Doctoral Thesis

Chemical Synthesis of Biomolecules Analogs Inositol Phosphate/Sulfate Hybrids And Fluorinated Carbohydrates

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Chemical Synthesis of Biomolecules Analogs:

Inositol Phosphate/Sulfate Hybrids

And

Fluorinated Carbohydrates

A thesis submitted to attain the degree of

DOCTOR OF SCIENCES of ETH ZURICH

(Dr. sc. ETH Zurich)

presented by

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Abstract

Inositol phosphates and carbohydrates are cyclic polyols biomolecules that play a number of key biological roles. Elucidation of their functions has been hampered by their structural diversity and the difficulty to isolate pure material. Chemical synthesis of these biomolecules and their analogues has not only contributed to illuminate their functional roles but also offers new therapeutic opportunities which are both presented in the Chapter 1. Nonetheless, chemical synthesis of well-defined structures of these classes of molecules remains a real challenge. A special focus was put on the stereoselectivity aspect, hence some current strategies in diastereoselective glycosylation are briefly introduced, and methodologies of chiral phosphorylation of inositols are described.

The design and synthesis of inositol phosphate/sulfate hybrids as potential drugs against *Clostridium difficile* infection (CDI) is presented in Chapter 2. CDI is an increasingly frequent and severe bacterial infection of the colon that requires additional treatment options. The therapeutic strategy is based on the preemptive induction of toxin B auto-processing in the lumen thus preventing cell penetration of the disease-causing enzymatic domain. The naturally occurring allosteric activator, inositol hexakisphosphate (InsP\(_6\)), is inactive in the presence of extracellular high calcium concentration, prompting us to design InsP\(_6\) analogues. At first, a synthesis strategy was developed to prepare meso inositol phosphate/sulfate hybrids, and a unified and streamlined synthesis delivered successfully three derivatives. One of the candidates, SSSPSP, showed allosteric activity in presence of calcium. Thus, an improved synthesis was designed to provide sufficient amount of material for further *in vivo* investigation. A key finding was that the O-sulfation of deprotected inositol phosphates was possible, significantly improving the synthesis of the challenging hybrids, enabling the synthesis of different racemic derivatives. Finally, a new chiral phosphoramidite agent allowing phosphorylation and chiral resolution of inositol intermediates was developed. This methodology now enables the synthesis of various chiral natural inositol phosphates, as well as phosphate/sulfate hybrids.

The Chapter 3 is dedicated to the investigation of fluorine-directed β-stereoselective glycosylation in the 2-fluorogalactopyranosyl donor. A systematic study showed that fluorine atom at the C2 position, together with electron donating nature of the protecting groups, were leading excellent level of β selectivity in glycosylation reactions. The effect of the glycosyl acceptor was probed and revealed that although the reaction is sensitive to steric hindrance, the presence of fluorine is still able to drive good β selectivity. Utility of this methodology was then illustrated in the synthesis of fluorinated analogues of commonly found disaccharide building blocks and of the glycosphingolipid β-galactose ceramide.
Abstract

To conclude, the development of the synthetic methods presented in this thesis provide useful tools to the synthesis of biomolecule analogues.
Résumé

Les inositol phosphates et les glucides sont des biomolécules polyhydroxylées cycliques qui jouent un rôle important dans un grand nombre de processus biologiques. L’élucidation de leurs fonctions a été ralentie du fait de leur diversité structurale et de la difficulté à isoler du matériel pur. La synthèse chimique de ces biomolécules, ainsi que de leurs analogues, a contribué à éclairer leur rôle fonctionnel et aussi à offrir de nouvelles opportunités thérapeutiques, qui sont présentées dans le Chapitre 1. Néanmoins la synthèse de structures bien définies de ces types de molécules reste un défi. Un intérêt particulier a été donné à l’aspect de la stéréosélectivité de la synthèse des glucides et des inositol phosphates. Alors que les méthodes courantes de glycosylation diastéréosélective sont brièvement introduites, les méthodologies de phosphorylation chirale des inositols sont décrites de manière plus détaillées.

