4, 74.7, 71.8, 71.7, 71.6, 67.0, 62.5, 62.0. NMR data are in accordance with literature values.[156]

Benzyl 4-*O*-(3-*O*-allyl-2,4,6-tri-O-benzyl-β-*D*-galactopyranosyl)-2,4,6-tri-*O*-benzyl-β-*D*-glucopyranoside (42)

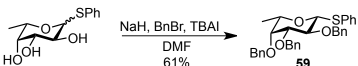
Coumpound **41** (1.54 g, 3.26 mmol) was dissolved in DMF (30 mL) and Bu_4NBr was added (15 mg, 0.047 mmol) followed by benzylbromide (6.1 mL, 52 mmol). NaH (2.04 g, 60% in an oil suspension, 51 mmol) was added at 0 °C and the mixture stirred at rt for 2 h, quenched with MeOH (1 mL), taken up in H_2O (150 mL) and extracted with ether (2×100 mL), dried over $MgSO_4$, concentrated and purified by flash column chromatography (SiO_2, EtOAc/heptane 2:3) resulting in a colorless amorphous solid

(2.33 g, 71%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.43–7.04 (m, 35H), 5.91 (ddt, J = 17.2, 10.5, 5.2 Hz, 1H), 5.31 (dq, J = 17.2, 1.7 Hz, 1H), 5.16 (dq, J = 10.5, 1.4 Hz, 1H), 5.00 (d, J = 10.7 Hz, 1H), 4.96 (d, J = 1.5 Hz, 1H), 4.94–4.85 (m, 2H), 4.79 (d, J = 11.2 Hz, 1H), 4.76–4.67 (m, 3H), 4.64 (d, J = 12.1 Hz, 1H), 4.57–4.50 (m, 2H), 4.48 (d, J = 7.5 Hz, 1H), 4.45–4.36 (m, 2H), 4.32 (d, J = 11.8 Hz, 1H), 4.22 (d, J = 11.8 Hz, 1H), 4.14 (ddd, J = 5.2, 2.6, 1.2 Hz, 2H), 3.93 (dd, J = 9.7, 8.7 Hz, 1H), 3.85 (d, J = 2.8 Hz, 1H), 3.80 (dd, J = 11.0, 4.3 Hz, 1H), 3.76–3.64 (m, 2H), 3.60–3.41 (m, 3H), 3.40–3.25 (m, 4H). NMR data are in accordance with literature values.[111] ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 139.3, 139.2, 139.0, 138.8, 138.6, 138.2, 137.7, 135.1, 129.2, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.2, 125.4, 116.5, 102.9, 102.6, 83.2, 82.5, 81.9, 80.0, 77.0, 75.5, 75.4, 75.4, 75.2, 74.8, 73.6, 73.5, 73.2, 73.1, 71.6, 71.1, 68.5, 68.2.

Benzyl 4-*O*-(2,4,6-tri-O-benzyl-β-*D*-galactopyranosyl)-2,4,6-tri-*O*-benzyl-β-*D*-glucopyranoside[155] (9)

Wilkinson's catalyst [$ClRh(PPh_3)_3$] (463 mg, 1.0 mmol, prepared from $RhCl_3{\cdot}3H_2O$) was dissolved in anhydrous THF (27 mL) and the solution was degassed. *n*-BuLi (0.75 mL, 1.6 M in hexane, 1.2 mmol) was added and the mixture was degassed for additional 10 min. A degassed solution of **42** (5.0 g, 4.9 mmol) in anhydrous THF (40 mL) was heated to reflux and the solution of the catalyst was added. The reaction mixture was refluxed for 2 h, whereupon ^{1}H-NMR revealed full conversion into the vinyl ether. It was cooled to 50 °C followed by addition of MeOH (150 mL) and Amberlite IR-120 H^+(15 mL). The resulting mixture was stirred for 48 h where TLC showed full conversion to the alcohol. The resin was filtered off, washed with CH_2Cl_2 and the filtrate was concentrated and purified by flash column chromatography (SiO_2, EtOAc/heptane 2:3) resulting in an amorphous colorless solid (3.3 g, 69%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.62–6.76 (m, 35H), 4.93 (d, J = 10.7 Hz, 1H), 4.85 (d, J = 8.5 Hz, 1H), 4.81 (d, J = 7.3 Hz, 1H), 4.76–4.44 (m, 8H), 4.43–4.23 (m, 4H), 4.16 (d, J = 11.8 Hz, 1H), 3.98–3.84 (m, 1H), 3.77–3.62 (m, 3H), 3.53–3.22 (m, 8H), 2.16 (s, 1H). NMR data are in accordance with literature values.[111] ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 139.1, 138.7, 138.6, 138.5, 138.3, 138.0, 137.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.6, 127.2, 102.7, 102.5, 82.9, 81.8, 80.6, 76.7, 75.9, 75.4, 75.2, 75.1, 75.1, 75.0, 74.1, 73.4, 73.2, 73.2, 71.0, 68.3, 68.0.

Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-*D*-fucopyranoside[157,158] (59)



Phenyl-1-thio-α/β-*L*-fucopyranoside (2.5 g, 0.0098 mol) was dissolved in DMF (40 mL) and NaH (2.33 g, 60% in an oil suspension, 0.059 mol) was added. After stirring for 10 min BnBr (7.0 mL, 0.059 mol) was added followed by TBAI (0.18 g, 0.49 mmol), and the mixture was stirred for 2 h. The reaction was quenched by addition of water (50 mL), extracted with a EtOAc:Heptane solution (1:1, 100 mL), the organic phase

was washed with water (3×50 mL), a saturated aqueous solution of NH_4Cl (50 mL), dried over $MgSO_4$ and evaporated under vacuum to a yellow oil, which crystallized overnight. Recrystallization from Et_2O:Hexane gave the benzylether as white crystals (3.1 g, 61%) M.p. 105.5-106 °C. M.p. lit. 107-109 °C.[157] ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.58–7.47 (m, 2H), 7.45–7.02 (m, 18H), 4.94 (d, J = 11.7 Hz, 1H), 4.72 (d, J = 10.2 Hz, 1H), 4.69–4.63 (m, 3H), 4.60 (d, J = 11.7 Hz, 1H), 4.53 (d, J = 9.6 Hz, 1H), 3.86 (dd, J = 9.6, 9.1 Hz, 1H), 3.56 (dd, J = 2.8, 0.8 Hz, 1H), 3.52 (dd, J = 9.1, 2.8 Hz, 1H), 3.49–3.40 (qd, J = 6.4, 0.8 Hz 1H), 1.19 (d, J = 6.4 Hz, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 138.8, 138.5, 138.5, 134.5, 131.6, 128.9, 128.6, 128.5, 128.5, 128.3, 128.1, 127.8, 127.8, 127.7, 127.6, 127.1, 109.9, 87.7, 84.6, 77.2, 75.7, 74.7, 74.7, 73.0, 17.5. NMR data are in accordance with literature values.[117]

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside[159] (44)

1) NaOMe, MeOH then Et_3N, phthalic anhydride
2) Ac_2O, pyridine
54%

D-Glucosamine hydrochloride (10.7 g, 0.0496 mol) was purred into a solution of NaOMe (1.1 g Na, 100 mL MeOH) at 0 °C, and the solution stirred for 10 min. The solution was filtered into phthalic anhydride (3.75 g, 0.025 mol) which had been heated to assure there was no phthalic acid present, and the filter cake was washed with MeOH (50 mL). The solution was stirred for 10 min followed by addition of Et_3N (7 mL). After an additional 5 min of stirring phthalic anhydride (3.7 g, 0.025 mol) was added and the solution was stirred for additional 3 hours. The solvent was evaporated under reduced pressure and the residue was treated with pyridine (50 mL) and acetic anhydride (60 mL) with stirring for 16 hours at rt. The reaction mixture was poured into ice water (250 mL) and stirred for 20 min, followed by extraction with CH_2Cl_2 (2×150 mL). The organic phases were washed with 2 M aqueous H_2SO_4 (2×100 mL), H_2O (2×75 mL), saturated aqueous solution of $NaHCO_3$ (75 mL) and H_2O (75 mL), dried over $MgSO_4$ and concentrated under reduced pressure. Purified by flash column chromatography (SiO_2, EtOAc/heptane 1:1) resulting in a white crystalline product of only the β-anomer (12.9 g, 54%). M.p. 107–109 °C. M.p. lit. 100–103 °C.[160] ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.86 (dd, J = 5.5, 3.0 Hz, 2H), 7.75 (dd, J = 5.5, 3.0 Hz, 2H), 6.51 (d, J = 8.9 Hz, 1H), 5.88 (dd, J = 10.6, 9.1 Hz, 1H), 5.21 (dd, J = 10.2, 9.2 Hz, 1H), 4.47 (dd, J = 10.6, 8.9 Hz, 1H), 4.37 (dd, J = 12.5, 4.4 Hz, 1H), 4.14 (dd, J = 12.2, 2.3 Hz, 1H), 4.02 (ddd, J = 10.3, 4.4, 2.1 Hz, 1H), 2.11 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.86 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.74, 170.11, 169.56, 168.72, 167.45, 134.60, 131.29, 123.89, 89.83, 72.70, 70.57, 68.34, 61.60, 53.56, 20.88, 20.85, 20.72, 20.50. NMR data are in accordance with literature values.[159]

Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside[161] (45)

PhSH, $BF_3 \cdot OEt_2$
CH_2Cl_2
78%

$BF_3 \cdot OEt_2$ (1.7 mL, 13.4 mmol) was added slowly to a solution of **44** (3.5 g, 7.3 mmol)

and thiophenol (1.0 mL, 9.8 mmol) in CH_2Cl_2 (35 mL) and the reaction mixture stirred for 20 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous NaOMe (3×50 mL), dried over $MgSO_4$ and evaporated *in vacuo* resulting in a yellow oil which crystallized overnight. The resulting white crystals were washed thoroughly with MeOH and was dried (3.0 g, 78 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.99–7.66 (m, 4H), 7.45–7.23 (m, 5H), 5.79 (dd, J = 10.2, 9.2 Hz, 1H), 5.71 (d, J = 10.6 Hz, 1H), 5.14 (dd, J = 10.2, 9.2 Hz, 1H), 4.48–4.08 (m, 3H), 3.90 (ddd, J = 10.2, 5.0, 2.4 Hz, 1H), 2.10 (s, 3H), 2.02 (s, 3H), 1.83 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.8, 170.2, 169.6, 168.0, 167.1, 134.6, 134.5, 133.4, 131.7, 131.3, 131.1, 129.0, 128.6, 123.9, 83.2, 76.0, 71.7, 68.8, 62.3, 53.7, 20.9, 20.8, 20.6. NMR data are in accordance with literature values.[159]

Phenyl 2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside[161] (46)

OAc AcO AcO O SPh NPhth 45 — NaOMe, MeOH, 99% → OH HO HO O SPh NPhth 46

Compound **45** (3.1 g, 5.9 mmol) was taken op in a solution of NaOMe (0.1 g Na in 50 mL MeOH) and the mixture stirred at rt for 20 min whereupon Amberlite IR-120 $H^+$was added and the mixture stirred for 1 h followed by filtration and evaporation under reduced presure. Purified by flash column chromatography (SiO_2, EtOAc) resulting in a white crystalline product (2.34 g, 99%). ^{1}H-NMR (300 MHz, CD_3OD): δ = 7.97–7.77 (m, 4H), 7.44–7.32 (m, 2H), 7.30–7.14 (m, 3H), 5.59 (d, J = 10.3 Hz, 1H), 4.26 (dd, J = 10.3, 8.2 Hz, 1H), 4.10 (t, J = 10.3 Hz, 1H), 3.94 (dd, J = 12.1, 1.9 Hz, 1H), 3.76 (dd, J = 12.1, 5.3 Hz, 1H), 3.53 - 3.39 (m, 2H). NMR data are in accordance with literature values.[161] ^{13}C-NMR (75 MHz, CD_3OD): δ = 169.7, 169.2, 135.6, 135.6, 134.4, 133.1, 132.9, 132.8, 130.0, 128.7, 124.5, 124.2, 85.4, 82.6, 73.8, 72.2, 62.8, 57.7.

Phenyl
4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside (30)

OH HO HO O SPh NPhth 46 — $PhCH(OCH_3)_2$, *p*-TsOH, MeCN, 88% → Ph O O HO O SPh NPhth 30

A mixture of **46** (9.0 g, 23 mmol), benzaldehyde dimethyl acetal (5.8 mL, 39 mmol) and p-toluenesulfonic acid monohydrate (0.25 g, 1.3 mmol) in acetonitril (150 mL) was stirred for 3 h. The reaction was quenched by addition of Et_3N until neutral medium and the mixture was concentrated to a yellow oil. Purified by flash column chromatography (SiO_2, EtOAc/heptane 2:3) resulting in a viscous oil (9.7 g 88 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.94–7.79 (m, 2H), 7.79–7.66 (m, 2H), 7.52–7.15 (m, 10H), 5.68 (d, J = 10.4 Hz, 1H), 5.56 (s, 1H), 4.69–4.56 (m, 1H), 4.38 (dd, J = 10.7, 5.0 Hz, 1H), 4.32 (t, J = 10.4 Hz, 1H), 3.81 (t, J = 10.0 Hz, 1H), 3.67 (td, J = 9.5, 4.6 Hz, 1H), 3.58 (t, J = 9.0 Hz, 1H), 2.69 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 168.4, 137.0, 134.4, 132.8, 131.9, 131.7, 129.9, 129.5, 129.1, 128.5, 128.3, 126.4, 124.0, 123.5, 102.1, 84.4, 82.0, 70.4, 69.8, 68.7, 55.6. NMR data are in accordance with literature values.[117]

Phenyl 3-*O*-allyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-*β*-*D*-glucopyranoside[162] (72) ♣

A Schlenk flask charged with **30** (1.50 g, 3.1 mmol), $Pd_2(dba)_3$ (71 mg, 0.078 mmol) and dppb (132 mg, 0.031 mmol) was degassed and the solids were dissolved in THF (5 mL). Efter stirring for 5 min allylethylcarbonate (404 mg, 3.1 mmol) in THF (2.5 mL) was added and the mixture was heated to 65 °C and stirred for 30 min whereupon additional allylethylcarbonate (404 mg, 3.1 mmol) in THF (2.5 mL) was added and the mixture stirred for 1 h. Cooled to rt, concentrated and purified by flash column chromatography (SiO_2, acetone/toluene 1:99) resulting in a viscous oil (1.49 mg, 92 %). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.88 (m, 2H), 7.81–7.72 (m, 2H), 7.52–7.44 (m, 2H), 7.43–7.33 (m, 5H), 7.31–7.21 (m, 3H), 5.68 (d, J = 10.4 Hz, 1H), 5.59 (s, 1H), 5.52 (m, 1H), 5.01 (dq, J = 17.2, 1.6 Hz, 1H), 4.84 (dt,J = 10.4, 1.6, 1.2 Hz, 1H), , 4.47–4.37 (m, 2H), 4.33 (t, J = 10.2 Hz, 1H), 4.26 (ddt, J = 13.1, 5.1, 1.4 Hz, 1H), 3.95 (ddt, J = 13.1, 6.3, 1.2 Hz, 1H), 3.88–3.80 (m, 1H), 3.78–3.66 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.5, 167.4, 137.4, 134.4, 134.3, 132.8, 131.9, 129.2, 129.1, 128.4, 128.2, 126.2, 124.0, 123.5, 117.3, 101.4, 84.4, 82.6, 76.3, 73.4, 70.6, 68.8, 55.0.

Phenyl 3-*O*-allyl-2-deoxy-2-phthalimido-1-thio-*β*-*D*-glucopyranoside (73) ♣

Compound **72** (1.5 g, 2.8 mmol) was suspended in MeOH (45 mL), and CSA (100 mg, 0.43 mmol) was added. After stirring for 16 h the reaction was quenched with Et_3N, concentrated and purified by flash column chromatography (SiO_2, toluene/EtOAc 1:1) resulting in a colorless foam (1.24 g, 99%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.98–7.82 (m, 2H), 7.82–7.70 (m, 2H), 7.43–7.32 (m, 2H), 7.31–7.17 (m, 3H), 5.73–5.51 (m, 2H), 5.04 (dq, J = 17.2, 1.5 Hz, 1H), 4.93–4.83 (m, 1H), 4.36–4.13 (m, 3H), 4.07–3.93 (m, 2H), 3.88 (dd, J = 12.0, 4.4 Hz, 1H), 3.78 (dd, J = 9.6, 8.4 Hz, 1H), 3.59 (ddd, J = 9.7, 4.4, 3.5 Hz, 1H), 2.79 (br, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 168.6, 167.5, 134.4, 132.3, 132.2, 131.6, 129.1, 128.0, 123.9, 123.5, 117.6, 83.8, 80.3, 79.6, 73.5, 71.4, 62.4, 54.8.

Phenyl 3-*O*-allyl-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-*β*-*D*-glucopyranoside (74) ♣

Compound **73** (1.9 g, 4.3 mmol) was dissolved in DMF, and the mixture was stirred with 4Å MS for 10 min. The mixture was cooled to 0 °C whereupon Ag_2O (4.25 g, 1.8 mmol), BnBr (1.6 mL, 13 mmol) and KI (1.7 g, 10 mmol) were added. After stirring for 10 min the icebath was removed and the mixture stirred at rt for 4 h. Diluted with CH_2Cl_2 (250 mL) and filtered through a pad of Celite, washed with 10%

aqueous solution of $Na_2S_2O_3$ (2×80 mL), H_2O (2×150 mL), dried over $MgSO_4$ and evaporated under reduced pressure. Purified using flash column chromatography (SiO_2, acetone/toluene 1:49) resulting in a colorless foam (2.2 g, 82%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.87–7.62 (m, 4H), 7.40–7.04 (m, 15H), 5.55–5.36 (m, 2H), 4.89 (dq, J = 17.2, 1.5 Hz, 1H), 4.77–4.65 (m, 2H), 4.59–4.43 (m, 3H), 4.26–4.17 (m, 2H), 4.12 (ddt, J = 12.7, 5.3, 1.3 Hz, 1H), 3.81 (ddt, J = 12.7, 6.4, 1.2 Hz, 1H), 3.76–3.56 (m, 4H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 168.5, 167.5, 138.4, 138.1, 134.3, 132.6, 132.4, 131.8, 128.9, 128.5, 128.5, 128.0, 127.9, 127.9, 127.8, 127.7, 123.9, 123.4, 117.5, 83.5, 80.5, 79.5, 79.0, 75.0, 74.0, 73.5, 69.0, 55.2.

Phenyl 4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-*β*-*D*-glucopyranoside[155] (61) ♣

OBn BnO O SPh NPhth 74 — $ClRh(PPh_3)_3$, n-BuLi, THF → 75 — H^+, MeOH, 64% → BnO HO SPh NPhth 61

Wilkinson's catalyst [$ClRh(PPh_3)_3$] (357 mg, 0.39 mmol) was dissolved in anhydrous THF (10 mL) and the solution was degassed. n-BuLi (0.29 mL, 1.6 M in hexane, 0.46 mmol) was added and the mixture was degassed for an additional 10 min. A degassed solution of **74** (1.2 g, 1.9 mmol) in anhydrous THF (15 mL) was heated to reflux and the solution of the catalyst was added. The reaction mixture was refluxed for 3 h, whereupon TLC revealed full conversion into the vinyl ether. It was cooled to 50 °C followed by addition of MeOH (60 mL) and Amberlite IR-120 H^+ (6 mL). The resulting mixture was stirred for 48 h where TLC showed full conversion to the alcohol. The resin was filtered off, washed with CH_2Cl_2 and the filtrate was concentrated and purified by flash column chromatography (SiO_2, acetone/toluene 1:19) resulting in a white foam (0.87 g, 77%) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.97–7.64 (m, 4H), 7.55–7.06 (m, 15H), 5.59 (d, J = 10.4 Hz, 1H), 4.76–4.63 (m, 3H), 4.58 (d, J = 12.0 Hz, 1H), 4.53–4.34 (m, 1H), 4.24 (t, J = 10.4 Hz, 1H), 3.96–3.76 (m, 2H), 3.77–3.53 (m, 2H), 2.17 (br, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 168.4, 167.8, 138.3, 138.2, 134.3, 132.6, 132.3, 131.7, 128.9, 128.7, 128.5, 128.1, 128.0, 127.9, 127.9, 127.7, 123.8, 123.5, 83.5, 79.3, 79.2, 74.9, 73.6, 73.0, 69.0, 55.7.

Phenyl 6-*O*-benzyl-4-fluorenylmethyloxycarbonyl-2-deoxy-2-phthalimido-1-thio-*β*-*D*-glucopyranoside (68) ♣

30 — FmocCl, DMAP, pyridine, 78% → 67 — Et_3SiH, TFA, TFAA, CH_2Cl_2, 80% → 68

Fluorenylmethyloxycarbonyl chloride (0.62 g, 2.4 mmol) was added to a solution of **30** (0.98 g, 2.0 mmol) and DMAP (cat.) in pyridine (18 mL) at 0 °C. The mixture was quenched with MeOH after stirring for 3 h, concentrated *in vacuo* and purified by flash column chromatography (SiO_2, EtOAc/heptane 1:3) resulting in a white foam (1.11 g, 78%). The residue was dissolved in CH_2Cl_2 (5 mL) whereupon trifluoroacetic anhydride (0.68 mL, 4.8 mmol) and triethylsilane (1.26 mL, 7.9 mmol) were added at 0 °C. After stirring for 5 min trifluoroacetic acid (0.58, 7.6 mmol) was added dropwise over a 2 min period. The mixture was diluted with EtOAc (100 mL) after it had stirred

for 4 h, washed with a saturated aqueous solution of $NaHCO_3$ (100 mL), H_2O (100 mL) and dried over $MgSO_4$. The residue was evaporated under reduced pressure and purified by flash column chromatography (SiO_2, EtOAc/heptane 1:2) resulting in a white foam (0.88 g, 80%) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.94–7.54 (m, 6H), 7.54–7.13 (m, 16H), 5.78 (d, J = 10.5 Hz, 1H), 5.63 (dd, J = 10.3, 8.9 Hz, 1H), 4.72–4.54 (m, 2H), 4.46 (t, J = 10.4 Hz, 1H), 4.23–4.05 (m, 2H), 4.03–3.75 (m, 5H), 3.15 (br, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 168.1, 167.2, 155.3, 143.2, 143.1, 141.2, 141.1, 137.8, 134.4, 134.2, 132.9, 131.7, 131.3, 129.0, 128.6, 128.2, 128.0, 127.9, 127.9, 127.3, 127.3, 125.3, 125.2, 123.8, 120.0, 83.4, 78.4, 78.1, 73.9, 71.0, 70.5, 70.2, 53.6, 46.5.

Phenyl 6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside (100)

Ph, O, O, HO, O, SPh, NPhth, **30** — Et_3SiH, TFA, TFAA / CH_2Cl_2 / 69% → OBn, HO, HO, O, SPh, NPhth, **100**

Trifluoroacetic anhydride (0.79 g, 5.6 mmol) and triethylsilane (1.47 mL, 9.2 mmol) were added to **30** (0.89 g, 1.8 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After stirring for 5 min trifluoroacetic acid (0.68 mL, 8.9 mmol) was added dropwise over a 2 min period. The mixture was diluted with EtOAc (100 mL) after it had stirred for 4 h, washed with a saturated aqueous solution of $NaHCO_3$ (100 mL), H_2O (100 mL) and dried over $MgSO_4$. The residue was evaporated under reduced pressure and purified by flash column chromatography (SiO_2, EtOAc/heptane 4:5) resulting in a white foam (620 mg, 69%) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.94–7.63 (m, 4H), 7.48–7.11 (m, 10H), 5.59 (d,J = 10.3 Hz, 1H), 4.70–4.50 (m, 2H), 4.34 (dd, J = 10.4, 8.1 Hz, 1H), 4.20 (t, J = 10.3 Hz, 1H), 3.90–3.73 (m, 2H), 3.73–3.52 (m, 2H), 2.96 (br, 2H). NMR data are in accordance with literature values.[167] ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 137.8, 134.3, 132.5, 132.3, 131.7, 129.0, 128.6, 128.0, 128.0, 127.9, 83.7, 78.0, 73.8, 73.5, 72.8, 70.5, 55.4.

Phenyl 4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside[168] (66) ♣

Ph, O, O, HO, O, SPh, NPhth, **30** — BH_3·THF, $Cu(OTf)_2$ / THF / 63 % → OH, BnO, HO, O, SPh, NPhth, **66**

Compound **30** (2.00 g, 4.09 mmol) was treated with BH_3·THF as a 1 M solution in THF (16 mL). After stirring at rt for 10 min $Cu(OTf)_2$ (73 mg, 0.20 mmol) was added at 0 °C. After stirring for 5 h the mixture was cooled down to 0 °C, and the reaction was quenched by sequential addition of triethylamine and methanol. The resulting mixture was concentrated followed by co-evaporation with methanol. The residue was purified by flash column chromatography (SiO_2, EtOAc/heptane 2:3) resulting in a white foam (1.27 mg, 63%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.85 (br, 2H), 7.76–7.65 (m, 2H), 7.44–7.18 (m, 10H), 5.63 (d, J = 10.5 Hz, 1H), 4.84–4.68 (m, 2H), 4.49 (dd, J = 10.3, 8.3 Hz, 1H), 4.21 (t, J = 10.4 Hz, 1H), 3.95 (d, J = 12.1 Hz, 1H), 3.78 (dd, J = 12.2, 2.3 Hz, 1H), 3.65–3.46 (m, 2H), 1.98 (br, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 138.03,

134.38, 132.51, 132.12, 131.75, 129.13, 128.83, 128.28, 128.15, 128.09, 123.91, 123.50, 83.59, 79.53, 78.94, 75.05, 72.78, 62.12, 55.83.

Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-*D*-galactopyranoside[173] (32)

Compound **47** (40 g, 0.10 mol) was dissolved in anhydrous CH_2Cl_2 (500 mL) followed by addition of thiophenol (13.7 mL, 134 mol) and the mixture was stirred for 10 min. Then $BF_3 \cdot OEt_2$ (30 mL, 24 mol) was slowly added to the solution. After stirring for 24 hours the reaction mixture was pourred into CH_2Cl_2 (300 mL), washed with saturated aqueous $NaHCO_3$ (2×150 mL), H_2O (2×150 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc/heptane 1:5) resulting in a white solid (30 g, 67%) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.59–7.40 (m, 2H), 7.38–7.27 (m, 3H), 5.41 (d, J = 3.3 Hz, 1H), 5.23 (t, J = 9.9 Hz, 1H), 5.04 (dd, J = 9.9, 3.3 Hz, 1H), 4.71 (d, J = 10.0 Hz, 1H), 4.23–4.05 (m, 2H), 3.98–3.89 (m, 1H), 2.11 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.5, 170.3, 170.1, 169.5, 132.6, 132.5, 129.2, 129.0, 128.2, 86.7, 74.5, 72.1, 67.3, 67.3, 61.7, 20.9, 20.8, 20.7, 20.7. NMR data are in accordance with literature values.[174]

2,3,4,6-Tetra-*O*-acetyl-α-*D*-galactopyranosyl bromide (33)

Compound **47** (0.10 g, 0.23 mmol) was dissolved in CH_2Cl_2 (1 mL) and Br_2 (0.23 mL, 1 M in CH_2Cl_2, 0.23 mmol) was added at 0 °C. The mixture stirred at rt for 30 min. Concentrated and purified by dry column chromatography (SiO_2, EtOAc/heptane, 0 to 72% in steps of 6%) resulting in a clear oil (0.83 mg, 88%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 6.68 (d, J = 4.0 Hz, 1H), 5.50 (dd, J = 3.3, 1.3 Hz, 1H), 5.39 (dd, J = 10.6, 3.3 Hz, 1H), 5.03 (dd, J = 10.6, 4.0 Hz, 1H), 4.47 (t, J = 6.7 Hz, 1H), 4.24–4.03 (m, 2H), 2.14 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.5, 170.2, 170.0, 169.9, 88.2, 71.2, 68.1, 67.9, 67.1, 61.0, 20.9, 20.8, 20.7, 20.7. NMR data are in accordance with literature values.[176]

Phenyl 1-thio-β-*D*-galactopyranoside[173] (i)

Compound **32** (30 g, 0.068 mol) was taken up in a solution of NaOMe (0.626 g, 0.027 mol Na in 200 mL MeOH) and stirred at room temperature for 16 hours whereupon Amberlite IR-120 H^+ was added and the mixture stirred for an additional 45 minutes followed by filtration. Evaporation under reduced pressure and recrystallization from EtOAc resulted in a white crystalline product (18 g, 99%). M.p. 105–109 °C. M.p.

lit. 105–107 °C.[175] ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ = 7.45 (d, J = 7.5 Hz, 2H), 7.38–7.15 (m, 3H), 5.14 (d, J = 5.8 Hz, 1H), 4.89 (d, J = 5.5 Hz, 1H), 4.64 (t, J = 4.9 Hz, 1H), 4.59 (d, J = 9.3 Hz, 1H), 4.49 (d, J = 4.4 Hz, 1H), 3.75–3.68 (m, 1H), 3.59–3.32 (m, 5H). ^{13}C-NMR (75 MHz, DMSO-d$_6$): δ = 135.6, 129.2, 128.9, 126.1, 87.8, 79.2, 74.3, 69.3, 68.4, 60.6. No NMR data reported in DMSO-d$_6$.