La conception et la synthèse d’inositols hybrides phosphate/sulphate en tant que molécule potentiellement active contre une infection à Clostridium difficile (ICD) sont présentées dans le Chapitre 2. L’ICD est une infection bactérienne du côlon qui est de plus en plus fréquente et de plus en plus sévère, ainsi il est donc nécessaire de trouver de nouveaux traitements. La stratégie thérapeutique proposée est basée sur l’induction de l’auto-protéolyse de la toxine B dans la lumière du gros intestin afin de prévenir l’absorption du domaine enzymatique causant la maladie. L’activateur allostérique naturel, l’inositol hexakisphosphate (InsP₆), est inactif en présence de concentrations élevées de calcium habituellement présentes dans l’intestin. Cette observation, nous a mené à concevoir des analogues de InsP₆. Dans un premier temps, une stratégie de synthèse a été développée pour préparer des inositols hybrides phosphate/sulphate meso. Une synthèse unifiée et profilée a permis de fournir avec succès trois dérivés. Un des candidats, SSSPSP, a montré une activité allostérique en présence de calcium. Ainsi, une synthèse améliorée a été conçue pour fournir une quantité suffisante de matériau pour pouvoir effectuer des études in vivo plus approfondies. Une découverte clé a été la réalisation de la sulfation sur les inositol phosphates entièrement déprotégés. Ce résultat a amélioré de manière significative la synthèse compliquée des inositols hybrides, donnant accès à la préparation de différents dérivés racémiques. Finalement, un nouveau phosphoramidite chiral, permettant phosphorylation et résolution chirale d’intermédiaires d’inositols a été développé. Cette méthodologie peut être utilisée dans la synthèse chirale d’inositols phosphates naturels, et aussi d’inositols hybrides phosphate/sulphate.

Le Chapitre 3 est dédié à l’étude de la glycosylation β-stéréosélective dirigée par l’atome de fluor du 2-fluorogalactopyranoside. Une étude systématique a montré que l’atome de fluor à la position C2, combiné à la nature électro-donneur des groupes protecteurs conduisaient à un excellent niveau de
Résumé

β sélectivité. L’effet du glucide accepteur a été sondé et a révélé que bien que la sélectivité de la réaction était sensible à l’encombrement stérique, la présence du fluor est toujours capable de mener une bonne sélectivité. L’utilité de cette méthodologie a été illustrée dans la synthèse d’analogues fluorés de disaccharides communément rencontrés et d’un glycosphingolipide β-galactose ceramide.
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>Ac</td>
<td>acetyl</td>
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<tr>
<td>ACN</td>
<td>acetonitrile</td>
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<tr>
<td>aq</td>
<td>aqueous</td>
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<tr>
<td>Bn</td>
<td>benzyl</td>
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<tr>
<td>Boc</td>
<td>tert-butyloxycarbonyl</td>
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<tr>
<td>CBP</td>
<td>carbohydrate-binding protein</td>
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<tr>
<td>cc</td>
<td>concentrated</td>
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<tr>
<td>CDI</td>
<td><em>C. difficile</em> infection</td>
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<tr>
<td>CIP</td>
<td>contact ion pair</td>
</tr>
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<td>CPD</td>
<td>cysteine protease domain</td>
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<tr>
<td>CT</td>
<td>cholera toxin</td>
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<tr>
<td>DAST</td>
<td>diethylaminosulfur trifluoride</td>
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<tr>
<td>DBU</td>
<td>1,8-diazabicycloundec-7-ene</td>
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<tr>
<td>DMAP</td>
<td><em>N,N</em>-dimethyl-4-aminopyridine</td>
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<td>DMF</td>
<td><em>N,N</em>-dimethylformamide</td>
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<tr>
<td>d.r.</td>
<td>diastereomeric ratio</td>
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<tr>
<td>EC$_{50}$</td>
<td>half maximal effective concen</td>
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<td>ee</td>
<td>enantiomeric excess</td>
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<tr>
<td><em>E. coli</em></td>
<td><em>Escherichia coli</em></td>
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<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>e.r.</td>
<td>enantiomeric ratio</td>
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<td>FDA</td>
<td>US Food and Drug Administration</td>
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<tr>
<td>Fm</td>
<td>bis-fluorenyl</td>
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<td>Gal</td>
<td>galactose</td>
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<tr>
<td>GI</td>
<td>gastro-intestinal</td>
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<td>Glu</td>
<td>glucose</td>
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<tr>
<td>GPI</td>
<td>glycophosphatidylinositol</td>
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<tr>
<td>Hib</td>
<td><em>Haemophilus Influenzae</em> type B</td>
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<tr>
<td>InsPs</td>
<td>inositol phosphate</td>
</tr>
<tr>
<td>InsP$_6$</td>
<td>inositol hexakisphosphate</td>
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<tr>
<td>InsS$_6$</td>
<td>inositol hexakissulfate</td>
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<tr>
<td><em>iPrOH</em></td>
<td>isopropanol</td>
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<tr>
<td>LG</td>
<td>leaving group</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>mCPBA</td>
<td>m-chloroperbenzoic acid</td>
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<tr>
<td>Me</td>
<td>methyl</td>
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<tr>
<td>Min</td>
<td>minute</td>
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<tr>
<td>m.p.</td>
<td>melting point</td>
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<tr>
<td>MPO</td>
<td>myeloperoxidase</td>
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<tr>
<td>MS 4 Å</td>
<td>molecular sieves 4Å</td>
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<tr>
<td>NMR</td>
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<tr>
<td>Ph</td>
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</tr>
<tr>
<td>PMB</td>
<td>p-methoxybenzyl</td>
</tr>
<tr>
<td>Pdt-InsPs</td>
<td>phosphatidylinositol phosphate</td>
</tr>
<tr>
<td>PP-InsPs</td>
<td>diphosphoinositol phosphate</td>
</tr>
<tr>
<td>RBD</td>
<td>receptor-binding domain</td>
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<tr>
<td>rt</td>
<td>room temperature</td>
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<tr>
<td>SGAG</td>
<td>sulfated glycosaminoglycan</td>
</tr>
<tr>
<td>sLe^a/x</td>
<td>sialyl Lewis^a/x</td>
</tr>
<tr>
<td>SSIP</td>
<td>solvent-separated ion pair</td>
</tr>
<tr>
<td>Stx</td>
<td>Shiga-like toxin</td>
</tr>
<tr>
<td>TBDMs</td>
<td>tert-butyldimethylsilyl</td>
</tr>
<tr>
<td>TEAB</td>
<td>triethylammonium bicarbonate</td>
</tr>
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<td>TMSBr</td>
<td>bromotrimethylsilane</td>
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<tr>
<td>TMSOTf</td>
<td>trimethylsilyl trifluoromethanesulfonate</td>
</tr>
<tr>
<td>TcDA</td>
<td>C. difficile toxin A</td>
</tr>
<tr>
<td>TcDB</td>
<td>C. difficile toxin B</td>
</tr>
<tr>
<td>Tf</td>
<td>trifluoromethanesulfonyl</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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1. Background and Purpose

1.1. Carbohydrates and inositol phosphates: intriguing biomolecules

Carbohydrates and inositol phosphates are ubiquitous biomolecules that play a number of key biological roles (Figure 1). The understanding of their functions at a molecular level is challenging and hampered by their structural diversity and the difficulty to isolate pure, well defined material in sufficient amount. Chemical synthesis of these biomolecules has strongly contributed to shed light on the vast realm they form. Synthesis gives access to pure and structurally well defined molecules but also to unnatural analogues or structures functionalized with moieties such as biotin, photocrosslinker, dye, etc. These constitute invaluable chemical tools to profile protein targets, evaluate activity and better understand the protein interactions at a molecular level. Despite recent progress, new synthetic methods are still required to advance the preparation of complex biomolecules and also to exploit their potential as therapeutics.