Phenyl 4,6-*O*-benzylidene-1-thio-*β*-*D*-galactopyranoside (ii)

PhCH(OCH$_3$)$_2$, *p*-TsOH, MeCN, 81 %

Benzaldehyde dimethyl acetal (2.7 mL, 18 mmol) was added to a suspension of **i** (3.0 g, 11 mmol) in acetonitrile (70 mL) followed by *p*-toluenesulfonic acid monohydrate (0.10 g, 0.53 mmol). After stirring for 6 h the reaction was quenched with Et$_3$N, evaporated under reduced pressure and recrystalized from EtOAc resulting in a white crystalline product (3.2 g, 81%). ^{1}H-NMR (300 MHz, CD$_3$OD): δ = 7.72–7.61 (m, 2H), 7.54–7.41 (m, 2H), 7.40–7.31 (m, 3H), 7.31–7.17 (m, 3H), 5.59 (s, 1H), 4.59 (d, J = 9.3 Hz, 1H), 4.28–4.16 (m, 2H), 4.10 (dd, J = 12.4, 1.7 Hz, 1H), 3.72–3.58 (m, 3H). NMR data are in accordance with literature values.[177] ^{13}C-NMR (75 MHz, CD$_3$OD): δ = 139.8, 133.8, 129.9, 129.8, 129.0, 128.4, 127.7, 102.4, 88.8, 77.6, 75.1, 71.3, 70.4, 69.6.

Phenyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-*β*-*D*-galactopyranoside (35)

Ac$_2$O, pyridine, 68 %

Acetic anhydride (20 mL, 0.21 mol) was slowly added to a suspension of **ii** (1.4 g, 3.8 mmol) in pyridine (20 mL) at 0 °C, and the mixture stirred at rt for 16 h. The reaction was quenched with MeOH at 0 °C, and diluted with EtOAc (60 mL). The organic mixture was washed with 1M HCl (50 mL), saturated aqueous NaHCO$_3$ (50 mL), brine (50 mL), dried over MgSO$_4$ and evaporated to dryness. The residue was purified by flash column chromatography (SiO$_2$, EtOAc/heptane 2:3) resulting in a white foam (1.2 g, 68%). ^{1}H-NMR (300 MHz, CDCl$_3$): δ = 7.69–7.55 (m, 2H), 7.45–7.19 (m, 8H), 5.47 (s, 1H), 5.35 (t, J = 9.9 Hz, 1H), 5.00 (dd, J = 9.9, 3.5 Hz, 1H), 4.71 (d, J = 9.9 Hz, 1H), 4.38 (m, 2H), 4.03 (dd, J = 12.5, 1.7 Hz, 1H), 3.60 (m, 1H), 2.09 (s, 3H), 2.03 (s, 3H). NMR data are in accordance with literature values.[177] ^{13}C-NMR (75 MHz, CDCl$_3$): δ = 170.9, 169.2, 137.6, 133.7, 131.4, 129.3, 128.9, 128.3, 128.2, 126.7, 101.3, 85.3, 73.6, 73.3, 69.9, 69.2, 66.9, 21.0.

2,3,4,6-Tetra-*O*-acetyl-*D*-galactopyranose[183] (iii)

Benzylamine (0.84 mL, 7.7 mmol) was added to a solution of **47** (2.0 g, 5.1 mmol) in THF (12 mL), and the reaction stirred for 16 h. The mixture was diluted with H_2O (40 mL) and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers was washed with 2 M HCl (2×50 mL), saturated aqueous $NaHCO_3$ (100 mL), and brine (75 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc/heptane 2:3) resulting in a clear oil (1.5 g, 85%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.50 (t, J = 3.6 Hz, 1H), 5.46 (dd, J = 3.4, 1.2 Hz, 1H), 5.44–5.35 (m, 1H), 5.14 (dd, J = 10.7, 2.8 Hz, 1H), 5.08–5.04 (m, 1H), 4.74–4.64 (m, 0.25H), 4.46 (dd, J = 7.6, 6.6 Hz, 1H), 4.15–4.05 (m, 3H), 3.98–3.91 (m, 0.25H), 3.66 (dd, J = 3.6, 1.1 Hz, 1H), 2.14 (s, 0.75H), 2.13 (s, 3H), 2.09 (s, 0.75H), 2.08 (s, 3H), 2.04 (s, 3.75 H), 1.98 (s, 3.75H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.7, 170.6, 170.4, 170.2, 96.1 (beta), 90.7 (alpha), 71.1 (beta), 70.5 (beta), 68.4 (alpha), 68.3 (alpha), 67.4 (alpha), 67.2 (beta), 66.3 (alpha), 61.9 (alpha), 61.6 (beta), 29.1, 21.0, 20.8, 20.8, 20.8, 20.7. NMR data are in accordance with literature values.[184]

2,3,4,6-Tetra-*O*-acetyl-*D*-galactopyranosyl *N*-phenyl trifluoroacetimidate (34)

Compound **iii** (0.50 g, 1.4 mmol) was dissolved in CH_2Cl_2 (15 mL) whereupon *N*-phenyl trifluoroacetimidoyl chloride (0.60 mL, 2.9 mmol) and Cs_2CO_3 (0.94 g, 2.9 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C followed by 1 h at rt. The suspension was filtered through Celite, concentrated and purified by flash column chromatography (SiO_2, EtOAc/heptane 2:3) resulting in an amorphous solid (0.65 g, 90%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.32 (t,J = 7.9 Hz, 2H), 7.14 (t,J = 7.4 Hz, 1H), 6.83 (d, J = 7.6 Hz, 2H), 5.74 (s, 1H), 5.53–5.35 (m, 2H), 5.07 (d, J = 8.5 Hz, 1H), 4.16 (d, J = 6.5 Hz, 2H), 3.96 (s, 1H), 2.19 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.1, 169.2, 143.1, 129.0, 124.8, 119.3, 95.1, 71.9, 70.7, 67.9, 66.8, 61.1, 20.8, 20.8, 20.7. NMR data are in accordance with literature values.[185]

2,3,4,6-Tetra-*O*-benzoyl-α-*D*-galactopyranosyl bromide[178] (36)

Compound **48** (6.0 g, 8.6 mmol) was dissolved in CH_2Cl_2 (20 mL) and a 30% solution of HBr in AcOH (6.6 mL) was added, and the mixture was stirred in the darkness at rt for 16 h. The reaction mixture was diluted with CH_2Cl_2 (35 mL) and poured over ice. The organic layer was washed with H_2O (2×50 mL), saturated aqueous $NaHCO_3$

(2×50 mL), brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was crystallized from Et_2O resulting in a white crystalline product (4.8 g, 84%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.08–7.87 (m, 6H), 7.78–7.68 (m, 2H), 7.64–7.26 (m, 10H), 7.26–7.12 (m, 2H), 6.91 (d, J = 4.0 Hz, 1H), 6.05 (dd, J = 3.4, 1.2 Hz, 1H), 5.99 (dd, J = 10.4, 3.4 Hz, 1H), 5.60 (dd, J = 10.4, 4.0 Hz, 1H), 4.91–4.80 (m, 1H), 4.57 (dd, J = 11.5, 6.8 Hz, 1H), 4.39 (dd, J = 11.5, 6.0 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 166.0, 165.7, 165.5, 165.4, 133.9, 133.5, 133.5, 130.1, 130.1, 129.9, 129.9, 129.3, 128.9, 128.9, 128.7, 128.6, 128.6, 128.5, 88.4, 71.9, 69.0, 68.7, 68.2, 61.9. NMR data are in accordance with literature values.[180]

2,3,4,6-Tetra-*O*-benzoyl-*D*-galactopyranose[178] (iv)

BzO OBz / BzO / BzO Br **36** — Ag_2CO_3, acetone/H_2O, 95% → BzO OBz / BzO / BzO OH **iv**

Compound **36** (1.4 g, 2.2 mmol) was dissolved in acetone (8 mL) and H_2O (0.3 mL) followed by addition of Ag_2CO_3 (0.31 g, 1.1 mmol). The reation mixture was stirred for 3 h, filtered through Celite and concentrated *in vacuo*, giving a white foam (1.2 g, 95%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.20–7.91 (m, 6H), 7.87–7.74 (m, 2H), 7.71–7.16 (m, 12H), 6.01 (dd, J = 3.4, 1.0 Hz, 1H), 5.74 (dd, J = 10.4, 3.4 Hz, 1H), 5.62 (dd, J = 10.4, 7.9 Hz, 1H), 5.05 (dd, J = 8.5, 7.9 Hz, 1H), 4.68 (dd, J = 11.0, 6.3 Hz, 1H), 4.52–4.32 (m, 2H), 4.01 (d, J = 8.6 Hz, 1H). NMR data are in accordance with literature values.[181] ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 167.3, 166.2, 165.6, 165.6, 133.8, 133.5, 133.5, 130.1, 130.1, 130.0, 129.9, 129.4, 129.1, 128.9, 128.8, 128.6, 128.6, 128.5, 96.5, 72.6, 71.8, 71.0, 68.3, 62.2. Anomer C-atom in accordance with literature values.[182]

2,3,4,6-Tetra-*O*-benzoyl-*D*-galactopyranosyl *N*-phenyl trifluoroacetimidate (37)

BzO OBz / BzO / BzO OH **iv** — Ph-N=C(Cl)CF$_3$, Cs_2CO_3, CH_2Cl_2, 90 % → BzO OBz / BzO / BzO O-C(=N-Ph)CF$_3$ **37**

Compound **iv** (0.59 g, 1.0 mmol) was dissolved in CH_2Cl_2 (10 mL) whereupon *N*-phenyl trifluoroacetimidoyl chloride (0.42 mL, 2.0 mmol) and Cs_2CO_3 (0.65 g, 2.0 mmol) were added at 0 °C. The reaction mixture stirred for 1 h at 0 °C followed by 1 h at rt. The suspension was filtered through Celite, concentrated and purified by flash column chromatography (SiO_2, EtOAc/heptane 1:3) resulting in a white foam (0.59 g, 78%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.19–7.93 (m, 6H), 7.88–7.76 (m, 2H), 7.74–7.18 (m, 11H), 7.11 (t, J = 7.4 Hz, 1H), 7.04 (d, J = 7.5 Hz, 0.1H), 6.72 (d, J = 7.4 Hz, 2H), 6.45 (s, 0.1H), 6.22–5.96 (m, 3H), 5.70 (d, J = 8.5 Hz, 1H), 4.89–4.78 (m, 0.1H), 4.66 (dd, J = 11.4, 7.1 Hz, 1H), 4.49 (dd, J = 12.0, 4.8 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 166.1, 165.6, 165.1, 143.1, 133.9, 133.7, 133.6, 133.4, 130.2, 129.9, 129.9, 129.4, 129.0, 128.9, 128.9, 128.8, 128.6, 128.6, 128.5, 124.7, 119.3, 95.3, 72.8, 71.6, 68.9, 68.0, 62.2.

Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-β-*D*-galactopyranoside (80)

Compound **60** (1.21 g, 2.02 mol) was taken up in a solution of NaOMe (0.10 g, 4.4 mmol Na in 25 mL MeOH) and stirred at 50 °C for 48 h whereupon Amberlite IR-120 H^+ was added and the mixture stirred for an additional 60 min followed by filtration and concentration. The residue was azeotropically distilled with toluene whereupon pyridine (24 mL) was added and the mixture was cooled to 0 °C. Acetic anhydride (24 mL) was added during 15 min and the mixture stirred for 3 h at rt. The reaction was quenched with MeOH, diluted with EtOAc (100 mL) and the reaction mixture was washed with 2M HCl (2×100 mL), H_2O(100 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (SiO_2, acetone/toluene 1:39) resulting in a buff solid (1.05 g, 97%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.46–7.20 (m, 15H), 5.42 (t, J = 9.8 Hz, 1H), 4.94 (d, J = 11.7 Hz, 1H), 4.68 (d, J = 12.2 Hz, 1H), 4.58 (d,J = 11.7 Hz, 1H), 4.53 (d, J = 12.2 Hz, 1H), 4.50–4.37 (m, 2H), 4.33 (d, J = 9.9 Hz, 1H), 3.99 (d,J = 2.7 Hz, 1H), 3.60 (br, 3H), 3.54 (dd, J = 9.6, 2.7 Hz, 1H), 2.81–2.58 (m, 2H), 2.04 (s, 3H), 1.23 (t, J = 7.5 Hz, 3H). NMR data are in accordance with literature values, but they are 3H short.[186] ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 169.8, 138.7, 138.1, 137.9, 129.1, 128.6, 128.5, 128.3, 128.1, 128.1, 128.0, 127.9, 127.6, 127.6, 83.8, 81.5, 77.6, 74.5, 73.7, 72.9, 72.0, 69.7, 68.6, 23.7, 21.2, 14.9.

Methyl 5-acetamido-3,5-dideoxy-β-*D*-*glycero*-*D*-*galacto*-2-nonulopyranosonate[187] (112)

A suspension of *N*-acetylneuraminic acid (10 g, 0.032 mol) in anhydrous MeOH (500 mL) containing Amberlite IR-120 H^+ resin (30 g) was stirred overnight at rt. The clear solution was filtered and the resin washed with MeOH. The filtrate was evaporated under reduced presure resulting in a white solid (9.0 g, 86%). M.p. 178–180 °C (decomp.). M.p. lit. 180–182 °C (decomp.).[188] $[\alpha]^{25}_{D}$ –28.4 (c 0.01, H_2O). Lit.$[\alpha]^{25}_{D}$ –28 (c 1, H_2O).[188] ^{1}H-NMR (300 MHz, CD_3OD): δ = 4.12–3.93 (m, 2H), 3.88–3.75 (m, 5H), 3.74–3.66 (m, 1H), 3.62 (dd, J = 11.0, 5.6 Hz, 1H), 3.48 (dd, J = 9.1, 1.2 Hz, 1H), 2.22 (dd, J = 12.9, 4.9 Hz, 1H), 2.02 (s, 3H), 1.89 (dd, J = 12.9, 11.4 Hz, 1H). ^{13}C-NMR (75 MHz, CD_3OD): δ = 175.1, 171.8, 96.6, 72.0, 71.6, 70.1, 67.8, 64.8, 54.3, 53.2, 40.7, 22.7. NMR data are in accordance with literature values.[187]

Methyl 5-acetamido-2-chloro-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-*D*-*glycero*-*D*-*galacto*-2-nonulopyranosonate[189] (95)

Freshly distilled AcCl (50 mL) was slowly added to an ice-cooled suspension of **112** (2.1 g, 6.5 mmol) in CH_2Cl_2 (20 mL) in a flask with screw cap capable of enduring high pressure. The ice-bath was removed and the reaction stirred overnight at rt. Anhydrous MeOH (9 mL) was added dropwise to AcCl (10 mL) with cooling in an ice water bath. The **112** AcCl solution was cooled on a icebath, and the cap was carefully taken off, whereupon the MeOH/AcCl mixture was added with moderate haste and the cap was quickly closed. The reaction mixture stirred overnight at rt, where NMR showed full conversion into the desired product, which was obtained by evaporation to dryness and co-evaporating with toluene (3×50 mL), resulting in a clear viscous oil (3.3 g, 100%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.58 (d, J = 10.1 Hz, 1H), 5.47 (dd, J = 6.9, 2.4 Hz, 1H), 5.39 (ddd, J = 11.2, 10.4, 4.8 Hz, 1H), 5.17 (ddd, J = 6.9, 5.9, 2.7 Hz, 1H), 4.43 (dd, J = 12.5, 2.7 Hz, 1H), 4.35 (dd, J = 10.7, 2.4 Hz, 1H), 4.21 (dd, J = 10.7, 10.4 Hz, 1H), 4.06 (dd, J = 12.5, 5.9 Hz, 1H), 3.89–3.84 (m, 3H), 2.78 (dd, J = 13.9, 4.8 Hz, 1H), 2.27 (dd, J = 13.9, 11.2 Hz, 1H), 2.12 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H), 1.90 (s, 3H). NMR data are in accordance with literature values.[190] ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 171.2, 170.9, 170.6, 170.2, 169.9, 165.8, 96.8, 74.1, 70.2, 69.0, 67.0, 62.3, 54.1, 48.9, 40.9, 23.4, 21.2, 21.1, 21.0, 21.0.

Methyl (4-nitrophenyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-*D*-*glycero*-*D*-*galacto*-2-nonulopyranosid)onate[145] (110)

Compound **95** (3.3 g, 6.5 mmol) was dissolved in CH_2Cl_2 (150 mL) and *p*-nitrophenol (4.4 g, 32 mmol) was dissolved in 0.1 M aqueous NaOH (150 mL). The two-phase system was refluxed (49 °C) for 0.5 h in the presence of benzyltriethylammonium chloride (3.2 g, 14 mmol) as phase-transfer catalyst. The organic phase was washed with aqueous 0.1 M NaOH saturated with NaCl (3×150 mL), brine (150 mL) and dried over $MgSO_4$. After evaporation *in vacuo* and the product was purified by flash column chromatography (SiO_2, EtOAc) and crystallized from EtOAc/heptane resulting in a white solid (2.14 g, 54%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.17 (d, J = 9.3 Hz, 2H), 7.13 (d, J = 9.3 Hz, 2H), 5.44–5.29 (m, 3H), 4.96 (ddd, J = 12.2, 10.4, 4.6 Hz, 1H), 4.60 (dd, J = 10.8, 1.4 Hz, 1H), 4.27–4.01 (m, 3H), 3.64 (s, 3H), 2.73 (dd, J = 13.1, 4.6 Hz, 1H), 2.36–2.22 (m, 1H), 2.17 (s, 3H), 2.10 (s, 3H), 2.04 (2 s, 6H), 1.91 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 171.0, 170.7, 170.4, 170.2, 170.1, 168.2, 159.1, 143.5, 125.8, 118.7, 99.6, 73.8, 68.5, 68.3, 67.1, 62.2, 53.5, 49.4, 38.8, 23.3, 21.1, 21.0, 20.9. NMR data are in accordance with literature values.[145]

Methyl (4-nitrophenyl-5-acetamido-3,5-dideoxy-α-*D*-*glycero*-*D*-*galacto*-2-nonulopyranosid)onate[146] (115)

AcO OAc CO2Me AcO AcHN AcO O O NO2 110 → NaOMe, MeOH 99% → HO OH CO2Me HO AcHN HO O O NO2 115

To a suspension of **110** (2.0 g, 3.2 mmol) in MeOH (250 mL) was added a NaOMe solution (2.5 mL, 1.6 M) added and the mixture stirred for 1 h whereupon Amberlite IR-120 H^+ was added at 0 °C. After stirring for 30 min the mixture was filtered and concentrated. Purified by flash column chromatography (SiO_2, MeOH/$CH_2Cl_2$1:9) resulting in a white solid (1.4 g, 99%). ^{1}H-NMR (400 MHz, CD_3OD): δ = 8.20 (d, J = 9.3 Hz, 1H), 7.34 (d, J = 9.3 Hz, 1H), 4.18 (dd, J = 10.5, 1.5 Hz, 1H), 3.91 (t, J = 10.5 Hz, 1H), 3.85–3.74 (m, 1H), 3.72 (s, 3H), 3.67–3.59 (m, 1H), 3.53 (dd, J = 9.2, 1.5 Hz, 1H), 2.78 (dd, J = 12.9, 4.6 Hz, 1H), 2.04 (dd, J = 12.9, 12.0 Hz, 1H), 2.02 (s, 3H). NMR data are in accordance with literature values.[146] ^{13}C-NMR (100 MHz, CD_3OD): δ = 175.2, 170.0, 160.9, 144.9, 126.3, 121.2, 101.9, 76.2, 71.9, 70.1, 67.9, 65.1, 53.6, 53.5, 42.2, 22.7.

***p*-Nitrophenyl-5-acetamido-3,5-dideoxy-α-*D*-*glycero*-*D*-*galacto*-2-nonulopyranosidonic acid[146] (109)**

HO OH CO2Me HO AcHN HO O O NO2 115 → 0.1 M NaOH 0.5 h ~95% → HO OH CO2H HO AcHN HO O O NO2 109

Compound **115** (50 mg, 0.11 mmol) was dissolved in a aqueous solution of NaOH (50 mL, 0.1 M) and the mixture stirred for 30 min. The reaction was quenched by addition of Amberlite IR-120 H^+ and the mixture stirred for 30 min, filtered and evaporated under reduced pressure yielding a yellow oil (45 mg, ~95%). Purified by reverse phase dry column chromatography (CH_2Cl_2/MeOH, 0 to 80% in steps of 5%) resulting in a clear amorphous solid (9.9 mg, 21%). ^{1}H-NMR (300 MHz, D_2O): δ = 8.17 (dd, J = 9.3, 1.1 Hz, 2H), 7.34–7.17 (m, 2H), 4.17 (dd, J = 10.4, 1.4 Hz, 1H), 3.95 (t, J = 10.1 Hz, 1H), 3.88–3.74 (m, 3H), 3.66–3.59 (m, 1H), 3.57 (dd, J = 8.9, 1.3 Hz, 1H), 2.82 (dd, J = 12.8, 4.6 Hz, 1H), 2.08–1.97 (m, 4H). NMR data are in accordance with literature values.[146] ^{13}C-NMR (75 MHz, D_2O): δ = 175.2, 173.0, 160.1, 143.1, 125.7, 120.0, 102.5, 73.8, 71.5, 68.3, 67.8, 62.9, 51.8, 41.0, 22.2.

Phenyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside (83) and ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside (84) ♣

BnO OBn BnO AcO O SEt 80 + OBn HO BnO O SPh NPhth 65 → NIS, AgOTf CH_2Cl_2 -30 °C ~84% → BnO OBn BnO AcO O O BnO OBn O SPh NPhth 83 (~58%) + BnO OBn BnO AcO O O BnO OBn O SEt NPhth 84 (~26%)

Compound **80** (113 mg, 0.21 mmol) and **65** (110 mg, 0.19 mmol) were dissolved in

CH_2Cl_2 (3 mL) and stirred under argon with 4 Å MS for 1 h. The mixture was cooled to -30 °C, NIS (51 mg, 0.23 mmol) and AgOTf (cat.) were added and the mixture was stirred for 15 min. Quenched with Et_3N and stirred for 15 min, then filtered through Celite and evaporated *in vacuo*. The residue was purified by dry column chromatography (SiO_2, acetone/toluene, 0 to 60% in steps of 3%) resulting in **84** (10 mg) and **83** (87 mg, 44%) and a mix of the two (7:11, 72 mg), further purification was not nessesary as both could be used in subsequent reactions. **83** ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.84–7.64 (m, 4H), 7.40–7.15 (m, 20H), 7.02–6.76 (m, 5H), 5.36 (dd, J = 10.0, 8.0 Hz, 1H), 5.22 (d, J = 10.0 Hz, 1H), 4.97–4.80 (m, 2H), 4.77–4.62 (m, 2H), 4.54–4.19 (m, 9H), 4.08–3.97 (m, 1H), 3.93 (d, J = 2.6 Hz, 1H), 3.85–3.71 (m, 2H), 3.59 (t, J = 9.4 Hz, 1H), 3.49–3.32 (m, 4H), 2.75–2.52 (m, 2H), 2.01 (s, 3H) 1.17 (t, J = 7.4 Hz, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 169.5, 168.0, 167.7, 138.9, 138.9, 138.3, 138.2, 138.1, 133.9, 132.6, 131.8, 128.9, 128.6, 128.5, 128.5, 128.2, 128.0, 127.9, 127.9, 127.8, 127.4, 127.4, 126.9, 123.5, 123.4, 100.8, 81.2, 80.4, 79.5, 78.0, 77.8, 74.8, 74.5, 73.6 (2×C), 73.4, 72.7, 72.1, 71.7, 68.2, 54.9, 24.0, 21.2, 15.1. **84** ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.81 (d, J = 6.7 Hz, 1H), 7.78–7.60 (m, 3H), 7.43–7.12 (m, 25H), 7.03–6.90 (m, 2H), 6.90–6.75 (m, 3H), 5.51 (d, J = 10.0 Hz, 1H), 5.36 (dd, J = 10.1, 7.9 Hz, 1H), 4.91 (d, J = 11.6 Hz, 1H), 4.84 (d, J = 12.2 Hz, 1H), 4.73–4.62 (m, 2H), 4.55–4.45 (m, 4H), 4.42 (d, J = 12.2 Hz, 1H), 4.36–4.21 (m, 4H), 4.02–3.95 (m, 1H), 3.93 (d, J = 2.5 Hz, 1H), 3.84–3.74 (m, 2H), 3.60 (d,J = 10.0 Hz, 1H), 3.49–3.33 (m, 4H), 2.00 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 169.4, 168.1, 167.5, 138.9, 138.8, 138.3, 138.2, 138.1, 134.0, 133.9, 132.6, 132.4, 131.8, 131.8, 128.9, 128.6, 128.5, 128.5, 128.2, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.5, 127.4, 126.9, 123.6, 123.5, 100.9, 83.6, 80.5, 79.5, 78.1, 77.8, 77.4, 77.2, 76.9, 74.9, 74.6, 73.6, 73.4, 72.8, 72.1, 71.8, 68.2, 54.9, 21.2.

Phenyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside (81) ♣

Compound **61** (150 mg, 0.26 mmol) and **80** (198 mg, 0.34 mmol) were dissolved in CH_2Cl_2 (3 mL) and stirred under argon with 4 Å MS for 1 h, whereupon MeOTf (0.13 mL, 1.2 mmol) was added at -10 °C and the mixture was left to stir for 6 h. The reaction was quenched with pyridine, evaporated *in vacuo* and purified by flash column chromatography (SiO_2, acetone/toluene 1:33) resulting in a clear oil (142 mg, 52%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.94–7.72 (m, 4H), 7.48–7.01 (m, 30H), 5.44 (d, J = 10.5 Hz, 1H), 5.31 (dd, J = 10.1, 7.9 Hz, 1H), 5.04 (d, J = 10.3 Hz, 1H), 4.92 (d, J = 11.3 Hz, 1H), 4.85–4.73 (m, 1H), 4.63 (d, J = 12.0 Hz, 1H), 4.60–4.18 (m, 8H), 4.14 (d, J = 7.9 Hz, 1H), 3.88 (d, J = 2.7 Hz, 1H), 3.85–3.77 (m, 2H), 3.71–3.60 (m, 2H), 3.48 (t, J = 10.0 Hz, 1H), 3.40–3.30 (m, 2H), 3.10 (dd, J = 10.1, 2.7 Hz, 1H), 2.02 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 169.9, 168.6, 167.2, 138.7, 138.4, 138.3, 138.0, 137.8, 134.4, 132.6, 132.0, 129.1, 128.9, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 128.0, 127.8, 127.7, 127.6, 127.5, 127.3, 127.2, 125.4, 123.6, 100.8, 83.7, 80.2, 79.4, 77.6, 76.7, 75.2, 74.7, 73.5, 73.5, 73.4, 72.6, 71.5, 71.3, 69.1, 67.7, 55.1, 21.0.

Phenyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside (77) ♣

Compound **60** (119 mg, 0.20 mmol) and **61** (97 mg, 0.17 mmol) were dissolved in CH_2Cl_2 (3 mL) and stirred under argon with 4 Å MS for 1 h, whereupon MeOTf (0.09 mL, 0.8 mmol) was added at -10 °C and the mixture was left to stir for 6 h. The reaction was quenched with pyridine, evaporated *in vacuo* and purified by dry column chromatography (SiO_2, acetone/toluene, 0 to 50% in steps of 2.5%) resulting in a clear oil (0.148 mg, 78%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.67 (d, J = 7.4 Hz, 2H), 7.62–7.56 (m, 3H), 7.53 (t, J = 7.4 Hz, 1H), 7.33 (t, J = 7.7 Hz, 3H), 7.29–6.94 (m, 28H), 6.91 (d, J = 7.3 Hz, 2H), 5.50–5.39 (m, 1H), 5.31 (d, J = 10.5 Hz, 1H), 5.02 (d, J = 10.7 Hz, 1H), 4.84 (d, J = 11.4 Hz, 1H), 4.78 (dd, J = 10.1, 7.8 Hz, 1H), 4.53–4.37 (m, 6H), 4.28–4.20 (m, 3H), 4.15 (d, J = 11.7 Hz, 1H), 3.85 (d, J = 2.3 Hz, 1H), 3.74 (d, J = 10.7 Hz, 1H), 3.69 (dd, J = 10.7, 4.3 Hz, 1H), 3.63–3.56 (m, 2H), 3.39 (t, J = 8.1 Hz, 1H), 3.34–3.25 (m, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 165.4, 138.7, 138.68, 138.4, 138.1, 137.5, 134.1, 133.0, 132.8, 131.8, 131.5, 130.4, 130.1, 128.9, 128.5, 128.4, 128.3, 128.3, 128.1, 128.1, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.3, 100.8, 84.0, 80.3, 79.4, 77.9, 77.3, 74.9, 74.8, 73.6, 73.5, 73.4, 72.9, 72.5, 71.7, 69.3, 67.9, 55.1. Phth peaks from C=O missing.

Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside (51) ♣

Compound **30** (100 mg, 0.20 mmol) and **37** (184 mg, 0.24 mmol) were dissolved in CH_2Cl_2 (2 mL) and stirred under argon with 4 Å MS for 1 h. The mixture was cooled to -40 °C, TESOTf (5.3 mg, 0.02mmol) was added and the mixture was stirred for 2h. Quenched with Et_3N and the mixture stirred for 15 min followed by evaporation *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc/heptane 1:1) resulting in a clear oil (146 mg, 68%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.02–7.98 (m, 2H), 7.91–7.86 (m, 2H), 7.66–7.09 (m, 34H), 5.81 (d, J = 3.0 Hz, 1H), 5.63 (s, 1H), 5.61–5.49 (m, 2H), 5.34 (dd, J = 10.3, 3.5 Hz, 1H), 4.97 (d, J = 8.1 Hz, 1H), 4.91–4.81 (m, 1H), 4.49 (t, J = 10.3 Hz, 1H), 4.41 (dd, J = 10.0, 4.3 Hz, 1H), 4.35–4.20 (m, 2H), 3.97 (t, J = 9.0 Hz, 1H), 3.88 (t, J = 10.1 Hz, 1H), 3.81–3.70 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 165.7, 165.6, 165.5, 164.7, 136.9, 133.9, 133.6, 133.3, 132.9, 132.8, 131.6, 130.3, 130.1, 129.8, 129.7, 129.6, 129.3, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 126.4, 126.2, 102.1, 100.8, 84.5, 81.3, 77.2, 72.0, 71.0, 70.6, 70.3, 68.8, 67.6, 61.1, 55.1. Phth peaks from C=O missing.

Phenyl 2,3,4,6-tetra-*O*-acetyl-*β*-*D*-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-*β*-*D*-glucopyranoside (49)

Compound **30** (100 mg, 0.20 mmol) and **34** (114 mg, 0.22 mmol) were dissolved in CH_2Cl_2 (2 mL) and stirred under argon with 4 Å MS for 1 h. The mixture was cooled to -30 °C, TESOTf (5.3 mg, 0.02 mmol) was added and the mixture was stirred for 1h. Quenched with Et_3N and the mixture stirred for 15 min followed by evaporation *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc/heptane 9:11) resulting in a clear oil (46 mg, 28%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.03–7.71 (m, 4H), 7.51–7.23 (m, 10H), 5.61–5.54 (m, 2H), 5.22–5.16 (m, 1H), 4.98 (dd, J = 10.4, 8.0 Hz, 1H), 4.81–4.70 (m, 2H), 4.55 (d, J = 8.0 Hz, 1H), 4.46–4.35 (m, 2H), 4.03 (dd, J = 11.0, 8.2 Hz, 1H), 3.92–3.78 (m, 3H), 3.72 (td, J = 9.7, 4.7 Hz, 1H), 3.50–3.42 (m, 1H), 2.07 (s, 3H), 1.92 (s, 3H), 1.84 (s, 3H), 1.53 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.2, 170.1, 169.0, 137.1, 132.9, 131.5, 129.5, 129.1, 128.5, 128.3, 126.2, 101.6, 100.5, 84.4, 80.9, 76.7, 71.1, 70.7, 70.4, 69.3, 68.7, 66.7, 60.9, 54.4, 20.7, 20.7, 20.6, 20.2. NMR data are in accordance with literature values.[191]

Phenyl 2,3,-di-*O*-acetyl-4,6-*O*-benzylidene-*β*-*D*-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-*β*-*D*-glucopyranoside (116) ♣

Compound **30** (92 mg, 0.19 mmol) and **35** (100 mg, 0.22 mmol) were dissolved in CH_2Cl_2 (2 mL) and stirred under argon with 4 Å MS for 1 h. The mixture was cooled to -30 °C, NIS (54 mg, 0.40 mmol) and TESOTf (5.3 mg, 0.02 mmol) were added and the mixture was stirred for 1h. Quenched with Et_3N and the mixture stirred for 15 min followed by evaporation *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc/heptane 1:1) resulting in a clear oil (40 mg, 26%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.99–7.76 (m, 4H), 7.46–7.21 (m, 15H), 5.59 (d, J = 10.6 Hz, 1H), 5.55 (s, 1H), 5.34–5.20 (m, 3H), 5.12 (dd, J = 10.6, 3.4 Hz, 1H), 4.84–4.75 (m, 1H), 4.47–4.34 (m, 2H), 4.19 (d, J = 2.4 Hz, 1H), 3.89–3.71 (m, 3H), 3.30 (s, 1H), 3.26–3.17 (m, 2H), 1.99 (s, 3H), 1.38 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.6, 169.8, 137.4, 136.8, 134.9, 132.9, 132.7, 131.7, 129.6, 129.2, 128.9, 128.5, 128.4, 128.3, 126.4, 126.3, 124.0, 102.0, 100.7, 99.3, 84.6, 81.6, 77.6, 73.7, 70.5 68.7, 68.4, 68.3, 67.1, 63.0, 55.0, 21.1, 20.1. Phth peaks from C=O missing.

Benzyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-*D*-glucopyranoside (64) ♣

Synthesized using the general one-pot glycosylation method, using **60** (124 mg, 0.21 mmol), **65** (110 mg, 0.19 mmol) and **9** (184 mg, 0.19 mmol). Purified by flash column chromatography (SiO_2, Acetone/toluene 1:19) resulting in a clear oil (270 mg, 72%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.99 (d, J = 7.4 Hz, 2H), 7.62 (t, J = 7.4 Hz, 2H), 7.48 (t, J = 7.7 Hz, 3H), 7.39–7.10 (m, 53H), 7.07 (t, J = 7.3 Hz, 2H), 6.94 (d, J = 6.8 Hz, 2H), 6.90–6.76 (m, 5H), 5.67 (dd, J = 9.8, 8.1 Hz, 1H), 5.30 (d, J = 8.3 Hz, 1H), 5.04 (d, J = 11.4 Hz, 1H), 4.99 (d, J = 11.6 Hz, 1H), 4.94 (d,J = 12.1 Hz, 1H), 4.91–4.81 (m, 3H), 4.73–4.62 (m, 3H), 4.60–4.52 (m, 4H), 4.52–4.19 (m, 14H), 4.16 (d, J = 11.9 Hz, 1H), 4.06 (t, J = 9.2 Hz, 1H), 4.04–3.97 (m, 2H), 3.95 (d, J = 1.8 Hz, 1H), 3.83 (t, J = 9.0 Hz, 1H), 3.70 (dd, J = 10.9, 3.3 Hz, 1H), 3.55 (dd, J = 10.1, 2.6 Hz, 1H), 3.53–3.24 (m, 14H), 2.92 (d, J = 7.6 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): 167.9, 167.8, 165.2, 139.5, 139.1, 139.0, 138.8, 138.7, 138.6, 138.4, 138.4, 138.3, 138.0, 137.8, 137.7, 133.5, 133.2, 131.3, 130.0, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.2, 127.1, 126.8, 126.7, 126.5, 123.1, 102.5 (2×C), 100.9, 99.7, 83.0, 82.1, 81.7, 79.9, 78.8 77.8, 77.4, 76.9, 76.1, 75.5, 75.1 (2×C), 74.8 (2×C), 74.6, 74.5, 74.1, 73.6 (2×C), 73.4, 73.3, 73.0, 73.0, 72.6, 72.6, 71.4, 70.9, 68.3, 68.1 (2×C), 67.7, 56.4.

Benzyl
2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-*D*-glucopyranoside (86) ♣

Synthesized by the general sequential glycosylation method, using **81** (270 mg, 0.256 mmol) and **9** (207 mg, 0.213 mmol). Purified by dry column chromatography (SiO_2, EtOAc/toluene, 0 to 33% in steps of 1.65%) resulting in a clear oil (240 mg, 58%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.72–7.52 (m, 2H), 7.47–7.01 (m, 60H), 6.96–6.80 (m, 2H), 5.31–5.21 (m, 2H), 5.08–4.99 (m, 2H), 4.94–4.79 (m, 5H), 4.69 (d, J = 10.9 Hz, 1H), 4.63–4.39 (m, 9H), 4.38–4.14 (m, 10H), 4.08 (d, J = 7.9 Hz, 1H), 4.01 (d, J = 2.8 Hz, 1H), 3.93–3.75 (m, 5H), 3.70 (ddd, J = 9.9, 4.6, 1.8 Hz, 1H), 3.66–3.60 (m, 1H), 3.53–3.43 (m, 4H), 3.43–3.24 (m, 8H), 3.04 (dd, J = 10.1, 2.7 Hz, 1H), 2.90 (ddd, J = 9.9, 3.4, 1.7 Hz, 1H), 1.92 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 169.9, 139.6, 139.2, 138.8, 138.8, 138.5, 138.4, 138.3, 138.1, 137.9, 137.8, 133.9, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 127.3, 127.2, 126.9, 126.4, 102.6, 102.5, 100.8, 99.5, 83.2, 81.7, 81.7, 80.4, 78.8, 77.1, 76.8, 76.3, 76.1,

75.6, 75.3, 75.2, 75.1, 75.0, 74.9, 74.8, 73.9, 73.8, 73.6, 73.4, 73.4, 73.3, 73.2, 72.7, 71.6, 71.4, 70.9, 69.5, 68.5, 67.8, 67.7, 56.6, 21.0.

Benzyl
2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside (85)

Synthesized by the general sequential glycosylation method, using a mixture of **83**/**84** (300 mg, ~0.284 mmol) and **9** (215 mg, 0.221 mmol). Purified by flash column chromatography (SiO_2, EtOAc/heptane 1:2) resulting in a clear oil (310 mg, 73%).
Synthesized by the general one-pot glycosylation method, using **80** (686 mg, 1.3 mmol), **65** (620 mg, 1.1 mmol) and **9** (1.00 g, 1.0 mmol). Purified by flash column chromatography (SiO_2, EtOAc/heptane 1:2) resulting in a clear oil (1.86 g, 94%).
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.42–7.04 (m, 58H), 6.94–6.76 (m, 6H), 5.37 (m, 2H), 5.07 (d, J = 11.4 Hz, 1H), 4.95–4.82 (m, 5H), 4.72–4.62 (m, 3H), 4.57–4.22 (m, 18H), 4.17 (d, J = 11.9 Hz, 1H), 4.06–3.98 (m, 3H), 3.93 (d, J = 2.7 Hz, 1H), 3.88–3.83 (m, 1H), 3.82–3.74 (m, 2H), 3.63–3.57 (m, 1H), 3.54 (dd, J = 9.8, 3.0 Hz, 1H), 3.53–3.31 (m, 12H), 2.98–2.88 (m, 1H), 2.01 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 169.4, 139.6, 139.2, 139.0, 138.9, 138.8, 138.6, 138.5, 138.5, 138.2, 138.2, 138.1, 137.7, 133.5, 128.6, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.2, 126.9, 126.8, 126.6, 102.6, 101.0, 99.9, 83.1, 82.1, 81.8, 80.5, 78.9, 78.0, 76.8 (2×C), 76.1, 75.6, 75.2, 75.2, 74.9 (2×C), 74.8, 74.6, 74.2, 73.8, 73.6, 73.4, 73.4, 73.2, 73.1, 72.8, 72.1, 71.8, 71.0, 68.4, 68.4, 68.2, 67.8, 56.5, 21.2. NMR data are in accordance with literature values.[93]

Benzyl
2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside (103) ♣

Synthesized by the general one-pot glycosylation method, using **60** (120 mg, 0.20 mmol), **100** (83 mg, 0.17 mmol) and **9** (164 mg, 0.17 mmol). Purified by flash column chromatography (SiO_2, EtOAc/heptane 1:2) resulting in a clear oil (183 mg, 57%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.09–7.94 (m, 2H), 7.69–6.81 (m, 62H), 5.65 (dd, J = 10.0, 8.0 Hz, 1H), 5.35 (d, J = 8.4 Hz, 1H), 4.98 (d, J = 11.4 Hz, 1H), 4.92 (d, J = 11.7 Hz, 1H), 4.89–4.75 (m, 3H), 4.68–4.36 (m, 11H), 4.31–4.13 (m, 8H), 4.12–3.99 (m, 3H), 3.96–3.84 (m, 3H), 3.84–3.75 (m, 1H), 3.67–3.25 (m, 17H), 2.94–2.84 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 167.9, 165.2, 139.5, 139.1, 138.7, 138.6, 138.5, 138.4,

138.3, 138.1, 137.7, 137.4, 137.4, 134.6, 133.7, 133.5, 133.4, 131.5, 130.2, 130.1, 129.9, 129.9, 129.1, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.2, 127.2, 127.1, 126.9, 126.6, 123.2, 102.5, 102.4, 102.1, 99.7, 83.0, 82.3, 82.1, 81.7, 79.8, 78.9, 76.8, 76.0, 75.5, 75.1 (C×2), 74.8, 74.6, 74.2, 74.0, 73.8, 73.7, 73.3, 73.1, 73.1, 73.1, 72.2, 72.1, 71.8, 70.9, 69.4, 68.7, 68.7, 68.4, 67.7, 56.6.

Benzyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-*D*-glucopyranoside (58) ♣

Synthesized by the general sequential glycosylation method, using **77** (120 mg, 0.11 mmol) and **9** (104 mg, 0.11 mmol). Purified by dry column chromatography (SiO_2, acetone/toluene, 0 to 75% in steps of 5%) resulting in a clear oil (170 mg, 80%).
Synthesized by the general one-pot glycosylation method, using **60** (124 mg, 0.21 mmol), **61** (110 mg, 0.19 mmol) and **9** (184 mg, 0.19 mmol). Purified by flash column chromatography (SiO_2, Acetone/toluene 1:19) resulting in a clear oil (27 mg, 7%).
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.68 (dd, J = 8.2, 1.2 Hz, 2H), 7.59–7.53 (m, 1H), 7.41–7.02 (m, 62H), 7.00–6.94 (m, 2H), 6.88–6.81 (m, 2H), 5.51 (dd, J = 9.9, 7.9 Hz, 1H), 5.22 (d, J = 8.4 Hz, 1H), 5.12 (d, J = 10.6 Hz, 1H), 4.99–4.81 (m, 6H), 4.69 (d, J = 10.9 Hz, 1H), 4.58–4.40 (m, 10H), 4.36–4.11 (m, 10H), 3.97 (d, J = 2.9 Hz, 1H), 3.94 (d, J = 2.6 Hz, 1H), 3.86–3.65 (m, 6H), 3.53–3.28 (m, 12H), 3.25 (d, J = 9.4 Hz, 1H), 2.92–2.84 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 165.5, 139.5, 139.1, 138.8, 138.7, 138.7, 138.7, 138.5, 138.3, 138.2, 138.1, 137.7, 137.6, 133.6, 132.7, 131.1, 130.3, 130.0, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.3, 127.2, 126.8, 126.4, 102.5, 102.5, 100.7, 99.6, 83.0, 81.7, 81.5, 80.3, 78.7, 77.5, 76.7, 76.3, 76.0, 75.5, 75.1, 75.1, 75.0, 75.0, 74.8, 74.8, 73.8, 73.7, 73.5, 73.4, 73.3, 73.3, 73.1, 72.9, 72.9, 71.7, 70.9, 69.5, 68.6, 67.9, 67.6, 56.4.

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

Synthesized by the general one-pot glycosylation method, using **80** (107 mg, 0.20 mmol), **100** (83 mg, 0.17 mmol) and **9** (145 mg, 0.15 mmol). Purified by flash column chromatography (SiO_2, EtOAc/heptane 1:2) resulting in a clear oil (140 mg, 51%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.70 (d, J = 6.8 Hz, 1H), 7.50 (t, J = 7.5 Hz, 1H), 7.41–7.02 (m, 55H), 6.96–6.87 (m, 2H), 5.45 (d, J = 8.4 Hz, 1H), 5.38 (dd, J = 10.0, 8.0

Hz, 1H), 5.07 (d, J = 11.4 Hz, 1H), 4.94–4.81 (m, 4H), 4.75–4.38 (m, 13H), 4.38–4.21 (m, 8H), 4.18 (d, J = 11.9 Hz, 1H), 4.10 (d, J = 11.9 Hz, 1H), 4.02 (d, J = 2.8 Hz, 1H), 3.92–3.80 (m, 2H), 3.77–3.65 (m, 4H), 3.63–3.29 (m, 13H), 2.95 (dd, J = 9.8, 1.8 Hz, 1H), 1.98 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 169.5, 167.9, 139.6, 139.1, 138.8, 138.6, 138.4, 138.4, 138.1, 137.8, 137.7, 137.4, 133.7, 133.6, 132.7, 131.5, 128.9, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.2, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.2, 127.2, 126.9, 126.7, 123.2, 102.5(C×2), 101.9, 99.7, 83.1, 82.1, 82.0, 81.8, 80.4, 79.0, 76.8, 76.1, 75.6, 75.2, 75.1, 74.9, 74.6, 74.3, 74.1, 73.9, 73.8, 73.7, 73.4, 73.1, 73.1, 72.3, 72.3, 71.3, 70.9, 69.3, 68.8, 68.6, 68.4, 67.8, 56.7, 21.1.

Benzyl 2,3,4-tri-*O*-benzyl-*α*-*L*-fucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-*D*-glucopyranoside (62)

NIS (112 mg, 0.50 mmol) and TESOTf (13 mg, 0.049 mmol) were added to a solution of **63** (711 mg, 0.38 mmol), **59** (253 mg, 0.48 mmol) and 4 Å MS in CH_2Cl_2 (7 mL) at -30 °C. After stirring for 20 min the reaction was quenched with Et_3N and the mixture stirred for 15 min, and was then filtered through Celite and evaporated *in vacuo*. Purification by flash column chromatography (SiO_2, Acetone/toluene 1:24) resulted in a clear oil (657 mg, 89%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.69 (br, 1H), 7.52 (br, 1H), 7.39–7.04 (m, 70H), 6.93–6.80 (m, 5H), 6.75 (t, J = 7.3 Hz, 2H), 5.73 (d, J = 3.8 Hz, 1H), 5.38 (d, J = 7.9 Hz, 1H), 5.10 (d, J = 11.4 Hz, 1H), 4.97 (d, J = 11.5 Hz, 1H), 4.94–4.16 (m, 34H), 4.15–3.99 (m, 4H), 3.95 (d, J = 2.5 Hz, 1H), 3.94–3.75 (m, 5H), 3.64 (dd, J = 9.7, 2.8 Hz, 1H), 3.59 (dd, J = 9.8, 2.9 Hz, 1H), 3.57–3.29 (m, 12H), 3.00–2.90 (m, 1H), 1.39 (d, J = 6.5 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 139.5, 139.1, 138.9, 138.9, 138.8, 138.7, 138.6, 138.6, 138.5, 138.4, 138.2, 138.2, 138.1, 137.7, 133.6, 131.3, 128.6, 128.6, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 129.0, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.3, 127.2, 127.0, 126.9, 126.5, 126.3, 123.2, 102.5, 102.5, 100.9, 100.2, 97.7, 84.1, 83.1, 82.5, 81.8, 79.4, 78.9, 78.2, 76.8, 76.8, 76.7, 76.1, 75.8 (2×C), 75.6, 75.3, 75.2, 75.0, 75.0, 74.7, 74.5, 74.3, 73.9, 73.7, 73.6, 73.5, 73.3, 73.2, 73.1, 72.7, 72.4, 72.3, 71.0, 70.9, 68.8, 68.5, 68.3, 67.8, 66.6, 56.5, 17.0. Phth peaks from C=O missing. NMR data are in accordance with literature values.[93]

Benzyl 2,3,4-tri-*O*-benzyl-*α*-*L*-fucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-*β*-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-*D*-glucopyranoside (91) ♣

NIS (17 mg, 0.077 mmol) and TESOTf (1.9 mg, 0.0077 mmol) were added to a solution of **87** (100 mg, 0.056 mmol), **59** (38 mg, 0.073 mmol) and 4 Å MS in CH_2Cl_2(1.5 mL) at -20 °C. The mixture was left to reach 10 °C, where the reaction was quenched with Et_3N and the mixture stirred for 15 min, and then filtered through Celite and evaporated *in vacuo*. Purification by flash column chromatography (deactivated with Et_3N) (SiO_2, Acetone/toluene 1:9) resulted in a clear oil (84 mg, 68%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.43–6.96 (m, 75H), 5.60 (d,J = 3.7 Hz, 1H), 5.06 (d, J = 11.1 Hz, 2H), 4.99 (d, J = 10.6 Hz, 1H), 4.95–4.34 (m, 31H), 4.26 (d, J = 11.8 Hz, 1H), 4.19 (dd, J = 9.5, 7.6 Hz, 1H), 4.05 (dd, J = 10.5, 2.5 Hz, 1H), 4.02–3.91 (m, 5H), 3.89 (d, J = 1.4 Hz, 1H), 3.87–3.37 (m, 18H), 3.33–3.22 (m, 1H), 1.63 (s, 3H), 1.20 (d, J = 6.5 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 169.3, 139.7, 139.6, 139.3, 139.2, 138.9, 138.7, 138.7, 138.4, 138.3, 138.3, 138.1, 137.6, 129.0, 128.8, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 127.3, 127.2, 127.2, 127.1, 126.4, 126.3, 102.7, 102.5, 102.0, 101.6, 98.2, 84.0, 83.0, 81.8, 81.5, 80.2, 79.3, 78.1, 78.0, 77.5, 77.2, 77.1, 76.8, 76.6, 76.4, 75.7, 75.6, 75.3, 75.3, 75.2, 75.1, 75.1, 75.0, 74.8, 74.7, 74.1, 73.7, 73.6, 73.6, 73.5, 73.5, 73.4, 72.8, 72.4, 72.0, 71.2, 71.0, 69.5, 68.9, 68.4, 68.3, 66.4, 55.8, 23.6, 16.8.

2-acetamido-

Benzyl 3,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-*D*-glucopyranoside (63)

Compound **85** (1.55 g, 0.81 mmol) was dissolved in CH_2Cl_2 (10 mL) whereupon a solution of NaOMe (40 mg, 1.74 mmol Na in 100 mL MeOH) was added. Amberlite IR 120 H^+ was added after the mixture had stirred at rt for 48 h, and the mixture stirred for an additional 2 h followed by concentration *in vacuo*, The residue was purified by flash column chromatography (SiO_2, EtOAc/heptane 1:3) resulting in a clear oil (1.23 g, 81%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.49–6.77 (m, 64H), 5.37 (d, J = 8.4 Hz, 1H), 5.06 (d, J = 11.4 Hz, 1H), 4.92–4.81 (m, 5H), 4.73–4.67 (m, 2H), 4.65–4.60 (m, 2H), 4.58–4.44 (m, 8H), 4.38 (d, J = 12.3 Hz, 1H), 4.35–4.11 (m, 10H), 4.08 (dd, J = 11.3, 3.5 Hz, 1H), 4.02–3.81 (m, 6H), 3.72 (ddd, J = 9.9, 3.2, 1.9 Hz, 1H), 3.59–3.26 (m, 13H), 2.92 (ddd, J = 9.9, 3.6, 1.7 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 139.5, 139.2, 139.0, 138.9, 138.8, 138.7, 138.5, 138.5, 138.3, 138.0, 138.0, 137.7, 133.5, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8,

127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 126.9, 126.8, 126.5, 123.2, 103.6, 102.6, 102.5, 100.0, 83.1, 82.1, 82.0, 81.8, 79.0, 78.4, 78.1, 76.8, 76.1, 75.6, 75.2, 75.2, 74.9, 74.8, 74.7, 74.6, 74.1, 73.8, 73.6, 73.6, 73.5, 73.2, 73.1, 72.9, 72.4, 72.3, 70.9, 68.8, 68.4, 68.4, 67.8, 56.6. Phth peaks from C=O missing. Synthesized, but NMR data not reported.[93]

Benzyl 3,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-*β*-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-*D*-glucopyranoside (87) ♣

1) EDA, ^{t}BuOH, reflux
2) Ac_2O, Et_3N, EtOH
72%

A mixture of **86** (301 mg, 0.162 mmol) and ethylenediamine (4.7 mL, 0.070 mmol) in *t*-BuOH (20 mL) was stirred at 100 °C for 16 h. The volatiles were removed *in vacuo* and co-evaporated with toluene (2×10 mL) then ethanol (10 mL). The residue was taken up in ethanol (4.5 mL), acetic anhydride (1.5 mL, 0.016 mol) and Et_3N (3 mL) were added at 0 °C and the mixture stirred at rt for 15 h. The residue was diluted with EtOAc (40 mL), then washed with a saturated aqueous solution of $NaHCO_3$ (2×20 mL), H_2O (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (deactivated with Et_3N) (SiO_2, EtOAc/heptane 2:3) resulting in a clear oil (209 mg, 72%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.40–7.11 (m, 60H), 5.09 (d, J = 8.6 Hz, 1H), 5.04–4.87 (m, 7H), 4.80–4.32 (m, 19H), 4.28–4.20 (m, 2H), 4.15 (d, J = 7.5 Hz, 1H), 4.03–3.85 (m, 5H), 3.85–3.58 (m, 9H), 3.57–3.43 (m, 6H), 3.42–3.34 (m, 2H), 3.34–3.27 (m, 2H), 1.55 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 171.2, 139.4, 139.4, 139.2, 139.0, 138.7, 138.6, 138.5, 138.3, 138.2, 138.2, 137.6, 128.7, 128.7, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.3, 126.5, 104.3, 102.6, 102.5, 101.5, 83.0, 81.8, 81.7, 81.4, 81.1, 80.8, 76.7, 76.5, 76.4, 75.6, 75.2, 75.2, 75.1, 75.1, 74.8 (C×2), 74.6, 73.9, 73.6 (C×2), 73.6, 73.6, 73.5, 73.5, 72.5, 71.8, 71.0, 69.4, 68.6, 68.4, 68.2, 56.1, 23.2.

Benzyl 2,3,4-tri-*O*-benzyl-*α*-*L*-fucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-*β*-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-*D*-glucopyranoside (90) ♣

1) EDA, ^{t}BuOH, reflux
2) Ac_2O, Et_3N, DMAP, CH_2Cl_2
74%

A mixture of **62** (475 mg, 0.207 mmol) and ethylenediamine (5.7 mL, 0.085 mmol) in *t*-BuOH (25 mL) was stirred at 100 °C for 16 h. The volatiles were removed *in vacuo* and co-evaporated with toluene (2×10 mL) then ethanol (10 mL). The residue was taken up in CH_2Cl_2(6 mL), and acetic anhydride (2 mL, 0.021 mol), Et_3N (4 mL) and DMAP (25 mg, 0.21 mmol) were added at 0 °C and the mixture stirred at rt for 15 h. The residue was diluted with EtOAc (50 mL), then washed with a saturated aqueous

solution of $NaHCO_3$ (2×30 mL), H_2O (40 mL), dried over $MgSO_4$ end concentrated *in vacuo*. The residue was purified by flash column chromatography (deactivated with Et_3N) (SiO_2, EtOAc/toluene 1:9) resulting in a clear oil (336 mg, 74%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.44–7.00 (m, 75H), 5.71 (d, J = 3.6 Hz, 1H), 5.10 (d, J = 8.3 Hz, 1H), 5.05–4.97 (m, 2H), 4.96–4.87 (m, 5H), 4.83–4.71 (m, 5H), 4.70–4.17 (m, 24H), 4.07–3.91 (m, 5H), 3.86 (d, J = 10.4 Hz, 1H), 3.81–3.33 (m, 19H), 3.30–3.23 (m, 1H), 1.47 (s, 3H), 1.27 (d, J = 6.4 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 170.1, 139.5, 139.2, 139.1, 138.9, 138.9, 138.8, 138.7, 138.6, 138.5, 138.3, 138.2, 138.0, 137.7, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 127.4, 127.3, 127.2, 126.9, 126.3, 102.7, 102.6, 101.8, 101.1, 97.6, 84.1, 83.0, 81.9 (C×2), 80.1, 79.4, 78.5, 78.1, 77.5, 77.2, 76.8, 76.6, 76.4, 75.9, 75.8, 75.7, 75.6, 75.3, 75.2, 75.0, 75.0, 74.8, 74.7, 74.3, 73.7, 73.6, 73.6, 73.5, 73.3, 73.3, 73.2, 72.7, 72.5, 72.4, 71.2, 71.0, 68.9, 68.3, 68.3 (C×2), 66.5, 56.4, 23.3, 17.0.