**Figure 1** Structure of bioactive carbohydrates and inositol phosphates. A) Sialyl Lewis-x (sLe-x): important selectin ligand (1). B) Isoglobotrihexosylceramide CD1d, involved in natural killer T-cell activation (2). C) Antithrombin pentasaccharide sequence: anticoagulant (3). D) Ins(1,4,5)P$_3$: second messenger in Ca$^{2+}$ release (4). E) Ptd-Ins(3,4,5)P$_3$: activates Akt (protein kinase B, PKB) which is involved in regulation of downstream processes that control apoptosis and cell survival (5). F) Glycosylphosphatidylinositol (GPIs) conserved core: anchor attached protein to cell membrane (6).
1.2. Carbohydrates

1.2.1. Biological role and therapeutic potential

Carbohydrates are considered the most abundant biomolecules on Earth (7). Soluble or membrane-anchored, they play a role in many biological processes including cell proliferation and differentiation, fertilization, embryogenesis, bacterial adhesion, viral infection, immune response, inflammation, and neural development (8). Carbohydrates can be found in a wide diversity of forms, from monosaccharides to oligo- and polysaccharides or conjugates such as glycoproteins, proteoglycans, glycolipids and glycosphatidylinositol (GPI). Indeed, glycosylation is the most important posttranslational modification. Furthermore, glycoconjugates are important constituents of cell membrane, where the carbohydrate moiety is surface-exposed and thus an important player in intracellular trafficking, cell-cell communication, cell-matrix, and cell-pathogen interactions. Glycosylation of soluble proteins also plays a role in protein folding and stability, protein clearance and other protein recognition processes as well as in their activity (9).

Synthetic saccharides and glycoconjugates are useful tools to decipher the function of these complex and diverse glycan structures (7b, 10). For example, glycan microarray is a very popular method to identify binding proteins and to investigate their binding specificities and properties (11). Carbohydrate analogues are also important synthetic tools in glycobiology. For instance, glycosyltransferase inhibitors that specifically block the synthesis of a glycoconjugate can provide a better understanding of its function (10, 12). The functionalization of glycans is a useful strategy to study the glycans in their living systems (13). Azido labeled glycans undergo Staudinger ligation or [3+2] cycloaddition in biological environments, so that a probe can be incorporated and allow in vivo visualization (14). Similarly, glycans functionalized with photoaffinity labels can be used to capture their binding partners by cross-linking them to enable their characterization (15).

Glycobiology has not only provided a better understanding of the function and role of carbohydrates but also led to the development of carbohydrate-based therapeutics. Some examples of different classes of glycotherapies are described below.

Primarily used as tool in glycobiology, glycosidase or glycosyl transferase inhibitors have been developed to understand glycan functions (7b) and later on became a paradigm in glycan small molecule drugs. Several inhibitors are currently on the market for the treatment of influenza (16), diabetes (17) or Gaucher disease (18). Prominent examples are the antiviral compounds zanamivir
1. Background and Purpose

(Relenza) (Figure 2.A) and oseltamivir (Tamiflu), which are inhibitors of the viral neuraminidase, an enzyme that cleaves sialic acid-containing glycans on host cell surface thus preventing virus infection.

Sulfated glycosaminoglycans (SGAG) are an important class of heavily sulfated polysaccharides. In particular, heparin and heparan sulfate are mediators of biological events through binding proteins involved in blood coagulation, cell proliferation and differentiation, as well as cell and pathogen interactions (19). Synthesis of pure and well defined GAGs is important to provide structural information and protein interactions details. Furthermore, it contributes to design therapeutics such as anticoagulant, anticancer, anti-inflammatory and antiviral agents (20). Heparin is one of the oldest drugs widely used as anticoagulant and is mainly isolated from porcine mucus. In 2008, batches contaminated with oversulfated chondroitin sulfate led to an international health crisis underlying the need to prepare pure and well defined products (21). Fondaparinux (ARIXTRA) (Figure 2, B) is a pentasaccharide heparin found on the market (22). Although fully synthetic, it suffers from a tedious chemical process, that spurred chemists to provide better and cost-effective preparation methods such as the chemoenzymatic synthesis recently published (23).

Selectins represent an important class of carbohydrate-binding proteins (CBP), or lectins, that act as cell adhesion molecules through the binding of the common glycosidic epitopes sialyl Lewis\(\alpha/x\) (sLe\(\alpha/x\)). Selectins are involved in the extravasation of cells from the blood stream and disregulation of this function is a common factor in inflammatory diseases and cancer (24). These findings have attracted considerable interests into the development of glycomimetic selectin inhibitors as therapeutic drugs and some candidates are currently in clinical trials such as Bimosiamose TBC-1269 for the treatment of asthma (Figure 2, C) (25) and GMI-1070 for the treatment of sickle cell crisis (26). Siglecs are another type of lectins present on the surface of immune cell that bind sialic acid and belong to the immunoglobulin superfamily. They are considered as a promising target for drug delivery and cancer therapies (27).

The adhesion of some bacteria to host cells is mediated by carbohydrate-binding proteins on the pathogen, or adhesins, and is a pivotal event in infection. One strategy against bacterial infection is to prevent adhesion of the pathogen with glycomimetics. With increasing bacterial resistance to antibiotics, anti-adhesion drugs represent an interesting alternative to antibiotics. The challenge of this approach is the presence of multiple lectins on the pathogen that encode for different carbohydrate binding partners and the low binding affinity of free carbohydrates. However progresses in carbohydrate chemistry and better understanding of pathogen mode of adhesion gave rise to important breakthroughs in this area, notably the synthesis of multivalent glycoconjugates such as polymers, dendrimers or nanoparticles which show promising results (28). For instance, urinary tract infection is a widespread and recurrent infection caused mainly by uropathogenic
1. Background and Purpose

*Escherichia coli*. While it is treated with antibiotics, there is a rise in the occurrence of resistance leading to treatment failure. Lately, much attention has turned to the development of molecules that can inhibit the adhesion of *E. coli*’s FimH lectins to epithelial cells (29). Based on the main natural ligands α-mannosides, FimH antagonists have been designed, including monovalent aromatic α-mannosides and multivalent glycoconjugates. The aromatic α-mannosides increase the binding affinity due to hydrophobic and π-π interactions with the “tyrosine gate” of the FimH binding pocket. Multivalent glycodendrimers bearing mannose residues were designed to enhance affinity through the cluster effect and some displayed low nanomolar affinities such as the G(0) pentaerythritol-based tetramer (30), the hyperbranched lysine-based 16-mer (31), the fullerene 12-mer (32) or a linear heptamer (33).

Another approach against bacterial infection is to target bacterial toxins which are sometimes the main virulence factor (34). Some toxins rely on binding to cell surface carbohydrate as their entry mechanism. Thus, an interesting approach to prevent pathogenesis is to hamper cell penetration by disrupting toxin-carbohydrate receptor binding with antagonists. Many interesting works have been reported especially on the shiga (35), cholera (36) and *Clostridium difficile* toxins (37). One of the most striking example is the STARFISH glycodendrimer tailored to bind the Shiga-like toxins I and II (Stx-1 and 2) (Figure 2, D)(35b). This dendrimer is composed of dendrons containing Gb3 trisaccharides analogues, a known ligand of Stx-1 and 2, attached to a glucose pentavalent core. This ligand revealed an in vitro inhibitory activity 1-10 million fold higher than any other ligands. The success of such strong affinity is due to the symmetry and size of the construct that precisely match the toxin’s B subunit homopentamer.

Identification of glycan epitopes on the surface of pathogens and malignant cells have also stimulated the development of carbohydrate-based vaccine against diseases caused by bacteria, virus, fungi, parasite and cancer (38). In the past, carbohydrate-based vaccine suffered from low immunogenicity, however, this problem could now be overcome by the use of adjuvants, integration into complex multi-components vaccines, and predominantly, covalent attachment to immunostimulants (conjugate vaccines) (39). The development of glycoprotein vaccines could benefit greatly from the design and synthesis of well-defined structures. The success of a glycoprotein vaccine does not only rely on antigen and carrier protein choice but also on its structure, which is determined by several parameters including the polysaccharide length and branching, the degree of conjugation to the protein, the type of spacer and the conjugation chemistry used (40). Currently, the only marketed synthetic carbohydrate-based vaccine is the Cuban conjugate vaccine against Haemophilus influenzae Type b (Hib) developed in collaboration with
1. Background and Purpose

Canada (Figure 2, E). The vaccine is composed of ribosylribitol pentasaccharides, based on Hib capsular polysaccharide antigen, and is conjugated to tetanus toxoid (41).