α-*L*-fucopyranosyl-(1→2)-β-*D*-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→3)-β-*D*-galactopyranosyl-(1→4)-β-*D*-glucopyranose (55)

A solution of **90** (140 mg, 0.064 mmol) in THF (5 mL) and MeOH (5 mL) was flushed thoroughly with argon, whereupon 5% Pd/C (78 mg, 0.037 mmol) and TFA (0.1 mL, 0.0013 mmol) were added. The mixture was subjected to a hydrogen atmosphere (balloon) at rt for 16 h, filtered through a pad of Celite, and concentrated *in vacuo*. The residue was purified by reverse phase dry column chromatography (CH_2Cl_2/MeOH, 0 to 100% in steps of 10%) resulting in an amorphous solid (53 mg, 99%). ^{1}H-NMR (400 MHz, D_2O): δ = 5.30 (d, J = 2.6 Hz, 1H, H-1””), 5.22 (d, J = 3.7 Hz, 0.4H, H-1α), 4.70 (d, J = 8.4 Hz, 1H, H-1”), 4.66 (d, J = 8.0 Hz, 0.6H, H-1β), 4.55 (d, J = 7.8 Hz, 1H, H-1”’), 4.44 (d, J = 7.8 Hz, 1H, H-1’), 4.21 (q, J = 6.6 Hz, 1H, H-5””), 4.14 (d, J = 3.2 Hz, 1H, H-4’), 4.01–3.54 (m, 25H), 3.50–3.42 (m, 1H), 3.32–3.23 (m, 0.6H, H-2β), 2.04 (s, 3H), 1.22 (d, J = 6.6 Hz, 3H, H-6””). NMR data are in accordance with literature values.[192,193] ^{13}C-NMR (100 MHz, D_2O): δ = 174.9, 102.9 (C-1’), 102.7 (C-1”), 100.2 (C-1”’), 99.4 (C-1””), 95.7 (C-1β), 91.8 (C-1α), 81.9, 78.3, 78.2, 76.4, 75.8, 75.2, 75.1, 74.8, 74.8, 74.3, 73.8 (C-2β), 73.5, 72.0, 71.6, 71.4, 71.1, 70.1, 70.0, 69.6, 69.1, 68.3 (C-4’), 68.2, 66.9 (C-5””), 61.1, 60.9, 60.0, 60.0, 55.4, 22.2, 15.3. NMR data are in accordance with literature values.[193]

α-*L*-fucopyranosyl-(1→2)-β-*D*-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→3)-β-*D*-galactopyranosyl-(1→4)-β-*D*-glucopyranose (54)

A solution of **91** (30 mg, 0.014 mmol) in THF (1 mL) and MeOH (1 mL) was flushed thoroghly with argon, whereupon 5% Pd/C (16 mg, 0.0075 mmol) and TFA (0.05 mL, 0.65 μmol) were added. The mixture was subjected to a hydrogen atmosphere (balloon) at rt for 16 h, was filtered through a pad of Celite and concentrated *in vacuo*. The residue was purified by reverse phase dry column chromatography (CH_2Cl_2/MeOH, 0 to 100% in steps of 10%) resulting in an amorphous solid (11.5 mg, 98%). ^{1}H-NMR (400 MHz, D_2O): δ = 5.22 (d, J = 3.7 Hz, 0.4H, H-1α), 5.19 (d, J = 4.0 Hz, 1H, H-1""), 4.70–4.58 (m, 2.6H, H-1β, H1"', H1"), 4.42 (d, J = 7.8 Hz, 1H, H-1'), 4.29 (q, J = 6.6 Hz, 1H, H-5""), 4.14 (d, J = 3.2 Hz, 1H, H-4'), 4.04–3.44 (m, 27H), 3.28 (t, J = 8.5 Hz, 0.6H, H-2β), 2.05 (s, 3H), 1.23 (d, J = 6.6 Hz, 3H).[194] ^{13}C-NMR (100 MHz, D_2O): δ = 174.2, 103.2 (C-1"), 102.9 (C-1'), 100.2 (C-1"'), 99.5 (C-1""), 95.7 (C-1β), 91.8 (C-1α), 81.5, 78.2, 78.1, 77.1, 76.6, 75.2, 75.0, 74.8, 74.3, 73.8, 73.5, 71.8, 71.3, 71.1, 70.2, 69.4, 69.1, 68.6, 68.4, 68.0, 66.5, 61.1, 60.9, 60.4, 60.0, 55.9, 22.1, 15.2. (35 carbon signals so 3 extra from α/β).

Table 1.3: Glycosylation between various galactose donors and 30

In all cases accept entry 5, prior to glycosylation a mixture of donor and acceptor **30** were co-evaporated with dry toluene (3×3 mL), and subjected to high vacuum 2-16 h (2 h for entry 7–10, 16 h for entry 4 and 6). The mixtures were then dissolved in CH_2Cl_2 and stirred under argon with 4 Å MS for 1 h.
Entry 4 See synthesis of **116**
Entry 5 Compound **30** (82 mg, 0.16 mmol) and **36** (100 mg, 0.22 mmol) were co-evaporated with dry toluene (3×3 mL) in separate flasks and subjected to high vacuum for 16 h. BSP, **36** and *sym*-collidine (0.1 mL) were dissolved in CH_2Cl_2 (1 mL) and Tf_2O (68 mg, 0.24 mmol) was added at -40 °C. A solution of **30** in CH_2Cl_2 (1 mL) was added after 15 min and the solution stirred for 2 h. Both donor and acceptor decomposed.
Entry 6 Same procedure as entry 4, using NBS (43 mg, 0.24 mmol) instead of NIS. Both donor and acceptor decomposed.
Entry 7 AgOTf (150 mg, 0.59 mmol) was added to a mixture of **33** (200 mg, 0.49 mmol) and **30** (171 mg, 0.35 mmol) in CH_2Cl_2 (5 mL) at -30 °C, and the mixture was left to reach rt (4 h). The reaction mixture was washed with 1M HCl (50 mL), H_2O (50 mL), saturated aqueous $NaHCO_3$ (50 mL), dried over $MgSO_4$ and evaporated to dryness. Donor decomposed and acceptor reisolated.
Entry 8 AgOTf (62 mg, 0.24 mmol) was added to a mixture of **36** (129 mg, 0.20 mmol) and **30** (80 mg, 0.16 mmol) in CH_2Cl_2 (2.5 mL) at -30 °C. The mixture was left to reach rt and stirred for 16 h. Filtered through Celite, washed with a 10% aqueous solution of $Na_2S_2O_3$ (20 mL), H_2O (30 mL), dried over $MgSO_4$ and concentrated un-

der reduced pressure. Purified by flash column chromatography (SiO_2, EtOAc/heptane 2:3). Donor decomposed and acceptor reisolated.
Entry 9 Ag_2CO_3 (74 mg, 0.27 mmol) was added to a mixture of **36** (129 mg, 0.20 mmol) and **30** (80 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) at -60 °C. The mixture was left to reach rt and stirred for 16 h. No reaction occured.
Entry 10 Ag_2O (70 mg, 0.3 mmol) was added to a mixture of **36** (129 mg, 0.20 mmol) and **30** (80 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) at -30 °C. The mixture was left to reach rt and stirred for 60 h. No reaction occured.
Entry 12 See synthesis of **49**
Entry 13 See synthesis of **51**

Table 1.4: Glycosylation between various galactose donors and 53

In all cases, prior to glycosylation a mixture of donor and acceptor **53** was co-evaporated with dry toluene (3×3 mL) and subjected to high vacuum for 16 h.
Entry 1 TfOH (11 mg, 0.07 mmol) was added to a mixture of **32** (169 mg, 0.27 mmol), **53** (107 mg, 0.22 mmol) and NIS (75 mg, 0.33 mmol) in CH_2Cl_2 (2 mL) at -30 °C. Quenched with Et_3N and purified by flash column chromatography (SiO_2, EtOAc/heptane 2:3). Donor reisolated and acceptor decomposed.
Entry 2 Me_2S_2-Tf_2O (0.45 mL, 0.45 mmol) was added to a mixture of **32** (169 mg, 0.27 mmol) and **53** (107 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) at -20 °C. The reaction mixture was quenched with Et_3N after 2 h of stirring and was washed with 1M HCl (50 mL), H_2O (50 mL), saturated aqueous $NaHCO_3$ (50 mL), dried over $MgSO_4$ and evaporated to dryness. Purified by dry column chromatography (SiO_2, EtOAc/heptane, 0 to 90% in steps of 6%). Product could be identified as a Gal-Glc dimer with a pentanyl in the reducing end.
Entry 3 Same procedure as entry 2, utilizing **31** (106 mg, 0.23 mmol) and **53** (75 mg, 0.19 mmol) and Me_2S_2-Tf_2O (0.23 mL, 0.23 mmol). Both donor and acceptor decomposed.
Entry 4 Same procedure as entry 7 in Table 1.3, employing AgOTf (62 mg, 0.24 mmol), **33** (100 mg, 0.24 mmol) and **53** (75 mg, 0.16 mmol) in CH_2Cl_2 (2.5 mL). Donor decomposed and acceptor reisolated.

Table 1.5 Glycosylation between 60 and 61 employing different promotors.

In all cases accept for entry 2, prior to glycosylation a mixture of donor **60** and acceptor **61** was co-evaporated with dry toluene (3×3 mL) and subjected to high vacuum for 16 h.
Entry 1 Compound **60** (118 mg, 0.20 mmol) and **61** (110 mg, 0.19 mmol) were dissolved in CH_2Cl_2 (2 mL) and stirred under argon with 4 Å MS for 1 h. The mixture was cooled to -40 °C, NIS (48 mg, 21 mmol) and AgOTf (cat.) were added. The solution was stirred for 1 h, quenched with Et_3N and stirred for 15 min followed by evaporation *in vacuo*. Purified by dry column chromatography (SiO_2, acetone/toluene, 0 to 50% in steps of 2.5%). Yield of **77**: 94 mg, 44%.
Entry 2 Compound **60** (118 mg, 0.20 mmol) and **61** (100 mg, 0.18 mmol) were co-

evaporated with dry toluene (3×3 mL) in separate flasks and subjected to high vacuum for 16 h. Compound **60** in CH_2Cl_2 (1 mL) was added over 5 min to a solution of **61**, NIS (48 mg, 21 mmol) and AgOTf (cat.) at -40 °C. Purified as entry 1. Yield of **77**: 44 mg, 22%.
Entry 3 Compound **60** (118 mg, 0.20 mmol) and **61** (100 mg, 0.18 mmol) were dissolved in CH_2Cl_2 (2 mL) and stirred under argon with 4 Å MS for 1 h. The mixture was cooled to -30 °C, NIS (48 mg, 0.21 mmol) and TESOTf (5.3 mg, 0.02 mmol) was added and the mixture was stirred for 80 min. Purified as entry 1. Yield of **77**: 68 mg, 32%.
Entry 4 TfOH (3.6 mg, 0.04 mmol) was added to a mixture of **60** (120 mg, 0.20 mmol), **61** (110 mg, 0.19 mmol) and NIS (51 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) at -50 °C. The mixture was left to reach -20 °C, and was purified as entry 1. Yield of **77**: 71 mg, 34%.
Entry 5 See synthesis of **77**
Entry 6 Same procedure as entry 5, but TTBP (49, 0.20 mmol) was added prior to addition of MeOTf. Yield: 61 mg, 33%.
Entry 7 Same procedure as entry 5, but the mixture was left to reach rt and stirred for 16 h. Decomposition of both donor and acceptor.
Entry 8 Same proceedure as entry 2 in Table 1.4, utilizing **60** (125 mg, 0.21 mmol), **61** (108 mg, 0.19 mmol) and Me_2S_2-Tf_2O (0.38 mL, 0.38 mmol). Both donor and acceptor decomposed.

Table 1.6: Glycosylation between 61 and 80 employing different promotors.

Entry 1 Same proceedure as entry 5 in Table 1.5, utilizing **61** (150 mg, 0.26 mmol) and **80** (198 mg, 0.34 mmol). Yield **81**: 142 mg, 52%.
Entry 2 Same proceedure as entry 3 in Table 1.5, utilizing **61** (195 mg, 0.34 mmol) and **80** (216 mg, 0.40 mmol). Yield **81**: 60 mg, 17%; mix **81** and **82**: 127 mg, ∼36%.
Entry 3 Same proceedure as entry 1 in Table 1.5, utilizing **61** (150 mg, 0.26 mmol) and **80** (167 mg, 0.31 mmol). Yield **81**: 116 mg, 42%; mix **81** and **82**: 94 mg, ∼34%.

Table 1.7: Attempts at synthesizing disaccharide 94

In all cases, prior to glycosylation a mixture of donor and acceptor **46** was co-evaporated with dry toluene (3×3 mL) and subjected to high vacuum for 2 h.
Entry 1 AgOTf (96 mg, 0.38 mmol) was added to a mixture of **46** (96 mg, 0.24 mmol) and **95** (128 mg, 0.25 mmol) in THF (2 mL) at -10 °C. The mixture was allowed to reach rt, and stirred at rt for 48 h. Quenched with Et_3N and starting materials were isolated after flash column chromatography.
Entry 2 Ag_2O (88 mg, 0.38 mmol) was added to a mixture of **46** (96 mg, 0.24 mmol) and **95** (128 mg, 0.25 mmol) in THF (2 mL) at -10 °C. The mixture was allowed to reach rt, and stirred at rt for 48 h. Quenched with Et_3N and starting materials were isolated after flash column chromatography.
Entry 3 To a solution of **46** (100 mg, 0.25 mmol) and **96** (175 mg, 0.32 mmol) in

THF (2 mL) were added NIS (76 mg, 0.34 mmol) and TESOTf (9 mg, 0.034 mmol) at -10 °C. The mixture was allowed to reach rt, and stirred at rt for 24 h. Both donor and acceptor decomposed.

Table 1.8: Ester hydrolysis of 115

A mixture of **115** (0.10 g, 0.23 mmol) and the base (0.01–0.10 M) in the solvent was stirred for 0.5–2.0 h cooled to 0 °C and neutralized with Amberlite IR-120 $H^+$by stirring for 20 min, filtered end concentrated. ^{1}H-NMR was recorded of the crude mixture.

CHAPTER 2

Synthesis of Potential Protein Bcl-X_L Antagonists by Regioselective Ring Opening Reactions of Enantiopure Oxabicycles

This chapter will introduce transition metal-catalyzed enantioselective and regioselective ring opening reactions of oxabicycles mainly by carbon-carbon bond formations. These methods will be utilized for synthesis of potential protein Bcl-X_L antagonists.

2.1 Antiapoptotic protein Bcl-X_L a potential drug target for cancer

Physiological or programmed cell death generally occurs by apoptosis, and when apoptosis is dysregulated it can contribute to many diseases, including cancer, autoimmunity and neurodegenerative disorders.[195] A reasonable estimate is that either too little or too much cell death contributes to half of the main medical illnesses for which adequate therapy is lacking. Consequently, a great interest in development of selective modulation of the apoptotic machinery has been the goal for more than two decades.[196]

The two apoptosis pathways of interest when looking for new drugs are the extrinsic pathway and the intrinsic pathway. The extrinsic pathway operates through cell-surface death receptors, and the intrinsic pathway is controlled by the Bcl-2 family of proteins and involves the disruption of mitochondrial membrane integrity in response to cellular insults or other danger signals. The Bcl-2 family of proteins consists of pro- and anti-apoptotic members, which regulate apoptosis by maintaining or disrupting the outer mitochondrial membrane. Upregulation of antiapoptotic members of this family including Bcl-X_L is observed in many cancers. This overexpression protects the cancer cells from the activation of apoptosis, favoring their proliferation and their survival to the anticancer compounds. Thus suppressing or inhibiting one or more of

the Bcl-2 family proteins may decrease cancer cell survival and cause cancer cell death instead.[197] A wide range af gene transfer, peptide inhibitor, antisense and other types of experiments have proven that Bcl-2 and Bcl-X$_L$ have a role in preventing tumor cell apoptosis.[198]

Experimental three-dimensional structures of some antiapoptotic proteins have shown the presence of a hydrophobic surface groove, formed by the Bcl-2 BH1, BH2 and BH3 regions (homology 1, 2 and 3), this is called the BH3 binding groove. This hydrophobic cleft constitutes the binding cavity for the proteins that promote apoptosis, such as Bax and Bak.[199] For proteins that promote cell death, only the BH3 region is required for activity.[200,201]

Several research groups have identified potent inhibitors of Bcl-2 and Bcl-X$_L$. One inhibitor worth mentioning is the natural compound Gossypol (see Scheme 2.1), a constituent of the cotton plant Gossypium.[202] However, several side effects of Gossypol limit its usefulness in using it as a proapoptotic drug. These side effects are probably due to Schiff's base-type reactions between the aldehydes on Gossypol and primary amines of proteins and nucleic acids making Gossypol less stable *in vivo*. Thus modifications of Gossypol were made to eliminate the undesired side effects one such analog is Apogossypol (see Scheme 2.1), which displays a proapoptotic activity in the hydrophobic groove on the surface of Bcl-X$_L$. The residues most affected by Apogossypol binding are located in the same pocket of Bcl-X$_L$, which is known to bind the BH3 peptides of endogenous Bcl-X$_L$ antagonist proteins. Apogossypol also showed cytotoxicity against primary leukemic cells freshly isolated from patients affected by chronic lymphocytic leukemia, which indicates potential applications of selective Bcl-2/Bcl-X$_L$ antagonists as chemosensitizers.[198]

Scheme 2.1: Structures of Gossypol and Apogossypol

The group of Andrea Basso have applied diversity orientated synthesis to a library of enantiopure oxabicyclic substrates creating a small library of compounds, which was subjected to a combined docking-NMR spectroscopy aterative approach leading to the identification of novel potential inhibitory scaffolds against Bcl-X$_L$.[199] Some of the most promissing results were found in products generated from metal catalyzed ring-opening of enatiopure oxabicycles. Initial docking studies of compound **1** in Figure 2.1 and similar coumpounds showed that the pyridine ring seems to be able to establish cation-π interactions with the Arg143 side chain, and the CH_2OH group seems to form hydrogen bonds with NH(CO) of Gly142 and with the Arg143 side chain of Bcl-X$_L$. As no hydrogen bonding between the pyridine nitrogen and the protein was observed, this functional group can be substituted by a phenyl ring. The benzyl group and/or phenyl ring in **1** was not directly involved in interactions with the protein, and these groups could therefore be removed and/or substituted with a different group without affecting

Figure 2.1: Structure of compound **1** used for docking studies

the activity but presumably resulting in compounds with increased solubility.[199]

This work will be further elaborated upon in section 2.2.3, as it was the starting point for the studies performed in the Mark Lautens group in collaboration with the Andrea Basso group.

2.2 Transition metal-catalyzed ring opening reactions of heterobicyclic alkenes

During the past 30 years there has been a drastic growth in the use of transition metal catalysts in synthetically important organic transformations.[203–206] One area, which has been widely explored since the end of the 1990th is the metal-catalyzed ring opening of heterobicyclic alkenes. These reactions can be carried out with a wide range of nucleophiles including hydride, stabilized and nonstabilized carbanions, alcohols, amines, and carboxylates. The transition metals employed in the ring-opening reactions are mainly copper, nickel, palladium, iridium and rhodium since these metals have led to a wide range of selective ring-cleaving reactions generating products in high yield with varying enantiomeric excess (ee) and diastereomeric ratio (dr).[206]

Three areas of research that have been investigated is, firstly, to utilize desymmetrization reactions of heterobicyclic meso compounds, secondly, asymmetric ring opening of racemic oxabicyclic alkenes and thirdly, regioselective ring opening of enantiopure oxabicycles. Much work have been done on the desymmetrisation reaction of meso compunds whereas the regioselective ring opening of enantiopure oxabicycles is a field which has hardly been explored.

The advances within the field of metal-catalyzed reactions have led to the discovery of new reactions that control the relative and absolute stereochemistry in cyclic and acyclic compounds. It is an efficient way to create new compounds containing many stereocenters in one step. These methods are of interest within drug discovery as many of the drugs known today contain alternating stereocenters.

2.2.1 Enantioselective ring opening desymmetrization reactions of meso compounds

The ring opening chemistry of oxabicyclic compounds underwent significant growth in the late 1970s as a result of the development of new methods to assemble the [3.2.1] core[207] and advances in the Diels-Alder reactions with furans.[208,209] Moreover, the oxabicyclic template has become increasingly common as a starting material in the preparation of both cyclic and acyclic compounds.[206,210,211] A lot of effort has been put into obtaining enantiomerically pure products from oxabicyclic precursors, including

Scheme 2.2: First reported enantioselective ring opening of an oxabicyclic alkene.

strategies of diastereo- or enantioselective cycloaddition with furan,[212,213] chemical and enzymatic esterification of meso oxabicycles,[214–216] and lastly, desymmetrization by ring opening of meso oxabicycles and azabicycles, which is the field of research explored in this thesis.

The first ring opening of the oxabicyclic system was reported in 1971, where Caple and co-workers opened an oxabenzonorbornadiene using BuLi and alkyl nucleophiles.[217] It was not before 1995 that examples of highly enantioselective ring opening reactions of oxabicyclic alkenes were reported. Here oxabicycle **2** was treated with a palladium(0)complex with various phosphine ligands (Scheme 2.2) and phenyl iodide or phenyl triflate as nucleophiles. The highest ee was observed using phenyl triflate as nucleophile with a Pd/BINAP complex. Here the minor product **3**, arising from carbopalladation and subsequent β-oxygen elimination was generated in 13% yield with a 96% ee. The major product **4**, arising from carbopalladation without following β-oxygen elimination, was obtained in 71% yield with a 64% ee. Using phenyl iodide and a Pd/(S,S)-Chiraphos complex the product **3** could be isolated as the only product, but in a low yield and close to be a racemic mixture of isomers.[218]

Since then asymmetric ring opening of oxabicycles has been well studied in many research groups giving the field a broad range of useful metals and nucleophiles. The

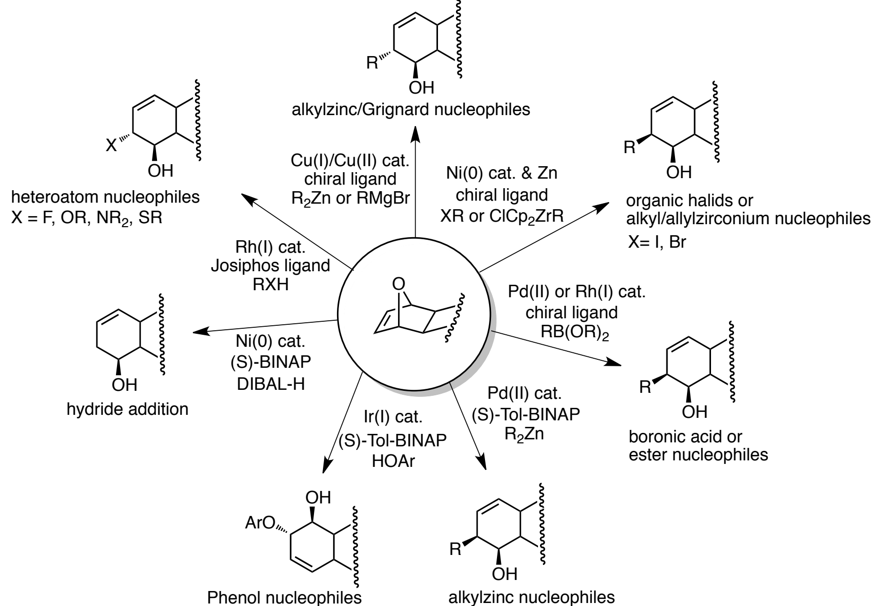


Figure 2.2: Catalysts and nucleophiles used for asymmetric ring opening of oxabicycles.

most efficient catalysts for the asymmetric ring opening of oxabicycles till date are copper,[219–222] iridium,[223] nickel,[224–228] palladium[206] and rhodium[206], as they each give rise to high yield and enantioselectivity with a given set of nucleophiles. The most utilized nucleophiles span from a hydride source,[224–226] through alcohols,[229–231] and amines,[229,232–234] to the recently employed fluoride[235] nucleophile. For products with C-C bond formation alkylzinc[219,236–238], and boronic acid/ester[239,240] nucleophiles have been most successful tried (Figure 2.2). As the desired nucleophiles for this study are boronic acid or ester nucleophiles, only prior work using these nucleophiles with palladium and rhodium catalysis will be further reviewed.

2.2.2 Asymmetric ring opening with boronic acids and boronic esters

Tremendous success has been achieved in the rhodium-catalyzed asymmetric 1,4-conjugate addition of organoboronic acids to electron-deficient olefins with significant advances gained since the first paper by Miyaura.[241] Highly enantioselective conjugate addition of boronic acids to olefins have been reported by Hayashi[242] where a common step is the carborhodation of the carbon-carbon double bond followed by hydrolysis of the organorhodium intermediate. This work established the basis for the rhodium catalyzed addition of an organoboron reagent to one of the two enantiotopic olefinic carbon atoms of an oxabicyclic substrate followed by β-elimination instead of hydrolysis.[239]

Excellent results were obtained for the addition of a wide variety of arylboronic acids to **5** in the presence of a $[Rh(COD)Cl]_2/PPF(^t Bu)_2$ catalytic system. It was found that the enantioselectivity of the asymmetric ring opening reaction increases with decreasing temperature. Also the reaction is faster when an excess of base is used, which is in agreement with the hypothesis that the use of base accelerates the transmetalation of the boronic acid to palladium in a Suzuki reaction.[243] The reaction requires a minimum amount of water for catalytic turnover and 1.2 eq. of $ArB(OH)_2$ is necessary for full conversion. They found the optimal conditions to be treatment of **5** with 2.5 mol% $[Rh(COD)Cl]_2$, 5.0 mol% $PPF(^t Bu)_2$, 1.2 eq. of the arylboronic acid, and 0.5 eq. of Cs_2CO_3 (5.0 M in H_2O) in THF at room temperature (scheme 2.3). Ring opening of oxabicycle **5** works equally well with both electronrich and electronpoor arylboronic acids, but it lacks the skill to perform the ring opening with ortho substituents on the boron-phenyl ring.

Applying the same conditions as used for **5** to the asymmetric ring opening of **7** leads to a complex mixture of products. Much better results were optained changing the arylboronic acid to the corresponding ethylene glycol ester. The yield was improved

Scheme 2.3: Asymmetric ring opening reactions of **5** with arylboronic acids.

Scheme 2.4: Asymmetric ring opening reaction of **7**.

from 20% to 78% using the ethylene glycol ester without loosing the selectivity (Scheme 2.4).[239]

As a challenge the amount of enantiopure oxabicycles provided by the Basso group was relatively small (30 mg - 500 mg), hence preliminary studies was carried out on 3 meso compounds to ensure that the same results as those described above could be achieved. Two of the oxabicycles attempted desymmetrized was the well described oxabicycles **5** (Scheme 2.3) and **7** (Scheme 2.4). The third oxabicycle (exo-cis-dimethyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate) **10** had not been exposed to desymmetrization reactions before and was chosen to ensure the reaction conditions would work in the presense of a methylester residue. For all reactions the boronic ester **8b** was used. In this case the ring opening of **5** and **7** gave similar results as those reported.[239] The ring opening of **10** proceeded faster than for both **5** and **7** with a ratio between the two enantiomers **11** and **12** of 4:1 (determined by ^1H-NMR) and in good yield (Scheme 2.5). These results proved that the reaction conditions could be used for oxabicycles containing an ester moiety.

The proposed mechanism for the asymmetric ring opening of oxabicyclic alkenes is given in scheme 2.6. The first step involves a transmetalation of the arylboronic acid to a rhodium(I)chloride or hydroxide to generate arylrhodium complex **13**, which then undergoes an exo-selective asymmetric carborhodation at the oxabicycle olefin to generate an organorhodium intermediate, **14**. Rhodium chelation of the olefin and the oxygen atom of the oxabicycle may help to contribute to the high exo selectivity. β-hydride elimination is not possible, as a result, β-oxygen elimination occurs to generate the ring opened rhodium alkoxide intermediate **15**, which upon hydrolysis liberates the product and regenarates the catalyst.[239]

Difficulties with the asymmetric ring opening of oxabicyclic alkenes using heteroarylboronic acids and a rhodium catalyst have been observed, as this leads to unopened addition products as well as oligomeric products.[240] An example of this is the ring opening of **7** with furan-3-boronic acid, which gave the desired product **16** in only

Scheme 2.5: Asymmetric ring opening of **10**.

Scheme 2.6: Propossed mechanism for rhodium catalyzed asymmetric ring opening reactions with arylboronic acid nucleophiles.

16% yield with 96% ee (Scheme 2.7). It might be possible to circumvent this problem using the corresponding ethylene glycol ester, however a palladium catalyst was used as an alternative in this case. Lautens and co-workers used a Pd(II) catalyst with Tol-BINAP as ligand to open **7** with furan-3-boronic acid, which enhanced the yield from 16 % to 82% yield but lowered the ee to 71% (Scheme 2.7). They observed poor enantioselectivities with the ferrocene-based ligands and a palladium catalyst, which gives excellent enantioselectivities in the analogous rhodium-catalyzed reactions.[240]

Scheme 2.7: Ring opening of **7** with furan-3-boronic acid using a Pd(II) or Rh(I) catalyst.

2.2.3 Stereoselective ring opening reactions of enantiopure oxabicycles

As can be seen from the previous sections enantioselective ring opening desymmetrization reactions of meso compounds have been widely explored, however regioselective ring opening of enantiopure oxabicycles is a field that is still to be investigated more thoroughly. The aim of the project in hand was to find a general method for regiose-

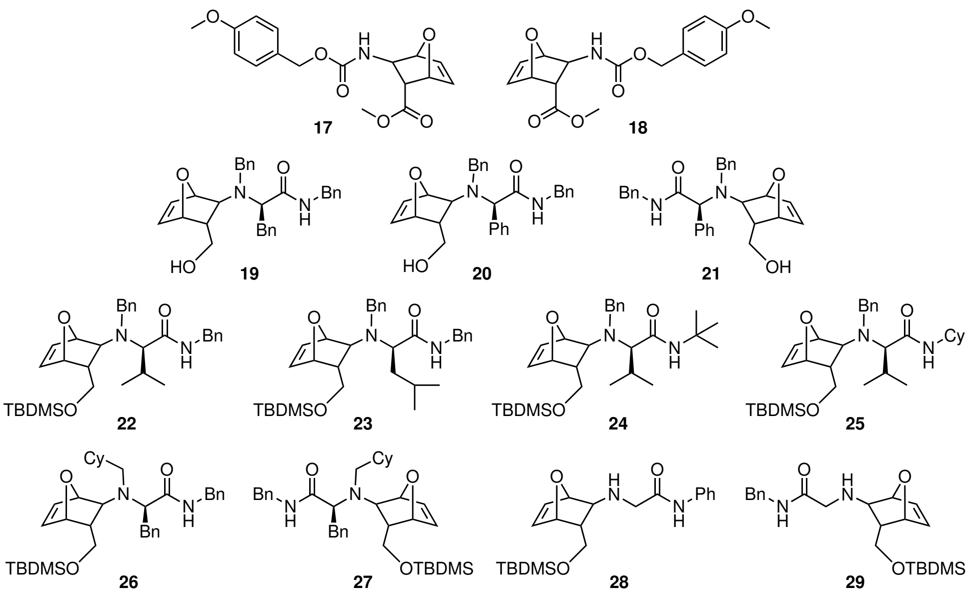


Figure 2.3: Enantiopure oxabicycles for stereoselective ring opening.

lective transition metal catalyzed ring opening of the enantiopure oxabicycles (shown i Figure 2.3) provided by the Basso group. Important features, which makes this work novel, are, firstly, the possibility for various functional groups attached to these substrates interfering with the reaction, secondly, as the bicyclic system is non symmetrical two distinct regioisomeric products should be possible to synthesize. In addition to this, because of the intrinsic chiral information in the substrates the need for an enantioselective catalyst should theoretically relinquish.

Prior to the collaboration between the Lautens group and the Basso group some work had been done by Basso *et al.*,[244] as they tried to open their synthesized enantiopure oxabicycles by metal catalysis. The ring opening was investigated using two different catalytic systems, one with a nickel/zinc catalyst and another using a palladium catalyst. When they used $Ni(PPh_3)_2Cl_2$, zinc dust, 3-iodoanisole in acetonitrile at 50 °C for the ring opening of **30**, they obtained a 60% yield of a 1:1 mixture of the

Scheme 2.8: Ring opening of **30** using a nickel catalyst.

Scheme 2.9: Ring opening of **34** using a palladium catalyst.

regioisomers **31** and **32** (Scheme 2.8). Besides the none excisting selectivity another problem is the stability of substrate **30** to the moderately high temperatures required, as the *retro* Diels-Alder adduct **33** was isolated in 10% yield.

To disfavor the *retro* Diels-Alder process the ester moiety was reduced to the alcohol, diminishing the strong electron withdrawing properties, thus kinetically disfavoring the *retro* cycloaddition process. Using the same conditions as used in scheme 2.8 on the altered oxabicycle **34** no reaction took place. Changing the catalytic system to $[Pd(C_6H_5CN)_2]Cl_2$, 1,3-bis(diphenylphosphino)propane, phenylboronic acid and an aqueous solution of Cs_2CO_3 in methanol at 60 °C resulted in the regioisomers **35** and **36** in 80% yield as a 7:3 mixture favoring **36** (Scheme 2.9).

These reaction conditions were utilized for similar oxabicycles and the outcome of these studies revealed that the reaction was highly substrate dependent. In one case only one regioisomer was isolated whilst other substrates yielded cyclic boronates as **37** (Figure 2.4) and similar, which could be hydrolyzed into the expected regioisomer. Using a 3-pyridylboronic acid as nucleophile instead of the phenylboronic acid did not result in a cyclic boronate. The explanation why cyclic boronates is only formed in some cases is supposedly the specific conformations adopted by the cyclohexenol derivative along with the reactivity of the boronic acids employed.

What is worth noting in these results is that the substituents of the bicyclic moiety could not direct the ring opening process to the selective formation of one regioisomer. Hence a solution might be to utilize an enantioselective catalyst.

Excellent results of regioselective ring opening of enantiopure oxabicycles were achieved by Webster *et al.*[246] using methanol as nucleophile. Here they employed a $Rh(COD)_2OTf$ catalyst and a josiphos ligand in THF at 80 °C resulting in the ring opening of **38**. One regioisomer was isolated exclusively in each case in high yield with retained ee (Scheme 2.10). When the (*R,S*)-PPF(^{t}Bu)$_2$ was employed **39** was the only product isolated and changing to (*S,R*)-PPF(^{t}Bu)$_2$ **40** was the only product isolated. These results imply that the enantiomeric ligand overrides the inherent preference of the substrate.

Figure 2.4: Structure of a cyclic boronate isolated after reactions where the same conditions as in Scheme 2.9 was used.

R,S-PPF-P(^{t}Bu)$_2$ (5.5 mol%)
Rh(COD)$_2$OTf (5 mol%)
MeOH, THF, 80 °C

38: 95% ee

39: 75% yield, 95% ee

S,R-PPF-P(^{t}Bu)$_2$ (5.5 mol%)
Rh(COD)$_2$OTf (5 mol%)
MeOH, THF, 80 °C

40: 90% yield, 94% ee

Scheme 2.10: Enantiopure oxabicycles for stereoselective ring opening.

In the present study oxabicycle **17** was chosen for the first attempts of stereoselective ring opening of oxabicycles **17**–**29**. As the ring opening of **10** showed encouraging results the same conditions were employed for the ring opening of **17**. Using the (S,R)-PPF(^{t}Bu)$_2$ ligand compound **41** was isolated in 61% yield as a crystaline compound, and a crystal structure was recorded to prove the stereochemistry, shown in Figure 2.5. Whereas using the (R,S)-PPF(^{t}Bu)$_2$ ligand compound **42** was isolated in 66% yield as an oil. To find the ratio between **41** and **42** a small sample of the crude reaction mixture was taken out for the two reactions. The two reaction mixtures were analyzed by HPLC giving the regioselectivities of the two diasteriomers shown in Scheme 2.11.

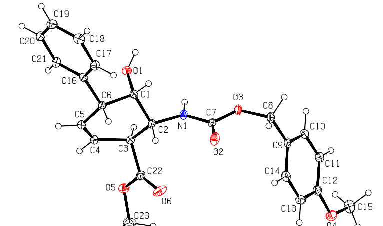


Figure 2.5: Crystal structure of **41**.

The ring opening occurred with *syn* stereoselectivity, as a result of exo attack of the nucleophile on the oxabicyclic unit, and is in accordance with the literature.[206] The selectivity of the attack with regard to the two carbon atoms in the alkene is the same as observed for other oxabicyclic ring opening reactions where a josiphos ligand is used. This result supports the discoveries made by Webster *et al.*[246] implying that the enantiomeric ligand overrides the inherent preference of the substrate.

Unfortunately implementing the same reaction conditions on the enantiomer **18** was unsuccesful. Alternating catalysts, ligands and solvents were employed to overcome this puzzling problem. As shown in Tabel 2.1, when using a Pd(II) catalyst and a Tol-BINAP ligand the regioselectivity is lovered remarkably, but the yield is increased (entry 7). Methanol was tried as a solvent since earlier studies had shown a faster reaction herein compared to THF,[240] thereby making it possible to run the reaction at lover temperatures and possibly giving higher enantioselectivity. This did not result in an improvement with the Rh catalyst as no product formation was observed (entry 3). The best results was obtained by using the initial reaction conditions as used for

Scheme 2.11: Regioselective ring opening of oxabicycle **17**.

17 while raising the temperature to 50 °C. Under these conditions the regioselectivity for each product (**43** and **44**) was 19:1 using either the (*R*,*S*)-PPF(^{t}Bu)$_2$ or (*S*,*R*)-PPF(^{t}Bu)$_2$ ligand (entry 9 and 10). As expected the enantiomer of **41** (compound **43**) was a crystaline product and was formed using (*R*,*S*)-PPF(^{t}Bu)$_2$ instead of (*S*,*R*)-PPF(^{t}Bu)$_2$. Similar findings was valid for the enantiomers **42** and **44**, which were both isolated as oils.

Table 2.1: Optimization of the regiooselective ring opening of **18**.

#	Catalyst	Ligand	% cat./lig	Temp.	Yield [%]	43:44
1[a]	[Rh(COD)Cl]$_2$	(*R*,*S*)-PPF(^{t}Bu)$_2$	5/10	rt	N.R.	–
2[a]	[Ir(COD)Cl]$_2$	(*R*,*S*)-PPF(^{t}Bu)$_2$	5/10	50 °C	N.R.	–
3[b]	[Rh(COD)Cl]$_2$	(*R*,*S*)-PPF(^{t}Bu)$_2$	5/10	rt	N.R.	–
4[a]	[Rh(COD)I]$_2$	(*R*,*S*)-PPF(^{t}Bu)$_2$	5/10	50 °C	Decomp.	–
5[a]	Pd(MeCN)$_2$Cl$_2$	(*R*,*S*)-PPF(^{t}Bu)$_2$	10/10	rt	**84**	**78:22**
6[b]	Pd(MeCN)$_2$Cl$_2$	(*R*,*S*)-PPF(^{t}Bu)$_2$	10/10	0 °C	Decomp.	–
7[b]	Pd(MeCN)$_2$Cl$_2$	*R*-Tol-BINAP	10/10	rt	**95**	**60:40**
8[a]	[Rh(COD)Cl]$_2$	*R*-Tol-BINAP	5/10	rt	N.R.	–
9[a]	[Rh(COD)Cl]$_2$	(*R*,*S*)-PPF(^{t}Bu)$_2$	5/10	50 °C	**69**	**95:5**
10[a]	[Rh(COD)Cl]$_2$	(*S*,*R*)-PPF(^{t}Bu)$_2$	5/10	50 °C	**65**	**5:95**

[a] THF [b] MeOH

Moving to oxabicycles with an alchohol moiety instead of an ester moiety on the oxabicyclic ring (**19**, **20** and **21**) turned out to be more challenging (table 2.2). No reaction was observed at room temperature independently on each sustrate and catalyst, entry 1, 4, 10 and 14 in Table 2.2. Running the reaction at 50 °C yielded **A** in low yield (29%) using the (*R*,*S*)-PPF(^{t}Bu)$_2$ ligand (entry 2) and **B** was isolated in 33% yield using the (*S*,*R*)-PPF(^{t}Bu)$_2$ ligand (entry 5). Unfortunately, the results were not reproducible, as a second reaction only resulted in isolation of starting material, which also turned out to be the case when running the reaction at 70 °C (entry 3). Changing the catalyst to [Rh(COD)OH]$_2$ made it possible to get reproducible ring opening of the oxabicycle **19**, unfortunately, still in very low yields (entry 7 and 8). This could

Table 2.2: Trials of stereoselective ring opening of **19**, **20** and **45**.

19: $R_1 = R_2 = Bn$
20: $R_1 = Ph$, $R_2 = Bn$
45: $R_1 = {}^iPr$, $R_2 = Cy$

8a or 8b; Catalyst (5 mol%), Ligand (10 mol%), THF, temp. → A + B

#	Sub.	Catalyst	Ligand	Temp.	Nu. (eq.)	yield [%]
1	**19**	$[Rh(COD)Cl]_2$	(R,S)-PPF$({}^t Bu)_2$	rt	**8b** (1.2 eq.)	N.R.
2	**19**	$[Rh(COD)Cl]_2$	(R,S)-PPF$({}^t Bu)_2$	50 °C	**8b** (1.2 eq.)	**29 (A)**
3	**19**	$[Rh(COD)Cl]_2$	(R,S)-PPF$({}^t Bu)_2$	70 °C	**8b** (1.2 eq.)	N.R.
4	**19**	$[Rh(COD)Cl]_2$	(S,R)-PPF$({}^t Bu)_2$	rt	**8b** (1.2 eq.)	N.R.
5	**19**	$[Rh(COD)Cl]_2$	(S,R)-PPF$({}^t Bu)_2$	50 °C	**8b** (1.2 eq.)	**33 (B)**
6	**19**	$[Rh(COD)Cl]_2$	(S,R)-PPF$({}^t Bu)_2$	50 °C	**8b** (2.2 eq.)	N.R.
7	**19**	$[Rh(COD)OH]_2$	(R,S)-PPF$({}^t Bu)_2$	50 °C	**8a** (1.2 eq.)	**20 (A)**
8	**19**	$[Rh(COD)OH]_2$	(R,S)-PPF$({}^t Bu)_2$	80 °C	**8a** (2.0 eq.)	**24 (A)**
9	**19**	$[Rh(COD)Cl]_2$	(R,S)-PPF$({}^t Bu)_2$	50 °C	**8b** (1.2 eq.)	Decomp.
10	**20**	$[Rh(COD)Cl]_2$	(S,R)-PPF$({}^t Bu)_2$	rt	**8b** (1.2 eq.)	N.R.
11	**20**	$[Rh(COD)Cl]_2$	(S,R)-PPF$({}^t Bu)_2$	35 °C	**8b** (1.2 eq.)	N.R.
12	**20**	$[Rh(COD)OH]_2$	(R,S)-PPF$({}^t Bu)_2$	rt	**8b** (1.2 eq.)	N.R.
13	**20**	$[Rh(COD)OH]_2$	(R,S)-PPF$({}^t Bu)_2$	80 °C	**8a** (2.0 eq.)	N.R.
14	**45**	$[Rh(COD)Cl]_2$	(R,S)-PPF$({}^t Bu)_2$	rt	**8b** (1.2 eq.)	N.R.
15	**45**	$[Rh(COD)Cl]_2$	(R,S)-PPF$({}^t Bu)_2$	50 °C	**8b** (1.2 eq.)	Decomp.

nevertheless, not be employed for ring opening of neither oxabicycle **20** nor **45**, hence it seemed impossible to use a rhodium catalyst here (entry 10–15). It was suspected that a problem might be interaction between the boron nucleophile and the alcohol moiety on the oxabicycle, however increasing the quantity of nucleophile to two eqvivalents did not result in a noteworthy increase in the yield (entry 6, 8 and 13). Since alcohols can be used as nucleophiles in asymmetric ring opening reactions with a rhodium catalyst,[229,230] a compeeting reaction could take place. The alcohol could either react inter- or intramolecular, as heteronucleophiles attack from the endo face and not from the exo face like phenylboron nucleophiles. Therefore the alcohol is placed in a position making an intramolecular ring opening possible creating a new oxabicyclic compound; an intramolecular attack, which to my knowledge has not been reported in literature with any rhodium catalyst.

These findings reinforced our suspicion that the alcohol moiety was problematic for the employed reaction conditions. Therefore, the research was continued by screening of the oxabicycles with a *t*-butyldimethylsilyl (TBDMS) protecting group on the alcohols (compounds **22-29** in Figure 2.3). It became evident after a few trials that a TBDMS protecting group was not suitable for the reaction conditions (Table 2.3). As none of the three different rhodium catalysts tried were able to catalyze the ring opening of **25** and **27**. Using $[Rh(COD)Cl]_2$ only starting material could be detected by NMR (entry 1) whereas $[Rh(COD)OH]_2$ and $Rh(COD)_2OTf$ also led to some decomposition of the starting material (entry 2-4). When using $[Rh(COD)OH]_2$ at 50 °C some TBDMS deprotected oxabicycle could be detected by NMR, while none was observed at 80 °C.

Table 2.3: Trials of stereoselective ring opening of **25** and **27**.

TBDMSO, Bn, O, N, Cy, H, **25**, or, Bn, N, H, O, Cy, N, O, Bn, OTBDMS, **27**, **8a** or **8b**, Catalyst (5 mol%), Ligand (10 mol%), THF, temp.

#	Sub.	Catalyst	Ligand	Temp.	Nu.(eq.)	Comment
1	**25**	$[Rh(COD)Cl]_2$	(R,S)-PPF(^{t}Bu)$_2$	50 °C	**8b** (1.2)	N.R.
2	**25**	$[Rh(COD)OH]_2$	(R,S)-PPF(^{t}Bu)$_2$	50 °C	**8b** (1.2)	decomp.[a]
3	**25**	$[Rh(COD)OH]_2$	(R,S)-PPF(^{t}Bu)$_2$	80 °C	**8a** (2.0)	decomp.
4	**27**	$Rh(COD)_2OTf$	(S,R)-PPF(^{t}Bu)$_2$	50 °C	**8b** (1.2)	decomp.

[a] TBDMS deprotection observed

As no promissing results could be achieved for oxabicycles **19**, **20** and **45** deprotection of the TBDMS-group is undesired. Another problem with the TBDMS-group is the size as it is a bulky group, which might prevent the ring opening to take place. Hence a new and smaller protecting group might circumvent the problem.

Modifications of the oxabicycles **19**, **20** and **45** were attempted by subjecting them to oxidizing conditions for aldehyde or acid formation. Mild oxidation conditions were chosen as the double bond in the oxabicycle easily could be cleaved under harsh oxidation conditions such as Cr(IV) or Mn(VII). Records of such, use Collins reagent (CrO_3, pyridine) on similar oxabicycles giving rise to opening of the ring at the alkene possition.[247] IBX, DMP and TEMPO was tried as oxidizing agents but none was succesfull. No conversion took place using TEMPO or IBX at rt, when the solvent was switched from DMSO to EtOAc and elevated temperatures (80 °C) resulted in decomposition in the case of IBX.

Exposing **45** to Dess-Martin periodinane at rt unfortunately resulted in decomposition although oxidation of a similar oxabicycle gave the aldehyde in 90% yield under the same reaction conditions.[248] What was possible to elucidate from crude NMR could be formation of an amine, which was formed by cleavage of the amine from the oxabicyclic ring. The crude NMR spectrum is shown in Figure 2.6 along with the predicted structure, the formation of the doublet structure for the two H-atoms of the benzyl group coupling with the amine H has been reported for similar structures.[249] All in all, it seems that the amine chain on the oxabicycles is to bulky to perform the oxidation under the conditions examined, and cleavage of the amine from the oxabicycle appear to be the only observed byproduct of the reactions tried. Other mild oxidizing agents might work but no more effort was put in this project.

Two new compounds were synthesized to overcome the problem with the hydroxy group; one with a methoxymethyl moiety (**46**) and one with an acetoxymethyl moiety (**47**). Oxabicycle **46** was synthesized because of the prior studies done on the desymmetrization of **5**.[239] Oxabicycle **47** was synthesized to introduce an ester group. This was done as good results were obtained for oxabicycles **17** and **18**.

The synthesis of **46** was more tedious than expected as the reaction did not run to compleetion at 0 °C and some decomposition took place at rt resulting in a modest yield of 22%. All product formed was subjected to the $[Rh(COD)OH]_2$ catalyst and

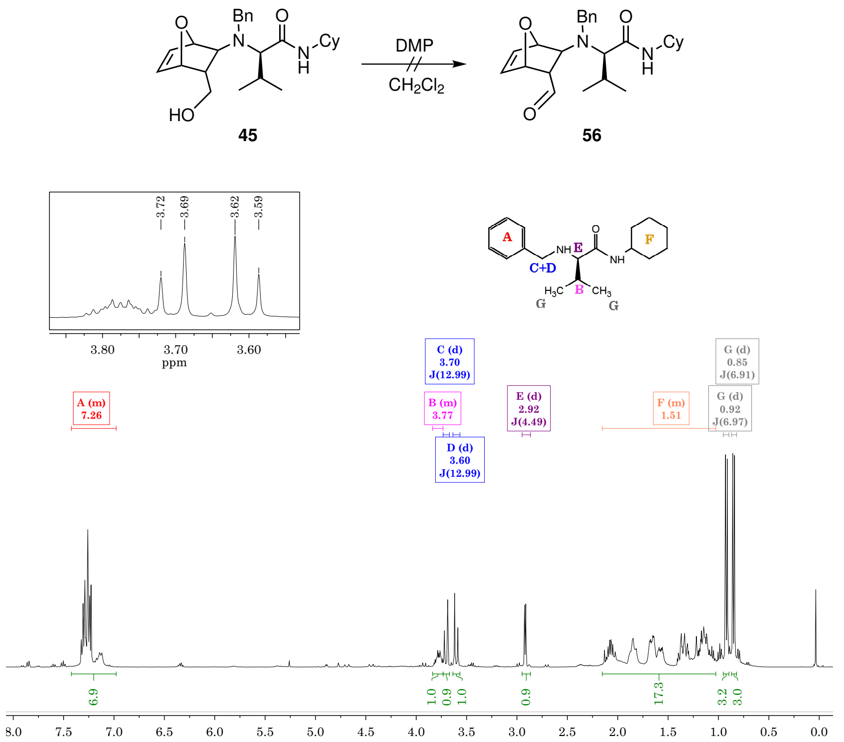


Figure 2.6: Crude ^{1}H-NMR spectrum from the oxidation of **45** using DMP.

the (R,S)-PPF(^{t}Bu)$_2$ only resulting in decomposition of the starting material, none of the starting material could be isolated (Scheme 2.12).

On the other hand, oxabicycle **47** was obtained after standard acetylation conditions in 90% yield and thereafter subjected to a $[Rh(COD)OH]_2$ or $[Rh(COD)Cl]_2$ catalyst. Good results were obtained using both catalysts as seen in Table 2.4. Using $[Rh(COD)OH]_2$ the reaction did not run to completion at rt after 15 h, nevertheless, only the starting material and **48** could be detected by NMR, and the amount of the mixture isolated after a short column corresponded to no loss of starting material. Heating the reaction to 65 °C gave a stereoselectivity of 1:9 favoring **48**, but the isolated yield of **48** was only 33% (entry 1 and 2). With a slight lowering of temperature and a change of ligand to the (S,R)-PPF(^{t}Bu)$_2$ ligand yielded a mixture of **48**

Scheme 2.12: Synthesis of **46** and conditions for the attempted ring opening of **46**.

Table 2.4: Ring opening of **47**.

#	Catalyst	Ligand	Temp.	Nu.(eq.)	Yield [%]	**47**:**48**:**49**[a]
1	$[Rh(COD)OH]_2$	(R,S)-PPF(^{t}Bu)$_2$	65 °C	**8a** (2.0)	**33** (**48**)	0:90:10
2	$[Rh(COD)OH]_2$	(R,S)-PPF(^{t}Bu)$_2$	rt	**8a** (2.0)	**100** (**47**+**48**)	60:40:0
3	$[Rh(COD)OH]_2$	(S,R)-PPF(^{t}Bu)$_2$	60 °C	**8a** (2.0)	**70** (**48**+**49**)	0:13:87
4	$[Rh(COD)Cl]_2$	(S,R)-PPF(^{t}Bu)$_2$	50 °C	**8b** (1.2)	**69** (**49**)	0:13:87

[a] predicted from crude NMR

and **49** in 70% with a ratio of 1:7 favoring **49** (entry 3). Replacing the catalyst with $[Rh(COD)Cl]_2$ and lowering the temperature to 50 °C resulted in ring opening with approximately the same regioselectivity, however it was possible to isolate **49** in 69% yield. The regioselectivities were estimated by ^{1}H-NMR.

Regrettably, my external stay in Toronto had come to an end just as these new discoveries were achieved. Newertheless the project was left at a stage where few alterations of the oxabicycles **19**–**29** would give access to a library of oxabicycles, which could be exposed to the same reaction conditions as for entry 4 in Table 2.4. Of course, first trying the conditions on one altered oxabicycle before make the changes on all oxabicycles assuring the conditions give similar results. Also a screening of temparature if the conditions work for the (R,S)-PPF(^{t}Bu)$_2$, and a different oxabicycle would be of interest to improve the regioselectivity.

While screening reaction conditions for oxabicycles **19**–**29** an attempt to increase the scope of nucleophiles for the ring opening of **17** and **18** using the pyridyl nucleophiles **50** and **51** was carried out. None of the reaction conditions employed led to ring opening of the oxabicycles **17** and **18** (Table 2.5). These findings were not totally un-

Table 2.5: Trials of regioselective ring opening of **17** and **18**, with pyridyl nucleophiles.

#	Sub.	Catalyst	Ligand	Temp.	Nu.(eq.)	Comment
1[a]	**17**	$[Rh(COD)Cl]_2$	(R,S)-PPF(^{t}Bu)$_2$	50 °C	**51** (1.3)	N.R.
2[a]	**18**	$[Rh(COD)Cl]_2$	(R,S)-PPF(^{t}Bu)$_2$	40 °C	**51** (1.3)	N.R.
3	**18**	$[Rh(COD)Cl]_2$	(R,S)-PPF(^{t}Bu)$_2$	40 °C	**50** (1.3)	N.R.
4	**18**	$[Rh(COD)OH]_2$	(S,R)-PPF(^{t}Bu)$_2$	80 °C	**50** (2.0)	N.R.
5	**17**	$Pd(MeCN)_2Cl_2$	(R,S)-PPF(^{t}Bu)$_2$	50 °C	**51** (1.2)	Decomp.[b]

[a] Cs_2CO_3 (5M) in H_2O(0.5 eq.) added [b] *retro* Diels-Alder pruduct observed

expected as Lautens and co-workers also had difficulties using a rhodium catalyst when employing heteronucleophiles.[240] What can be concluded from these results, which is of new knowledge, is the fact that changing to a pyridylboronic acid ethyleneglycol ester did not result in the opening of the oxabicycles. Using a palladium catalyst however did not result in the desired oxabicycle, only decomposition of the starting material took place and the *retro* Diels-Alder adduct was the only product identified. Thus changing to heteroatom containing nucleophiles would be a time consuming project as it would require screening of new catalysts, hense no more work was done on this part.

2.3 Summary and Outlook of Chapter 2

In an attempt to create a library of potential Bcl-X_L antagonists a range of enantiopure oxabicyles (**17**–**29** in Figure 2.3) were desired attempted for a regioselective ring opening. Enatiopure oxabicycles **17** and **18** were subjected to asymmetric ring opening reactions using a rhodium catalyst and a josiphos ligand yielding two sets of enantiomers in excellent regioselectivity and good yield.

Applying similar conditions to perform the ring opening of the oxabicycles with an alcohol moiety was not successful. Changing to different rhodium catalysts did not result in a satisfactory result, as the reactions were low yielding and not reproducible in all cases. Two plausible explanations for the lack of reactivity is either coordination of the hydroxy groups with the boron forming a new 6-membered cycle with the ring opened product, which is only possible for one of the two diastereomers. The other explanation for the lack of reactivity is that the alcohol might be a competing nucleophile.

Ring opening of oxabicycles with a TBDMS-protecting group on the alcohol moiety did not solve the problem with the reactivity. The general trend for these reactions was a mixture of starting material and decomposition of the starting material, and for one reaction deprotection of the TBDMS-group was observed, thus generating the undesired alcohol *in situ*.

These findings made it obvious that a different group than an alcohol was necessary, hence attempts were made to prepare three new oxabicycles, one with a methoxymethyl moiety **46**, one with a acetoxymethyl moiety **47** and one with a methylester moiety (unsuccessful synthesis). One trial of the ring opening on **46** only led to decomposition. Whereas asymmetric ring opening of **47** employing similar reaction conditions as for the ring opening of **18** yielded one regioisomer in good yield and regioselectivity. Whilst the other regioisomer was isolated in modest yield and good regioselectivity employing a $[Rh(COD)OH]_2$ catalyst.

Ring opening of **17** and **18** was attempted using a pyridylboronic acid nucleophile but none of the desired product could be isolated.

It was not possible to create a general asymmetric ring opening method for the enantiopure oxabicycles **17**–**29**, but for further studies it would be of interest to examine the reaction conditions found to work on **47** on other oxabicycles with the same acetoxymethyl moiety. If this was possible it would lead to a library of potential Bcl-X_L antagonists containing acetyl-groups, which could be hydrolysed into the alcohol leading to an even bigger library.

2.4 Experimental

General Experimental Conditions. Unless otherwise noted, reactions were carried out under argon atmosphere, in 2-5 mL vials or single-necked, round bottom flasks, with magnetic stirring. Air- or water-sensitive liquids and solutions were transferred via syringe. Organic solutions were concentrated by rotary evaporation at 23–40 °C under 53 mbar (house vacuum). Analytical thin layer chromatography (TLC) was performed with aluminum sheets pre-coated with silica gel 60F (Merck 5554). Visualization was done under a 254 nm UV light source and generally by immersion in a ceriumammoniumsulfate solution (1% cerium(IV)sulphate and 2.5% hexaammonium molybdate in a 10% sulfuric acid solution). Purification of reaction products was done by flash column chromatography on silica gel (SilicycleTM Ultra-Pure 230-400 mesh).
Reagents. Solvents were purchased from Sigma-Aldrich, Inc. Tetrahydrofuran and toluene were purified by distillation under N_2 from Na/benzophenone prior to use. Rhodium(I)catalysts, $Pd(MeCN)_2Cl_2$ and Tol-BINAP were purchased from Strem Chemicals Inc. and used as received. Josiphos ligands ((*S,R*)-PPF(^{t}Bu)$_2$ and (*R,S*)-PPF(^{t}Bu)$_2$) were generously provided by Solvias Inc. Compound **8b** was synthesized according to the procedure described in Meiland *et. al.*[250]
Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H-NMR) and carbon nuclear magnetic resonance spectra (^{13}C-NMR) were recorded with a Varian Mercury 400 (400 MHz/100 MHz) NMR spectrometer equipped with a Nalorac4N-400 probe, or a Bruker AvanceIII (400 MHz/100 MHz) NMR spectrometer equipped with BBOF probe using the residual solvent as the internal standard. Recorded chemical shifts are reported in parts per million (δ = scale) downfield from tetramethylsilane, and and all coupling constants (J) are expressed in Hz. High resolution mass spectras (HRMS) were obtained from Joel AccuTOF Direct Analysis in Real Time Time-of-Flight (DART). Melting points were obtained using Fisher-Johns melting point apparatus and are uncorrected. High performance liquid chromatography (HPLC) analyses were carried out on a HP 1100 Series modular system from Agilent Zorbax, using a gradient from H_2O/MeCN 9:1 to pure MeCN in 15 min (flow 0.8 mL/min) with a Zorbax SB-C18 column (4.6 mm × 50 mm, 3.5 μm). The crystal structure was obtained using Computer-controlled Nonius Kappa-CCD system, Oxford Cryostream variable temperature apparatus. Compounds with clubs (♣) are new.

4-Pyridylboronic acid ethyleneglycol ester (51)[250]

Ethyleneglycol (25 μL, 4.5 mmol) was added to a suspension of 4-pyridylboronic acid (50 mg, 0.41 mmol) in toluene (4.5 mL) and the reaction mixture stirred overnight at 150 °C with a Dean-Stark apparatus. The precipitate was filtered off and washed with toluene, which resulted in a light rosa solid product (44.9 mg, 74%). ^{1}H-NMR (400 MHz, D_2O): δ = 8.45 (d, J = 4.6 Hz, 2H), 8.06 (d, J = 4.6 Hz, 2H), 3.68 (s, 4H) ^{13}C-NMR (100 MHz, D_2O): δ = 138.1, 129.1, 62.5.

Exo-cis-dimethyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (10)[251]

H_2SO_4 / MeOH; 52 → 10 (CO_2Me, CO_2Me)

Compound **10** (2.0 g, 12 mmol) was dissolved in a solution of H_2SO_4 (1 mol eq.) in MeOH (20 mL) and stirred overnight. After evaporation of a few mL MeOH, a precipitate was observed and the reaction mixture was left to crystallize. The white crystals were washed thoroughly with MeOH and dried under vacuum (1.9 g, 73%). M.p. 116–117 °C. M.p. lit. 119 °C.[251] ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 6.44$ (t, J = 1.0 Hz, 2H), 5.25 (t, J = 1.0 Hz, 2H), 3.70 (s, 6H), 2.81 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): $\delta = 172.0, 136.7, 80.5, 52.4, 47.1.$[252]

***R*-2-(*N*-Benzyl-*N*((1*R*,2*S*,3*R*,4*S*)-3-(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-yl)amino)-*N*-(cyclohexyl)-3-metyltetraamide (45) ♣**

TBAF / THF, 0 °C; 25 (TBDMSO, Bn, N, Cy, H) → 45 (HO)

TBAF (1 M in THF, 0.15 mL, 0.15 mmol) was added to a solution of **25** (52.7 mg, 0.10 mmol) in THF (1.35 mL) at 0 °C. The reaction was stirred for 1 h (TLC showed full conversion of S.M.), solvent evaporated and the product was purified by flash column chromatography (SiO_2, EtOAc/Hexanes 1:2), which resulted in a clear glassy oil (40.4 mg, 98%). ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 7.40$–7.14 (m, 5H), 6.36 (qd, J = 5.8, 1.5 Hz, 2H), 5.87 (d,J = 7.9 Hz, 1H), 4.92 (d, J = 4.5 Hz, 1H), 4.80 (d, J = 1.4 Hz, 1H), 4.48 (d,J = 15.7 Hz, 1H), 3.90–3.71 (m, 1H), 3.65 (d, J = 15.7 Hz, 1H), 3.48 (dd, J = 10.8, 7.1 Hz, 1H), 3.22 (dd, J = 10.9, 7.5 Hz, 2H), 2.89 (d, J = 3.2 Hz, 1H), 2.74 (d, J = 10.3 Hz, 1H), 2.38 (dd, J = 7.0, 3.5 Hz, 1H), 2.21–2.07 (m, 1H), 1.97 (dd,J = 12.1, 3.5 Hz, 1H), 1.85 (dd, J = 12.5, 3.6 Hz, 1H), 1.78–1.50 (m, 3H), 1.47–1.26 (m, 2H), 1.26–0.97 (m, 6H), 0.83 (d, J = 6.5 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): $\delta = 172.5$, 140.6, 136.0, 135.6, 128.5, 128.1, 126.8, 84.8, 79.6, 68.7, 65.5, 62.3, 52.1, 47.9, 43.5, 33.3, 33.1, 27.9, 25.6, 25.0, 24.9, 20.6, 19.9. MS (DART, *m/z*): calcd. ($C_25H_37N_2O_3^+$) 413.2799, found 413.2805.

***R*-2-(*N*-Benzyl)-*N*-((1*R*,2*S*,3*R*,4*S*)-3-(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-yl)amino)-*N*-benzyl-3-metyltetraamide (53) ♣**

TBAF / THF, 0 °C; 22 (TBDMSO, Bn, N, Bn, H) → 53 (HO)

TBAF (1 M in THF, 0.45 mL, 0.45 mmol) was added to a solution of **22** (188 mg, 0.307 mmol) in THF (2.5 mL) at 0 °C. The reaction mixture was stirred for 15 min (TLC showed full conversion of S.M.), solvent evaporated and the product was purified by flash column chromatography (SiO_2, EtOAc/Hexanes 2:3), which resulted in a clear

glassy oil (129 mg, 84%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.46–7.11 (m, 10H), 6.37 (s, 1H), 6.33 (dd, J = 5.9, 1.8 Hz, 1H), 6.28 (dd, J = 5.9, 1.4 Hz, 1H), 4.77 (s, 1H), 4.69–4.52 (m, 2H), 4.45 (d, J = 15.6 Hz, 1H), 4.27 (dd, J = 14.3, 5.1 Hz, 1H), 3.60 (d, J = 15.6 Hz, 1H), 3.42 (dd, J = 11.0, 6.7 Hz, 1H), 3.03 (s, 1H), 2.87 (d, J = 10.1 Hz, 1H), 2.77 (d, J = 3.3 Hz, 1H), 2.27–2.14 (m, 1H), 2.14–2.04 (m, 1H), 1.91 (s, 1H), 1.12 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 173.2, 140.3, 138.6, 136.0, 135.5, 128.9, 128.8, 128.5, 128.2, 127.8, 126.8, 84.3, 79.6, 68.1, 65.5, 62.2, 52.1, 43.7, 43.5, 27.9, 20.7, 20.0.

***R*-2-(*N*-Benzyl)-*N*-((1*R*,2*S*,3*R*,4*S*)-3-(acetoxymethyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-yl)amino)-*N*-benzyl-2-phenylethanamide (47) ♣**

Acetylchloride (60 μL, 0.84 mmol) was added dropwise to a cold solution (0 °C) of **20** (50 mg, 0.110 mmol) and pyridine (50 μL) in CH_2Cl_2 (2 mL). The icebath was removed and the mixture stirred at rt for 3 h, solvent evaporated and the product was purified by flash column chromatography (SiO_2, EtOAc/Hexanes 1:3) which resulted in a clear glassy oil (49.0 mg, 90%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.83 (t, J = 5.8 Hz, 1H), 7.50–7.13 (m, 15H), 6.26 (dd, J = 5.8, 1.4 Hz, 1H), 6.12 (dd, J = 5.8, 1.8 Hz, 1H), 4.79 (d, J = 4.3 Hz, 1H), 4.62–4.49 (m, 2H), 4.41 (dd, J = 14.6, 5.4 Hz, 1H), 4.26 (d, J = 14.6 Hz, 1H), 4.08 (dd, J = 11.4, 4.6 Hz, 1H), 3.70–3.45 (m, 3H), 2.74 (d, J = 4.6 Hz, 1H), 2.35 (m, 1H), 2.03 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 171.4, 170.8, 139.0, 138.5, 137.1, 135.4, 134.6, 130.5, 128.9, 128.9, 128.8, 128.6, 128.3, 127.9, 127.6, 127.5, 79.8, 78.9, 66.8, 66.1, 64.1, 50.6, 43.5, 42.8, 21.0.

***R*-2-(*N*-Benzyl)-*N*-((1*R*,2*S*,3*R*,4*S*)-3-(methoxymethyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-yl)amino)-*N*-benzyl-2-phenylethanamide (46) ♣**

NaH (6.0 mg, 60% suspension in oil, 0.15 mmol) was weighed into an oven dried flask and covered by dry THF (0.5 mL). Compound **20** (50 mg, 0.11 mmol) in THF (0.5 mL) was added dropwise by syringe at 0 °C and the mixture was stirred for 1 h whereupon MeI (0.0075 mL, 0.12 mmol) was added. The mixture was stirred for an additional hour and TLC revealed a lot of starting material left. Additional NaH (6.0 mg, 60% suspension in oil, 0.15 mmol) was added and the mixture stirred for 15 min, where MeI (0.010 mL, 0.16 mmol) was added. Additional MeI (0.010 mL, 0.16 mmol) was added after stirring for 1 h and the mixture was left to stir overnight at rt. No starting material observed, product purified by flash column chromatography (SiO_2, EtOAc/Hexanes 1:4) resulting in a clear oil (11.5 mg, 22%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.45–8.17 (m, 1H), 7.59–7.03 (m, 15H), 6.15 (dd, J = 5.8, 1.4 Hz, 1H),

6.08 (dd, J = 5.8, 1.8 Hz, 1H), 4.88–4.64 (m, 2H), 4.54 (s, 1H), 4.39–4.15 (m, 2H), 3.56 (d, J = 14.8 Hz, 1H), 3.38 (s, 1H), 3.29 (dd, J = 9.0, 5.7 Hz, 1H), 3.16 (s, 3H), 3.08 (t, J = 9.0 Hz, 1H), 2.90 (d,J = 4.2 Hz, 1H), 2.40 (td, J = 9.6, 4.5 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 171.7, 139.3, 139.0, 137.3, 136.2, 134.2, 130.5, 128.9, 128.8, 128.7, 128.7, 128.2, 127.5, 127.4, 127.3, 80.3, 78.8, 75.6, 67.4, 65.0, 59.3, 50.6, 43.2, 42.8.

Ring opening of exo-cis-dimethyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (11 + 12)

A 5 mL flask was charged with $[Rh(COD)Cl]_2$ (2.9 mg, 0.0059 mmol), *R,S*-PPF(^{t}Bu)$_2$ (6.4 mg, 0.012 mmol) and **10** (50 mg, 0.236 mmol). The flask was flushed with argon for 15 min whereupon phenylboronic acid ethyleneglycol ester (42 mg, 0.283 mmol) as a THF solution (2 mL) was added by syringe. This was followed by Cs_2CO_3 (5 M) in H_2O (24 μL) and the reaction mixture was stirred at rt overnight. The solvent was evaporated and the product was purified by flash column (SiO_2, EtOAc/Hexanes 1:4) resulting in a mixture of two diasteriomers with a 1:4 ratio between minor and major product (49 mg, 71%). Compound **11**: ^{1}H-NMR (400 MHz, $CDCl_3$): 7.44–7.17 (m, 5H), 6.05 (ddd, J = 10.1, 4.8, 2.8 Hz, 1H), 5.90 (ddd, J = 10.1, 3.3, 2.0 Hz, 1H), 4.61–4.51 (m, 1H), 3.75 (s, 3H), 3.72–3.67 (m, 4H), 3.61 (td, J = 5.1, 2.8 Hz, 1H), 3.51 (d, J = 5.6 Hz, 1H), 3.13 (dd, J = 6.4, 1.9 Hz, 1H). Compound **12**: ^{1}H-NMR (400 MHz, $CDCl_3$): 7.44–7.17 (m, 5H), 5.97 (dt, J = 10.2, 2.6 Hz, 1H), 5.83–5.73 (m, 1H), 4.50–4.43 (m, 1H), 3.92–3.82 (m, 1H), 3.77 (s, 3H), 3.72 (s, 3H), 3.30 (d, J = 10.8 Hz, 1H), 1.55 (dd, J = 3.0, 1.2 Hz, 1H).

Regioselective ring opening of enantiopure oxabicycles

(1*S*,4*S*,5*S*,6*R*)-Methyl 5-hydroxy-6-((((4-methoxy)benzyloxy)carbonyl)amino)-4-phenyl-cyclohex-2-enecarboxylate (41) ♣

A 3 mL flask was charged with $[Rh(COD)Cl]_2$ (2.2 mg, 0.0045 mmol), (*S,R*)-PPF(^{t}Bu)$_2$ (4.9 mg, 0.009 mmol), oxabicycle **17** (50 mg, 0.150 mmol) and flushed with argon for 30 min. Compound **8b** (27 mg, 0.18 mmol) dissolved in THF (1.5 mL) was added by syringe, followed by Cs_2CO_3 (5 M) in H_2O (15 μL). The mixture was stirred at rt overnight whereupon TLC showed full conversion of starting material. Crude HPLC showed 3% of minor exo-diastereomeric product **42**. Purification by flash column chromatography (SiO_2, EtOAc/Hexanes 3:7) resulted in a white crystalline product (38

mg, 61%). Structure verified by x-ray. Mp: 134–135 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.37 (t, J = 7.3 Hz, 2H), 7.33–7.26 (m, 3H), 7.21 (d, J = 7.2 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 5.86 (d, J = 10.2 Hz, 1H), 5.79 (d, J = 10.2 Hz, 1H), 5.25 (d,J = 8.9 Hz, 1H), 5.03 (s, 2H), 4.35 (t, J = 10.0 Hz, 1H), 4.08 (s, 1H), 3.84 (s, 1H), 3.80 (s, 3H), 3.71 (s, 3H), 3.43–3.34 (m, 1H), 1.46 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 173.5, 159.7, 156.0, 139.6, 130.1, 129.0, 128.7, 128.7, 128.0, 127.6, 125.2, 114.0, 71.5, 66.8, 55.4, 53.0, 52.5, 47.6, 44.5. MS (DART, *m/z*): calcd. ($C_{23}H_{26}NO_6^+$) 412.1755, found 412.1773

(1*R*,2*S*,5*R*,6*R*)-Methyl 6-hydroxy-2-((((4-methoxy)benzyloxy)carbonyl)amino)-5-phenyl-cyclohex-2-enecarboxylate (42) ♣

A 3 mL flask was charged with $[Rh(COD)Cl]_2$ (2.2 mg, 0.0045 mmol), (*R,S*)-PPF(^{t}Bu)$_2$ (4.9 mg, 0.009 mmol), oxabicycle **17** (50 mg, 0.150 mmol) and flushed with argon for 30 min. Compound **8b** (27 mg, 0.18 mmol) as a THF solution (1.5 mL) was added by syringe followed by Cs_2CO_3 (5 M) in H_2O (15 μL). The mixture was stirred at rt overnight whereupon TLC showed full conversion of starting material. Crude HPLC showed 5% of minor exo-diastereomeric product **41**. Purification by flash column chromatography (SiO_2, EtOAc/Hexanes 3:7) resulted in a clear oil (41 mg, 66%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.37 (t, J = 7.2 Hz, 2H), 7.33–7.27 (m, 3H), 7.25 (d, J = 9.1 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 5.92 (d, J = 9.8 Hz, 1H), 5.80 (d, J = 8.5 Hz, 1H), 5.35 (d, J = 9.1 Hz, 1H), 5.15–4.95 (m, 2H), 4.73 (s, 1H), 4.33 (m, 1H), 3.80 (s, 3H), 3.75–3.57 (m, 4H), 2.80 (t, J = 7.5 Hz, 1H), 1.68 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 172.4, 159.7, 155.8, 137.7, 130.1, 130.0, 128.8, 128.7, 128.6, 128.1, 127.8, 114.0, 69.2, 66.8, 55.4, 52.3, 50.0, 48.7, 45.2.

(1*R*,4*R*,5*R*,6*S*)-Methyl 5-hydroxy-6-((((4-methoxy)benzyloxy)carbonyl)amino)-4-phenyl-cyclohex-2-enecarboxylate (43) ♣

A 2 mL flask was charged with $[Rh(COD)Cl]_2$ (1.1 mg, 0.00225 mmol), (*R,S*)-PPF(^{t}Bu)$_2$ (2.5 mg, 0.0045 mmol), oxabicycle **18** (25 mg, 0.075 mmol) and flushed with argon for 30 min. Compound **8b** (13.5 mg, 0.090 mmol) dissolved in THF (1 mL) was added by syringe followed by Cs_2CO_3 (5 M) in H_2O (8 μL). The mixture was stirred at rt overnight whereupon TLC showed full conversion of starting material. Crude HPLC showed 5% of minor exo-diastereomeric product **44**. Purification by flash column chro-

matography (SiO_2, EtOAc/Hexanes 3:7) resulted in a white crystalline product (21 mg, 69%). Mp: 133.5–135 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.36 (t, J = 7.2 Hz, 2H), 7.32–7.24 (m, 3H), 7.20 (d, J = 7.2 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 5.85 (d, J = 10.2 Hz, 1H), 5.78 (d, J = 10.2 Hz, 1H), 5.31 (d, J = 9.3 Hz, 1H), 5.02 (s, 2H), 4.35 (t, J = 9.9 Hz, 1H), 4.07 (s, 1H), 3.83 (s, 1H), 3.80 (s, 3H), 3.70 (s, 3H), 3.44–3.32 (m, 1H), 1.53 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 173.5, 159.7, 156.0, 130.1, 129.1, 128.7, 128.1, 127.6, 125.2, 114.1, 71.5, 66.8, 55.5, 53.0, 52.5, 47.6, 44.6.

(1*S*,2*R*,5*S*,6*S*)-Methyl 6-hydroxy-2-((((4-methoxy)benzyloxy)carbonyl)amino)-5-phenyl-cyclohex-2-enecarboxylate (44) ♣

A 2 mL flask was charged with $[Rh(COD)Cl]_2$ (1.1 mg, 0.00225 mmol), (*S,R*)-PPF(^{t}Bu)$_2$ (2.5 mg, 0.0045 mmol), oxabicycle **18** (25 mg, 0.075 mmol) and flushed with argon for 30 min. Compound **8b** (13.5 mg, 0.090 mmol) dissolved in THF (1 mL) was added by syringe followed by Cs_2CO_3 (5 M) in H_2O (8 μL). The mixture was stirred at rt overnight whereupon TLC showed full conversion of starting material. Crude HPLC showed 5% of minor exo-diastereomeric product **43**. Purification by flash column chromatography (SiO_2, EtOAc/Hexanes 3:7) resulted in a clear oil (20 mg, 65%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.37 (t, J = 7.2 Hz, 2H), 7.34–7.28 (m, 3H), 7.25 (d, J = 8.8 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 5.93 (d, J = 10.0 Hz, 1H), 5.80 (d, J = 8.4 Hz, 1H), 5.28 (d, J = 9.0 Hz, 1H), 5.13–4.98 (m, 2H), 4.73 (s, 1H), 4.34 (dd, J = 13.2, 6.1 Hz, 1H), 3.81 (s, 3H), 3.75–3.57 (m, 4H), 2.80 (t, J = 7.4 Hz, 1H), 1.59 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 172.3, 159.8, 155.8, 137.8, 130.2, 130.0, 128.9, 128.7, 128.6 128.1, 127.9, 114.1, 69.2, 66.8, 55.5, 52.4, 50.0, 48.6, 45.2.

General procedure for ring opening of oxabicycles containing free alcohols, TBDMS-protected alcohols and acetyl-protected alcohols using $[Rh(COD)Cl]_2$ as catalyst

A 2 mL flask charged with $[Rh(COD)Cl]_2$ (1.0 mg, 0.0020 mmol) and Josiphos ligand (2.2 mg, 0.0040 mmol), was flushed with argon for 5 min whereupon THF (0.5 mL) was added and the mixture stirred for 5 min under argon atmosphere. The oxabicycle (0.0333 mmol) and a phenylboronic acid ethyleneglycol ester (1.2 eq.) was dissolved in THF (1 mL) and the cat./ligand THF solution was added followed by Cs_2CO_3 (5 M) in H_2O (0.5 eq.) The mixture was left to stir overnight at varying temperatures.

(1S,2R,5S,6S)-2-(Benzyl((R)-2-(benzylamine)-2-oxo-1-phenylethyl)amino)-6-hydroxy-5-phenyl-cyclohex-2-ene methyl acetate (49) ♣

Colorless oil (13 mg, 69%) (HPLC of crude showed 13 % of **48**). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.50–7.44 (m, 2H), 7.43–7.17 (m, 16H), 7.17–7.10 (m, 2H), 6.92 (s, 1H), 5.85 (ddd, J = 10.2, 4.5, 2.1 Hz, 1H), 5.79 (d, J = 10.4 Hz, 1H), 4.67 (s, 1H), 4.43–4.29 (m, 2H), 4.24 (dd, J = 11.2, 2.4 Hz, 1H), 4.15 (dd, J = 14.5, 5.4 Hz, 1H), 4.08 (d, J = 14.5 Hz, 1H), 3.87–3.76 (m, 1H), 3.74–3.64 (m, 2H), 3.61 (dd, J = 9.7, 1.9 Hz, 1H), 2.05 (d, J = 7.4 Hz, 1H), 1.87 (s, 3H), 1.79 (tt, J = 10.2, 2.7 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 172.0, 171.7, 139.9, 138.1, 138.0, 137.1, 130.4, 130.2, 130.0, 128.9, 128.8, 128.8, 128.7, 128.5, 128.4, 128.1, 127.6, 127.4, 68.0, 61.1, 56.8, 53.8, 46.4, 43.6, 40.3, 21.1.

General procedure for ring opening of oxabicycles containing free alcohols, TBDMS-protected alcohols and acetyl-protected alcohols using $[Rh(COD)OH]_2$ as catalyst

A 2 mL flask charged with $[Rh(COD)OH]_2$ (0.9 mg, 0.0020 mmol) and Josiphos ligand (2.2 mg, 0.0040 mmol), was flushed with argon for 5 min whereupon THF (0.5 mL) was added and the mixture stirred for 5 min under argon atmosphere. The oxabicycle (0.0333 mmol) and a phenylboronic acid ethyleneglycol ester (1.2 eq.) or phenylboronic acid (2.0 eq.) was dissolved in THF (1 mL) and the cat./ligand THF solution was added. The mixture was left to stir overnight at varying temperatures.

(1R,4R,5R,6S)-6-(Benzyl((R)-2-(benzylamine)-2-oxo-1-phenylethyl)amino)-5-hydroxy-4-phenyl-cyclohex-2-ene methyl acetate (48) ♣

Colorless oil (6.3 mg, 33%) (HPLC of crude showed 10 % of **49**). Only ^{1}H-NMR spectrum recorded. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.51–7.35 (m, 5H), 7.35–7.15 (m, 13H), 6.97 (d, J = 7.1 Hz, 2H), 6.86 (t, J = 5.6 Hz, 1H), 5.87–5.79 (m, 1H), 5.56 (dd, J = 10.1, 1.9 Hz, 1H), 4.65 (s, 1H), 4.55 (dd, J = 10.9, 3.6 Hz, 1H), 4.40–4.29 (m, 3H), 4.26 (dd, J = 10.8, 6.1 Hz, 1H), 3.74 (d, J = 14.3 Hz, 1H), 3.44 (s, 1H), 3.36 (d, J = 10.9 Hz, 1H), 3.24 (s, 1H), 3.06–2.97 (m, 1H), 2.00 (s, 3H), 1.11 (d, J = 3.2 Hz, 1H).

General procedure using 4-pyridylborinic acid or ester as nucleophile

A 2 mL flask charged with catalyst (5 mol%) and Josiphos ligand (10 mol%) was flushed with argon for 5 min whereupon THF (0.5 mL) was added and the mixture stirred for 5 min under argon atmosphere. The oxabicycle (0.0375 mmol) and **50** or **51** was dissolved in THF (1 mL) and the cat./ligand THF solution was added followed by Cs_2CO_3 (5 M) in H_2O (0.5 eq.) when $[Rh(COD)Cl]_2$ and **51** were used. The mixture was left to stir overnight at varying temperatures.

Representative procedure for the in situ exchange of chloride to iodide ligands:[232]

To a flame-dried flask under argon atmosphere was added $[Rh(COD)Cl]_2$ (2.5 mg, 0.005 mmol), (*R,S*)-PPF(^{t}Bu)$_2$ (6.0 mg, 0.011 mmol) and THF (2 mL), the mixture stirred at rt for 5 min resulting in a dark red solution. In a separate flame-dried flask was added AgOTf (5.5 mg, 0.02 mmol). The rhodium-phosphine solution was transferred to the flask containing AgOTf via syringe resulting in white precipitate. This mixture was stirred at rt for 5 min prior to its transfer to a flame-dried rb flask containing TBAI (11 mg, 0.03 mmol). After stirring for additional 5 min the red-brown solution was ready for use.

Attempted synthesis of compound 43 and 44

Compound **18** (12.5 mg, 0.0375 mmol) and **8b** (6.7 mg, 0.045 mmol) was dissolved in THF (0.5 mL) and stirred under argon atmosphere for 5 min whereupon the $[Rh(COD)I]_2$-josiphos solution (0.8 mL) was added, followed by Cs_2CO_3 (5 M) in H_2O (0.5 eq.). The mixture was heated to 50 °C and it turned brown after 10 min, the mixture was left to stir overnight at 50 °C. TLC revealed S.M. and biproduct. No product could be detected by NMR.

Attempted synthesis of compound 43 and 44

Compound **18** (12.5 mg, 0.0375 mmol) and **8a** (6.0 mg, 0.049 mmol) was dissolved in THF (0.5 mL) and stirred under argon atmosphere for 5 min whereupon $[Rh(COD)I]_2$-

josiphos solution (0.8 mL) was added. The mixture was heated to 50 °C and it turned orange after 10 min, the mixture was left to stir overnight at 50 °C. TLC revealed S.M. and biproduct. No product could be detected by NMR.

Failed attempts on oxidizing oxabicycles

Attempted synthesis of compound 54[253]

Compound **20** (10 mg, 0.0022 mmol) was dissolved in DMSO (0.1 mL) and IBX was added (6 mg, 0.021 mmol) at 0 °C. The mixture was stirred for 15 min, at which time TLC showed no conversion of S.M. The mixture was stirred for additional 4 h at rt, but still no reaction. Filtered through a pad of silicagel and washed with ether before the starting material was isolated.

Attempted synthesis of compound 55

Compound **19** (48 mg, 0.102 mmol) was dissolved in EtOAc (4 mL) and IBX (31 mg, 0.11 mmol) was added. The mixture was refluxed for 3 h, after which it had turned brown and NMR showed no product formation, but decomposition of starting material.

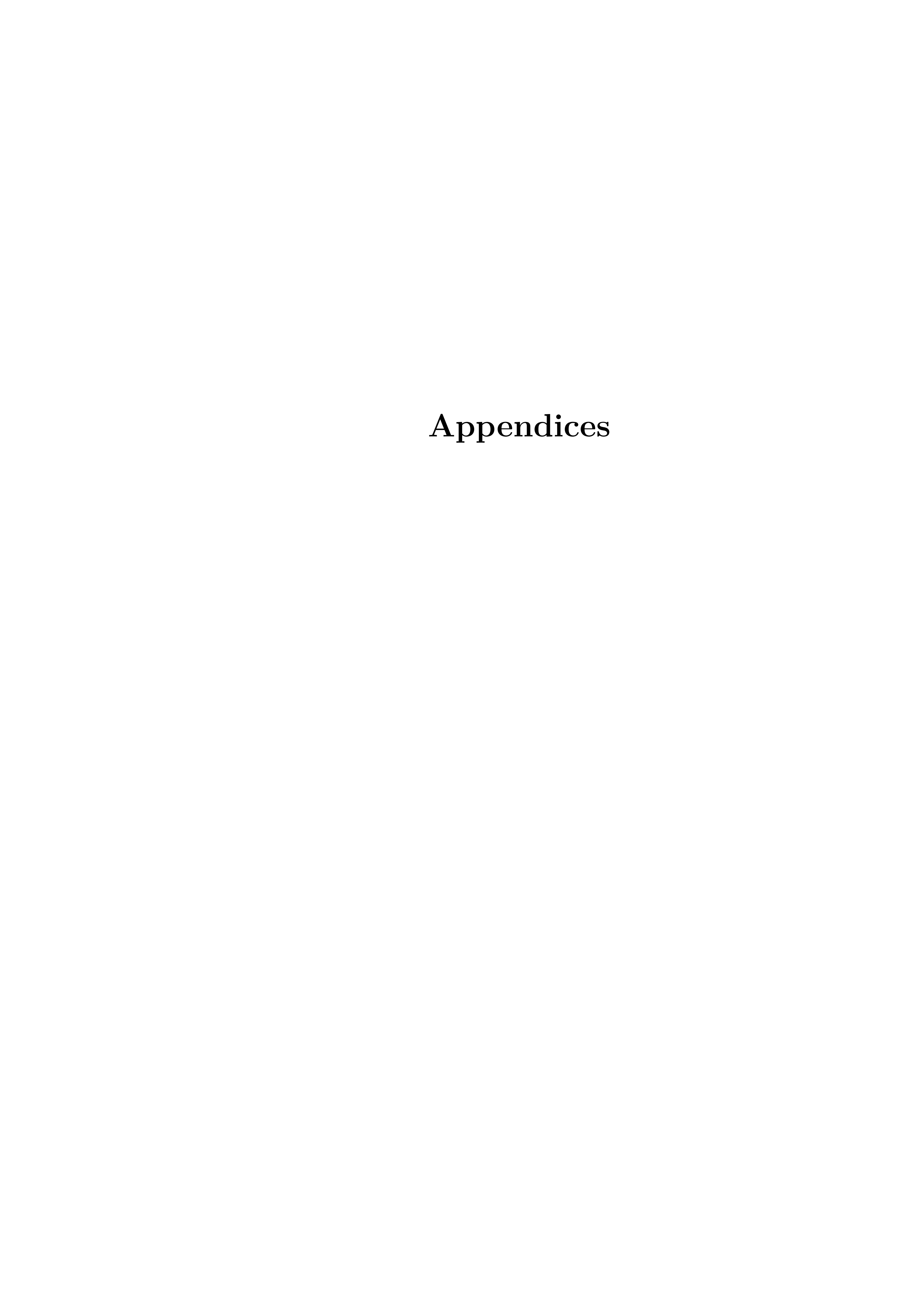
Attempted synthesis of compound 56[254]

To a solution of **45** (36 mg, 0.087 mmol) in CH_2Cl_2 (1 mL) was added Dess-Martin periodinane (0.056 g, 0.132 mmol). After stirring for 5 min the solution had changed color from clear to dark brown. After 15 min a saturated solution of $Na_2S_2O_3$ (1 mL) was added, this stirred for 15 min, whereupon Et_2O (2 mL) and 1M NaOH (1 mL) was added. The organic phase was callected and washed with brine (0.5 mL), dried over $MgSO_4$ and concentrated under reduced pressure. Only biproduct isolated.

Attempted synthesis of compound 57[255]

Compound **19** (56.2 mg, 0.120 mmol) was dissolved in MeCN (0.61 mL), a sodiumphosphate buffer (0.45 mL, pH = 6.7) and TEMPO (1.3 mg, 0.0083 mmol) was added. When

the reaction mixture had reached 35 °C one fourth of a $NaClO_2$ solution (0.027 g in 0.12 mL H_2O, 0.24 mmol) was added followed by one fourth of a $NaClO_2$ solution (3.5 μL in 0.07 mL H_2O, 0.0024 mmol). This was repeated every 15 min till all $NaClO_2$ was added. The mixture stirred for 1 h at 35 °C and cooled to rt, whereupon H_2O (10 mL) was added. A 10 % NaOH solution was added untill pH = 8. The reaction was quenched by pouring it into a 0 °C cold solution of Na_2CO_3 (3.7 mg in 0.6 mL H_2O). Precipitation after 2 min blocked the stirring of the mixture, therefore the the temperature was raised to rt and the mixture was stirred for 30 min. The mixture was extracted with Et_2O (10 mL), acidified with 10% HCl untill pH = 3 and extracted with Et_2O (3×10 mL). The combined organic phases was washed with brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Only starting material was isolated.



Appendices

List of Abbreviations

Abreviation	Full name	Structure
Ac	acetyl	
APT	attached proton test	
aq.	aqueous	
Ar	aromate	
Arg	Arginine	
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl	
Bn	benzyl	
br	broad	
BSP	1-benzenesulfinyl piperidine	
Bu	butyl	
Bz	benzoyl	
cat.	catalyst	
Chiraphos	2,3-Bis(diphenylphosphino)butane	
CMP	Cytidine monophosphate	
COD	cyclooctadiene	
conc.	concentration	

continues...

Abbreviation	Full name	Structure
COSY	correlation spectroscopy	
Cp	cyclopentadienyl	
CSA	camphorsulfonic acid	
Cy	cyclohexyl	
d	dublet	
DART	direct analysis in real time	
dba	dibenzylideneacetone	
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene	
DC-SIGN	dentritic cell-specific ICAM3-grabbing non-integrin	
dd	dublet of dublets	
decomp.	decomposition	
DIBAL-H	diisobutylaluminium hydride	
DIPEA	*N,N*-diisopropylethylamine	
DMAP	4-dimethylaminopyridine	
DMF	dimethylformamide	
DMM	*N*-dimethylmaleoyl	
DMP	Dess-Martin periodinane	
DMSO	dimethyl sulfoxide	
DMTST	dimethylthiomethylsulfonium trifluoromethane-sulfonate	
dppb	1,4-bis(diphenylphosphino)butane	
dppp	1,3-bis(diphenylphosphino)propane	
dr	diastereomeric ratio	
E. coli	*Escherichia coli*	
ee	enantiomeric excess	
eq.	equivalents	
Et	ethyl	
EtOAc	ethylacetate	
Fmoc	fluorenylmethyloxycarbonyl	
Fuc	fucose	

continues...

Abbreviation	Full name	Structure
Gal	galactose	
Glc	glucose	
GlcNAc	*N*-acetylglucosamine	
Gly	glycine	
h	hour(s)	
HIV	human immunodeficiency virus	
HMBC	heteronuclear multiple bond correlation	
HMO	human milk oligosaccharide	
HRMS	high resolution mass spectrometry	
HSQC	heteronuclear single quantum correlation	
IBX	2-iodoxybenzoic acid	HO, O, I, O, O
IDCP	iodonium di-*sym*-collidine perchlorate	N, I, N, ClO_4^-
IR	Infrared	
Josiphos	(*S*,*R*)-PPF(^{t}Bu)$_2$ or (*R*,*S*)-PPF(^{t}Bu)$_2$	
LG	leaving group	
LNFP	Lacto-*N*-fucopentaose	
LNnFP	Lacto-*N*-neofucopentaose	
LNnT	Lacto-*N*-neotetraose	
LNT	Lacto-*N*-tetraose	
m	multiplet	
M	molar (mol/L)	
Me	methyl	
MeOTf	methyl trifluoromethanesulfonate	
min	minutes	
mp.	melting point	
MS	molecular sieves, mass spectrometry	
MU	4-methylumbelliferyl	O, O
NEC	necrotizing enterocolitis	
Neu5Ac	*N*-acetylneuraminic acid	
NBS	*N*-bromosuccinimide	
NIS	*N*-iodosuccinimide	
NMR	nuclear magnetic resonance	
o	*ortho*	
p	*para*	
PG	protecting group	
Ph	phenyl	
Phth	phthaloyl	O, N, O
p-NP	*para*-nitrophenyl	
ppm	parts per million	

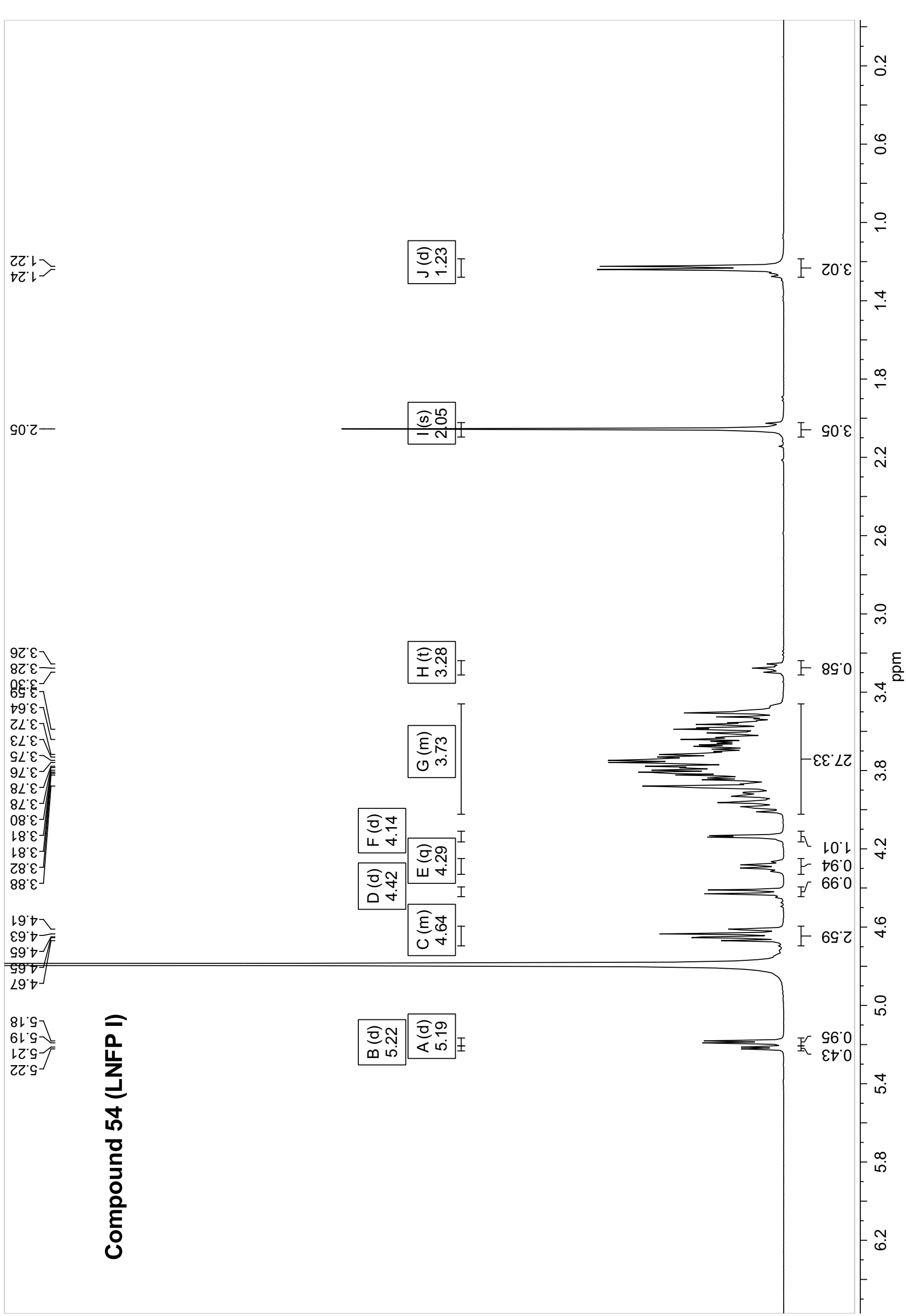
continues...

Abbreviation	Full name	Structure
py	pyridine	
q	quartet	
RRV	relative reactivity value	
(*R*,*S*)-PPF(^{t}Bu)$_2$	(*R*)-1-[(*S*)-2-(diphenylphosphino)ferrocenyl]-ethyldi-*tert*-butyl-phosphine	
rt	room temperature	
s	singlet	
(*S*,*R*)-PPF(^{t}Bu)$_2$	(*S*)-1-[(*R*)-2-(diphenylphosphino)ferrocenyl]-ethyldi-*tert*-butyl-phosphine	
sat.	saturated	
t	*tert*	
t	triplet	
TBAB	tetrabutylammonium bromide	
TBAF	tetrabutylammonium fluoride	
TBAI	tetrabutylammonium iodide	
TBDMS	tert-butyldimethylsilyl	
TCP	tetrachlorophthaloyl	
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl	
TES	triethylsilyl	
TFA	trifluoroacetic acid	
TFAA	trifluoroacetic anhydride	
TfO	trifluoromethansulfonate	
TfOH	trifluoromethanesulfonic acid	
THF	tetrahydrofuran	
TLC	thin layer chromatography	
TMS	trimethylsilyl	
Tol	tolyl	
Troc	trichloroethoxycarbonyl	
Ts	Tosyl, toluenesulfonyl	
TTBP	2,4,6-tri-*tert*-butylpyrimidine	
UDP	uridine diphosphate	
UV	ultraviolet	

Publications

"Synthesis of Human Milk Oligosaccharides by One-Pot Glycosylation Approach" Jennum, C. A.; Fenger, T. H.; Bruun, L. M.; Madsen, R. *in preparation*

NMR data for LNFP I and LNnFP I



Compound 54 (LNFP I)
J (d) 1.23
I (s) 2.05
H (t) 3.28
G (m) 3.73
F (d) 4.14
E (q) 4.29
D (d) 4.42
C (m) 4.64
B (d) 5.22
A (d) 5.19
3.02
3.05
0.58
27.33
1.01
0.94
0.99
2.59
0.95
0.43
1.22
1.24
2.05
3.26
3.28
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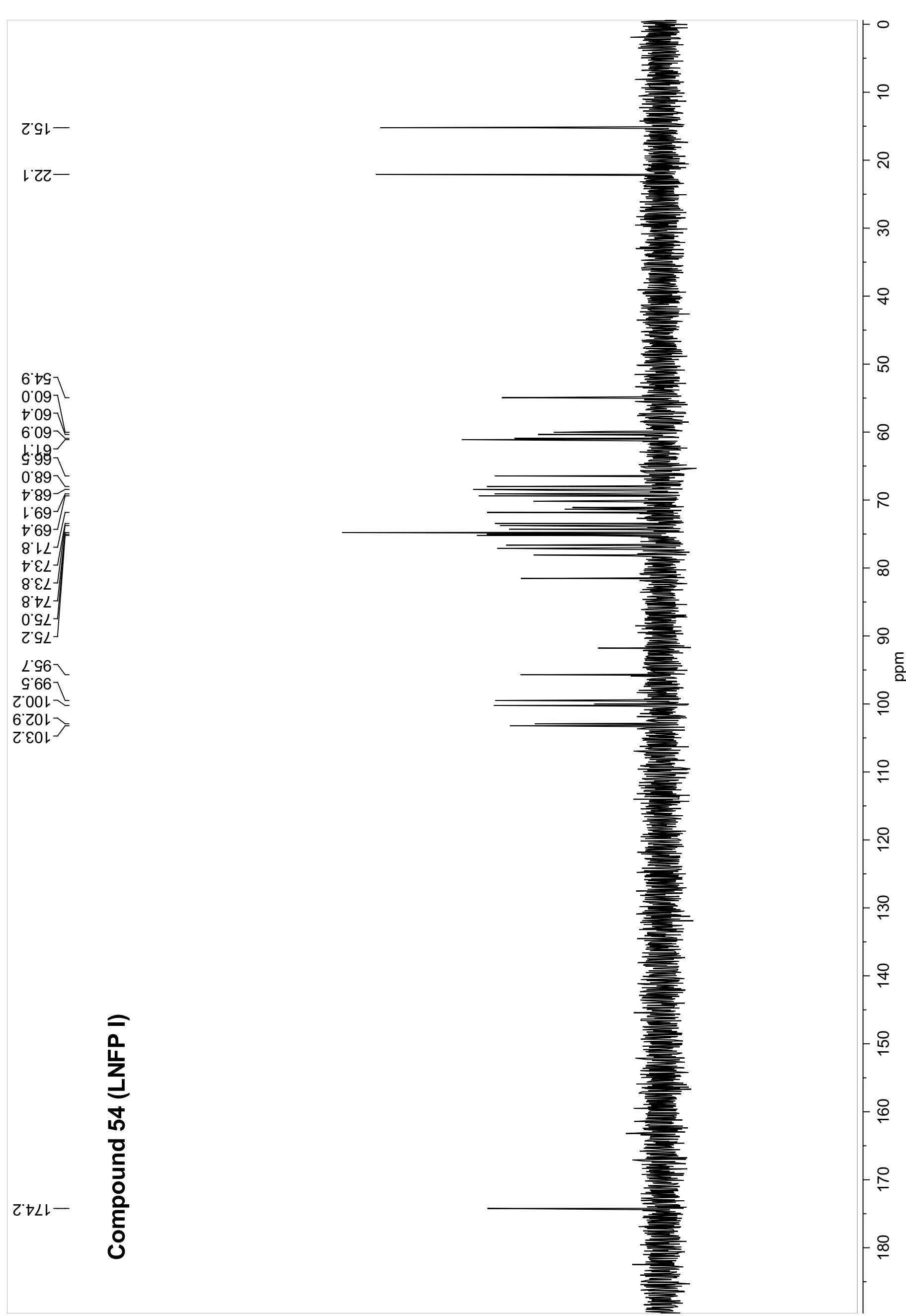
5.21
5.22
ppm


Compound 54 (LNFP I)
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22.1
54.9
60.0
60.4
60.9
61.1
66.5
68.0
68.4
69.1
69.4
71.8
73.4
73.8
74.8
75.0
75.2
95.7
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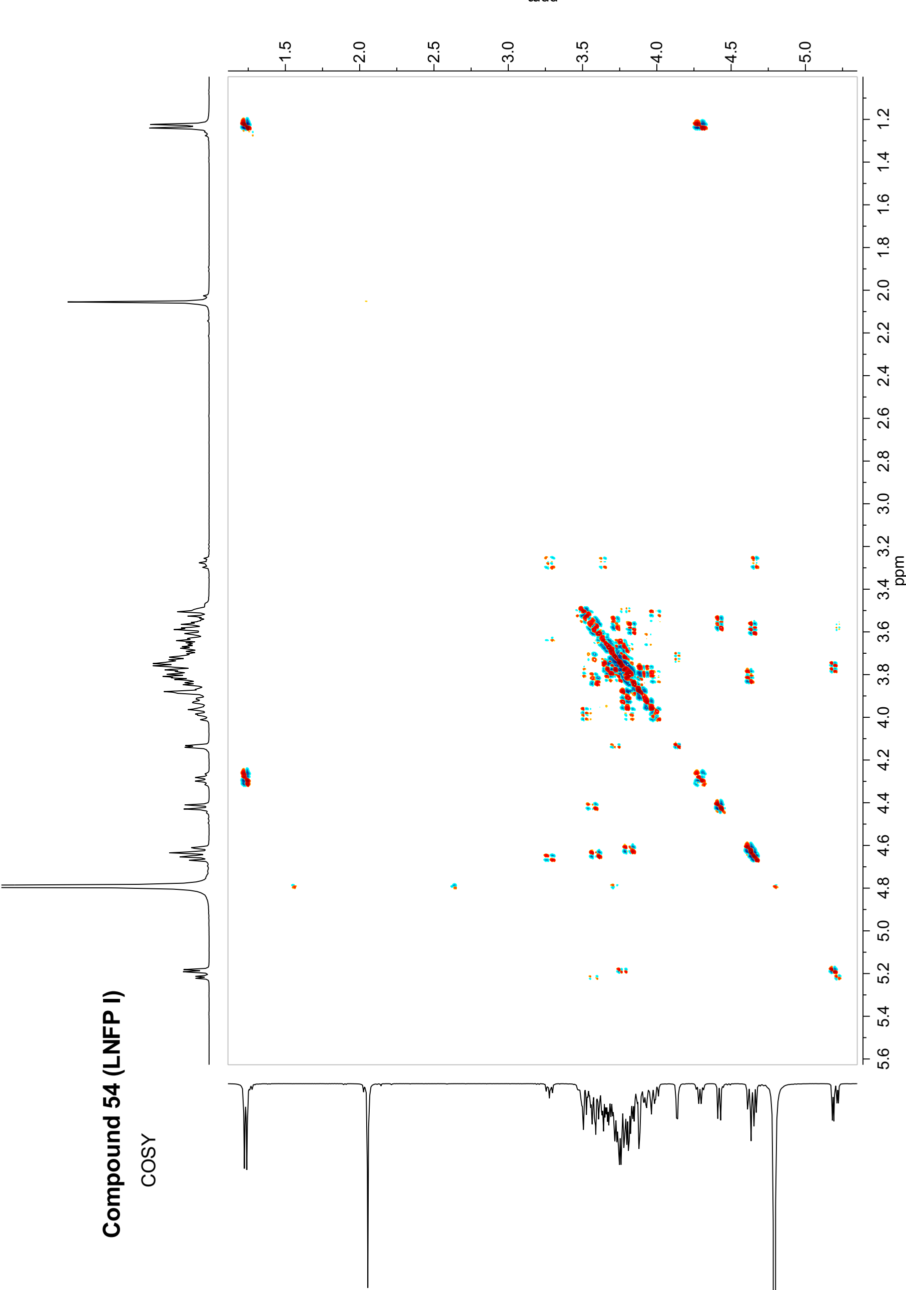
100.2
102.9
103.2
174.2
ppm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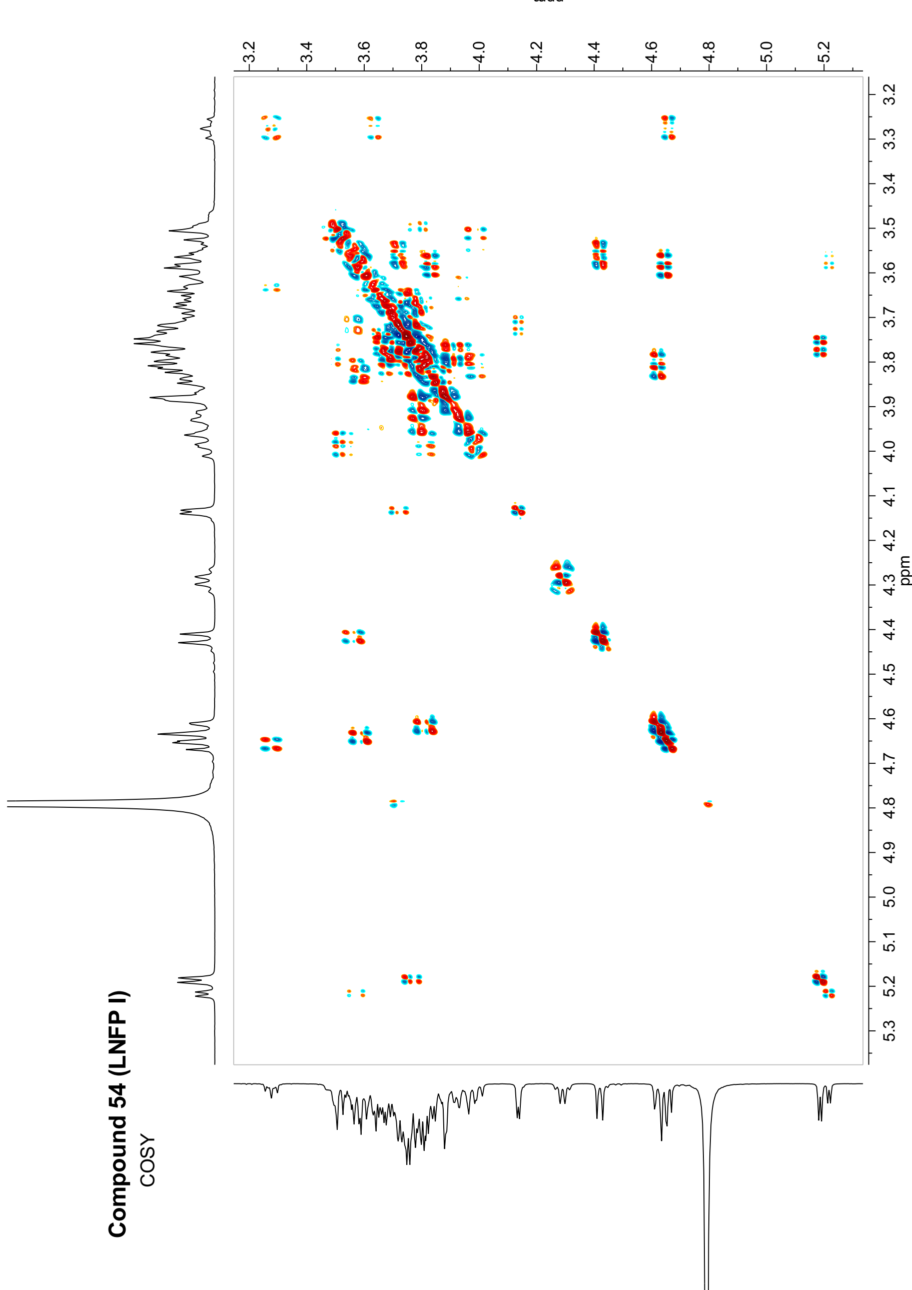
ppm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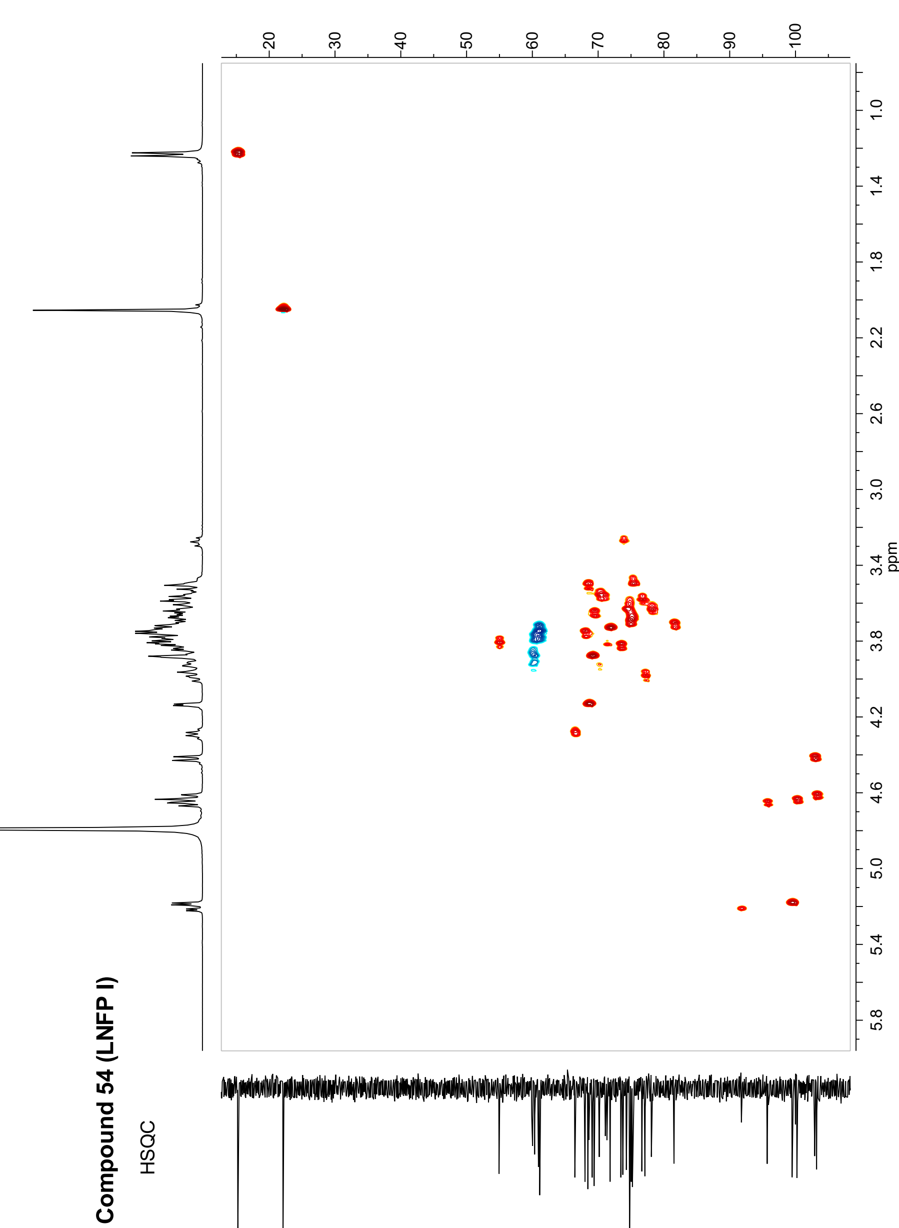
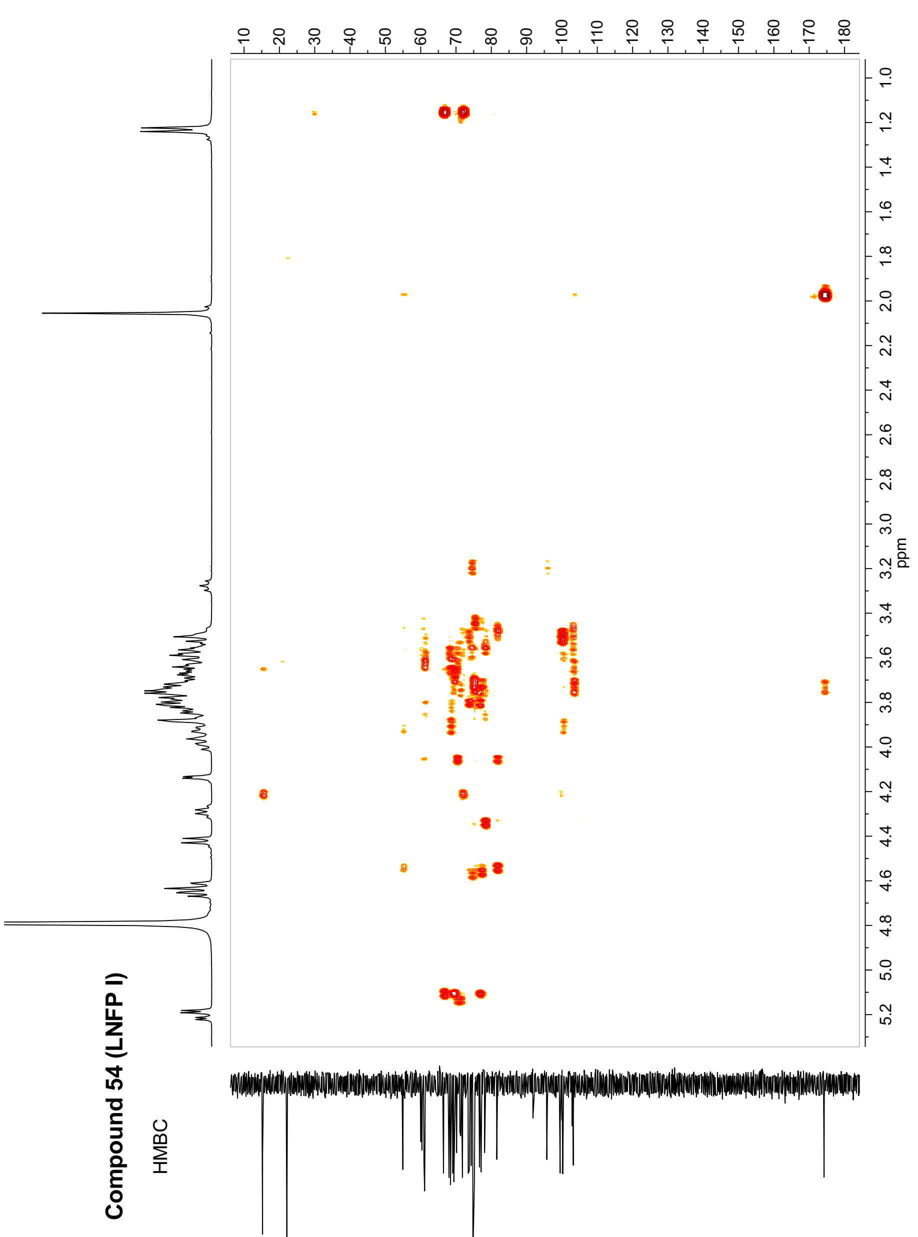
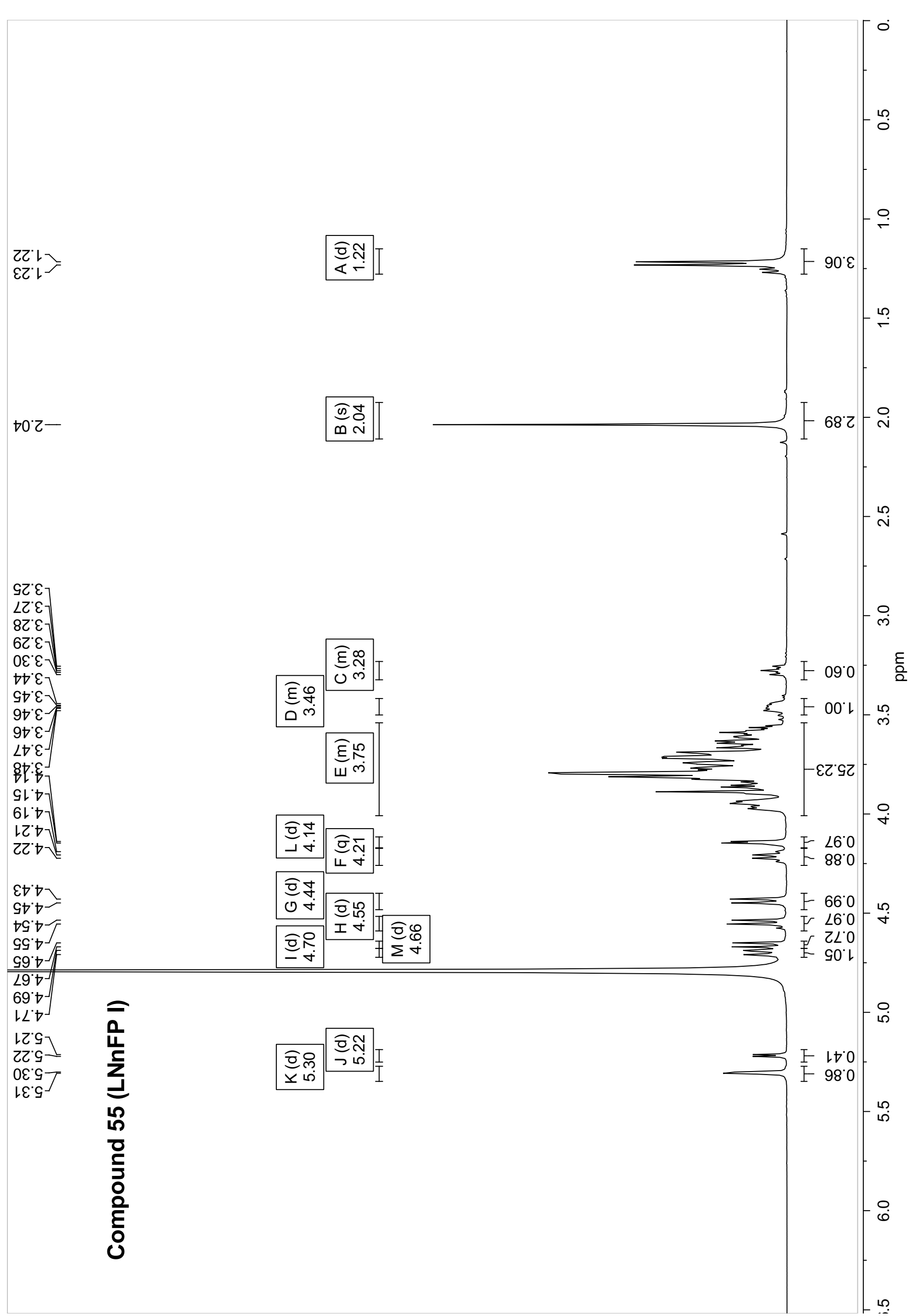
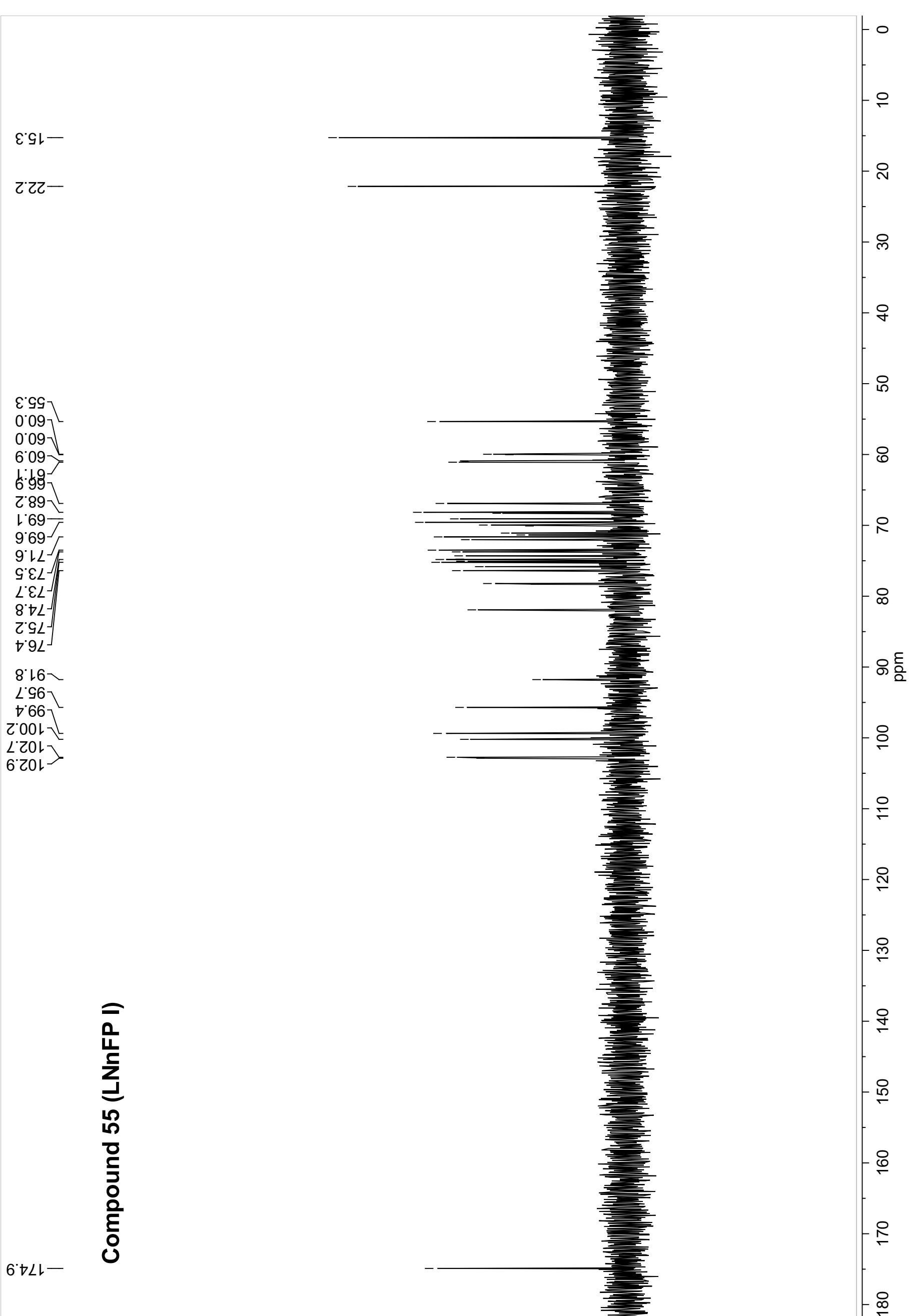


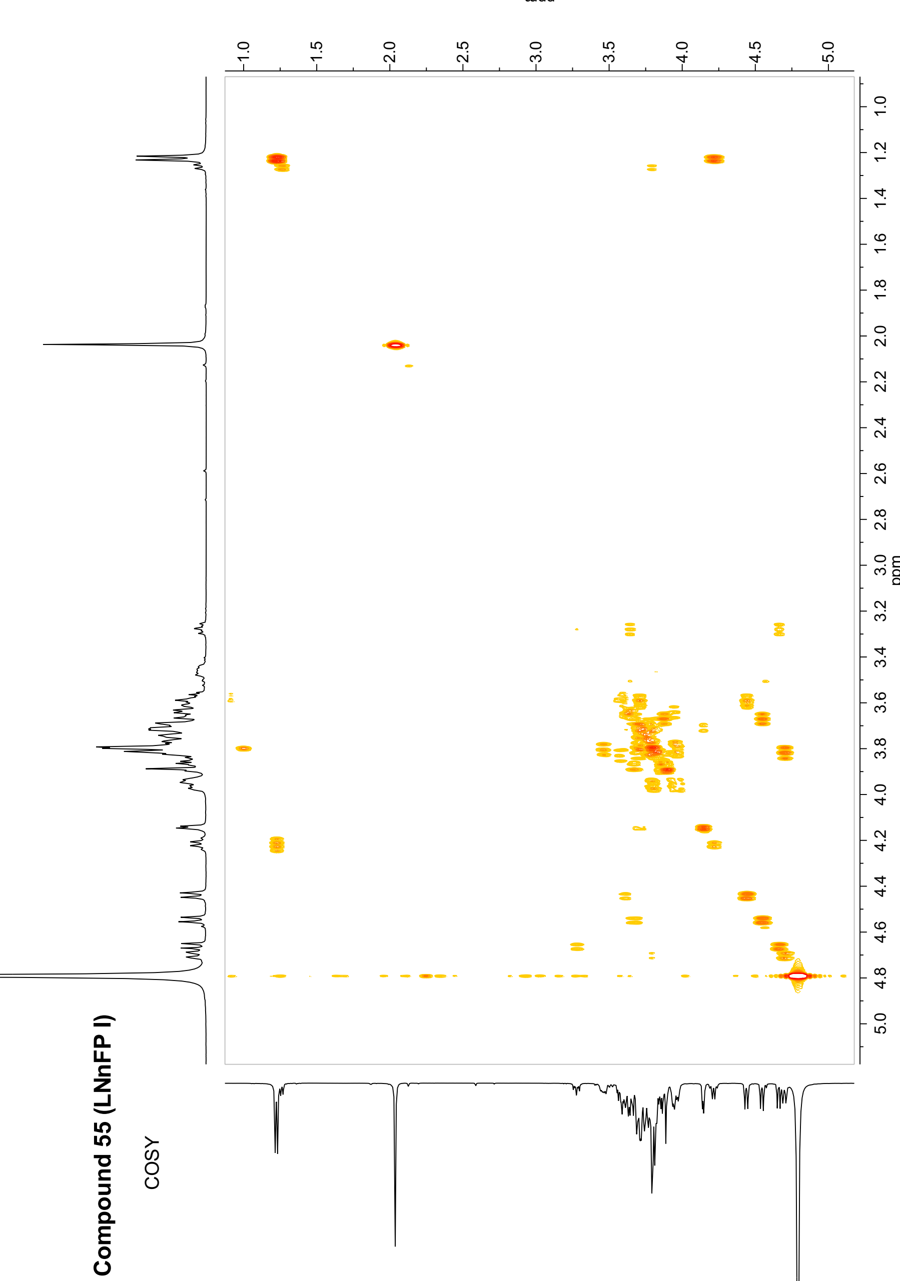
Compound 54 (LNFP I)
HSQC
ppm
ppm

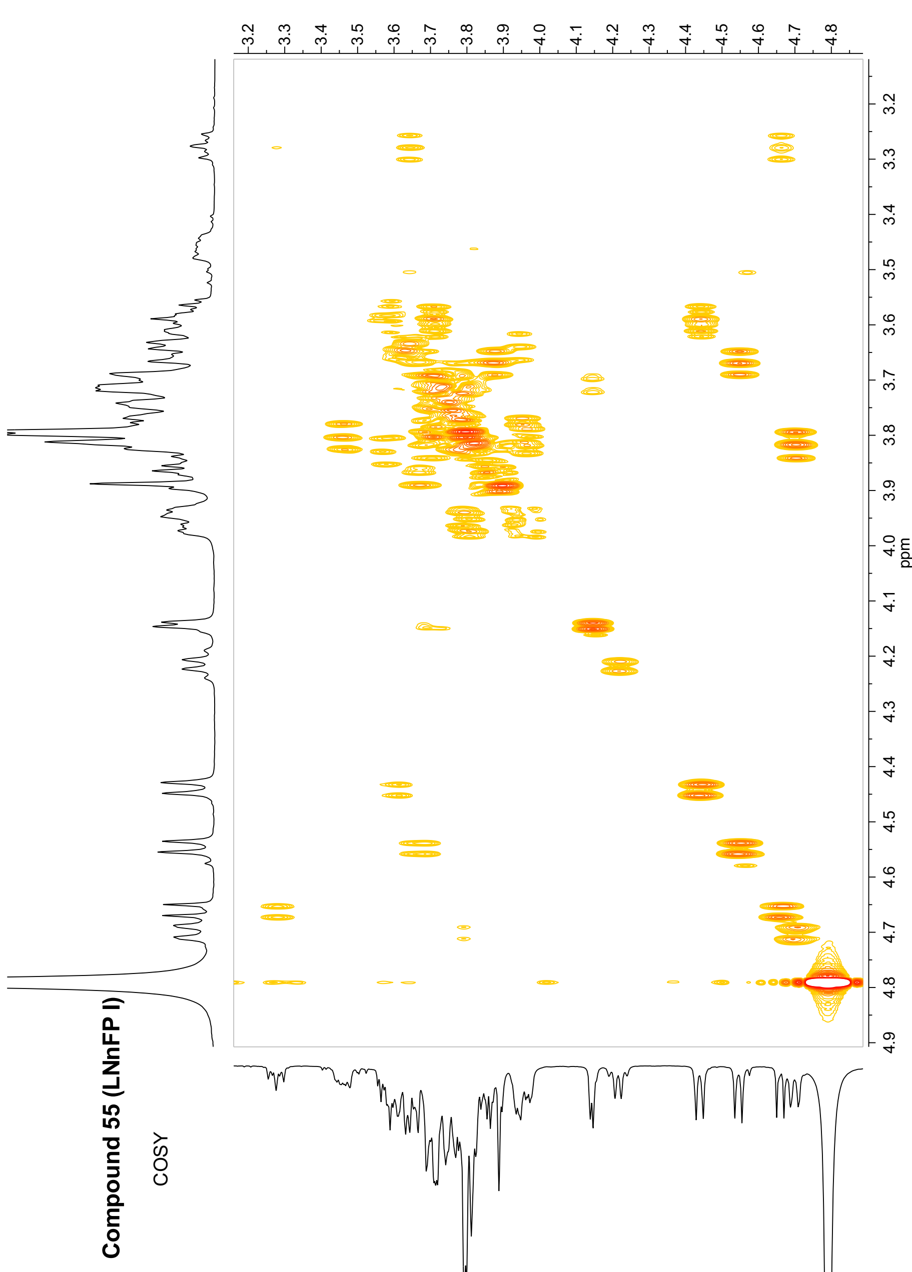


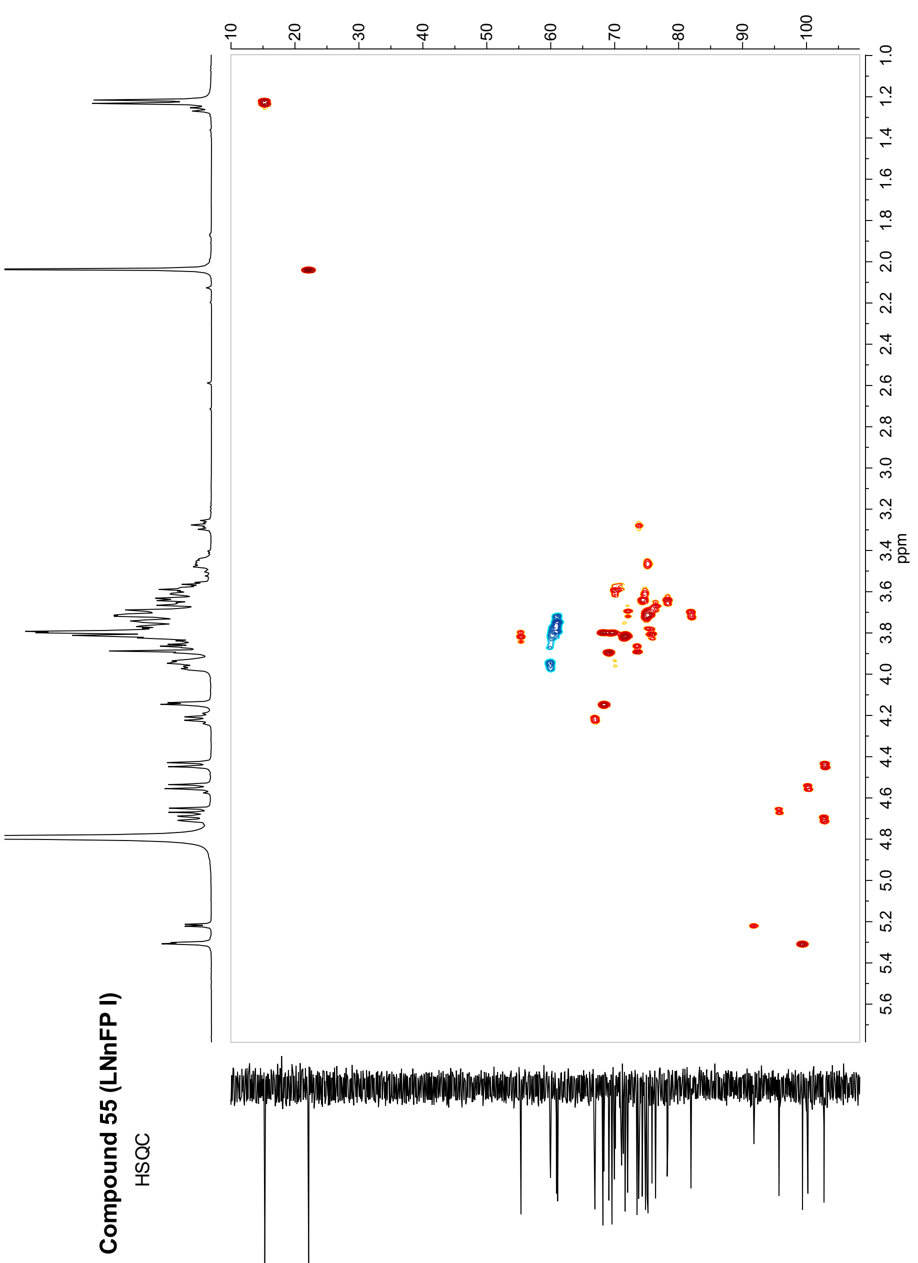
Compound 54 (LNFP I)
HMBC
ppm
ppm

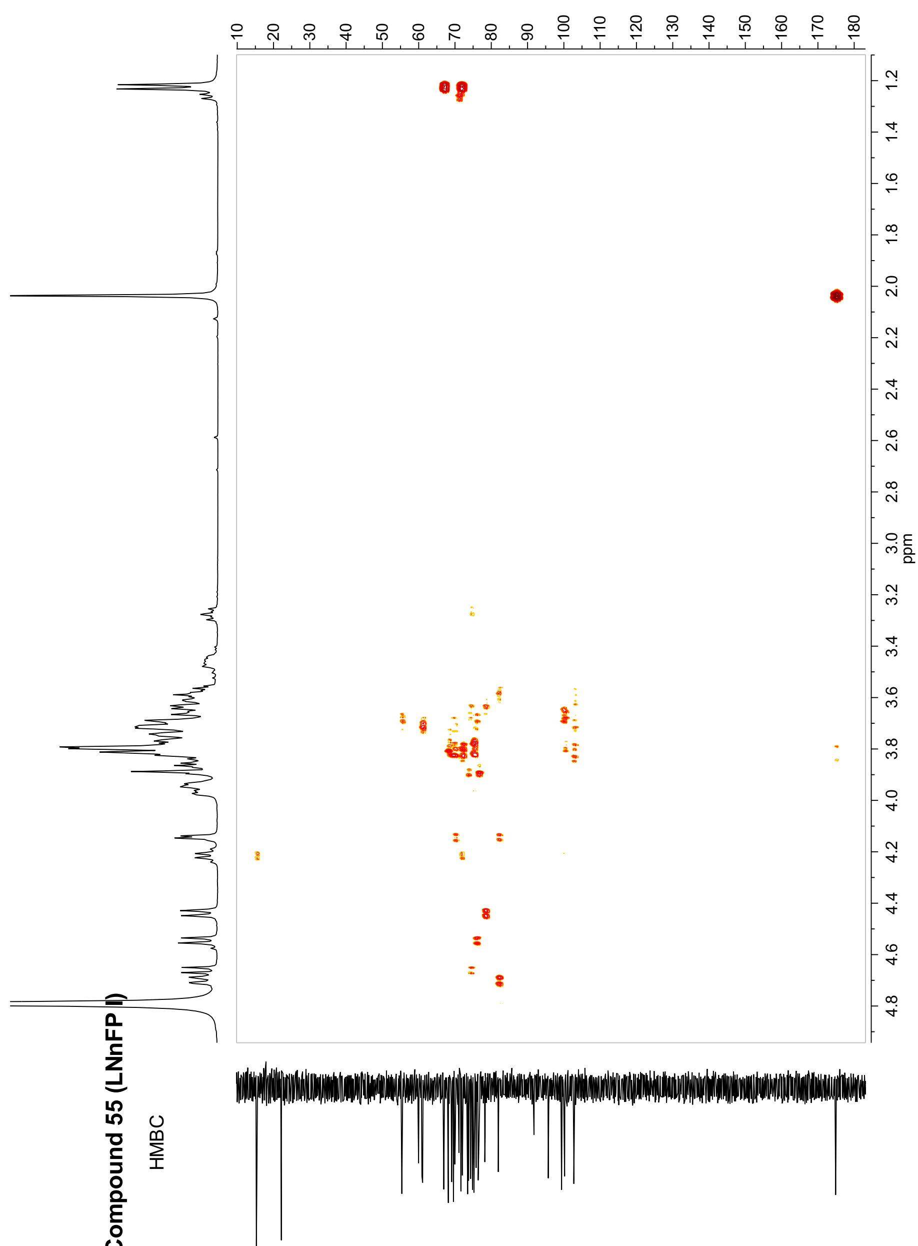

Compound 55 (LNnFP I)
A (d) 1.22
B (s) 2.04
C (m) 3.28
D (m) 3.46
E (m) 3.75
L (d) 4.14
F (q) 4.21
G (d) 4.44
H (d) 4.55
I (d) 4.70
M (d) 4.66
K (d) 5.30
J (d) 5.22
3.06
2.89
0.60
1.00
25.23
0.97
0.88
0.99
0.97
0.72
1.05
0.41
0.86
ppm


Compound 55 (LNnFP I)
15.3
22.2
55.3
60.0
60.0
60.9
61.1
66.9
68.2
69.1
69.6
71.6
73.5
73.7
74.8
75.2
76.4
91.8
95.7
99.4
100.2
102.7
102.9
174.9
ppm


Compound 55 (LNnFP I)
COSY
ppm
ppm


Compound 55 (LNnFP I)
COSY
ppm
ppm


Compound 55 (LNnFP I)
HSQC
ppm
ppm



Compound 55 (LNnFP I)
HMBC
ppm
ppm

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