Figure 2 Structure of carbohydrate-based drugs. A) Zanamivir (Relanza): glucosyltransferase inhibitor, influenza antiviral, on the market (16a). B) Fondaparinux (Arixtra): SGAG, antithrombotic, on the market (22). C) Bimosiamose: selectin antagonist, asthma and psoriasis, clinical trial (25a). D) STARFISH: multivalent carbohydrate ligand, Shiga toxin-producing Escherichia coli STEC infection (35b). E) Haemophilus influenza type b: vaccine, influenza virus, on the market (41).

1.2.2. Challenges in synthesis

In the last three decades, there have been several important advances in carbohydrate chemistry that allowed the preparation of ever more complex complex glycan structures. Notably, one-pot glycosylation (42) and solid-supported synthesis (43) are two approaches that considerably streamlined the oligosaccharide assembly by reducing the number of purification steps required. However, the complexity behind the preparation of well-defined oligosaccharides, including regioselectivity, stereoselectivity and branching issues, has so far hampered chemists to establish a routine process akin to that of peptide and oligonucleotide synthesis.

Central to carbohydrate chemistry is the glycosylation reaction, which consists in the assembly of a glycosyl donor with a glycosyl acceptor: upon activation of the glycosyl donor’s leaving group, the anomic position undergoes nucleophilic substitution by a glycosyl acceptor. The major challenge in the glycosidic bond formation is to control the stereoselectivity which is modulated by numerous
factors. The configuration, protecting groups and leaving group of the glycosyl donor represent the most crucial parameters that influence the stereoselectivity outcome of the reaction. In addition, the activation method, reaction conditions, and glycosyl acceptor properties also play a role. (44) As a result, a very large number of methods have been developed (45) and good control on the stereoselectivity can nowadays be achieved (46).

A few relevant examples of stereoselective glycosylation are presented here to highlight the main advances in the control of the anomic stereoselectivity, more details can be found in reviews focusing on oligosaccharides synthesis (47), including the excellent review from Zhu and Schmidt (45a).

A prevalent strategy in the formation of the 1,2-trans glycosides is the neighboring group participation. In this type of reaction, the oxocarbenium species formed after activation of the leaving group will be trapped by the 2-O acyl protecting group to form the more stable cis-dioxolenium ion species 2 (Scheme 1, A). Thus, the subsequent S_N2 displacement by the alcohol at the anomic center will be possible only from one face giving the 1,2-trans glycoside, resulting in the β-product for the glucosyl-type glycoside and the α-product for the mannoside-type. More recently, a new neighboring group participation method has emerged to give access to the 1,2-cis glycosides by formation of a trans sulfonium ion 5 (Scheme 1, B) (48).

Alternatively, control of the anomic stereoselectivity can be achieved via electronic and steric effects of the protecting groups. Conformation-constraining protecting groups have shown great performance in stereoselectivity. For instance the 4,6-O-benzylidene protecting group 7 introduced by Crich et al. led to excellent selectivity in the challenging cis glycosylation of mannose (Scheme 1, C) (49). It is proposed that the oxocarbenium ion is disfavored, resulting in the formation of the α-triflate intermediate 8 (pool of contact ion pair CIP and solvent-separated ion pair SSIP). Thus the α-face is shielded and subsequent nucleophilic attack by the glycosyl acceptor gives the β-mannoside. In-depth investigations into the mechanism concluded that the disfavored formation of the oxocarbenium ion is the consequence of torsional and electronic effects (47b). The torsional strain of the fused ring in the glycoside donor chair-chair conformation is increased in the chair-half chair or chair-boat conformation of the oxocarbenium ion intermediate. Also, Bols et al. pointed out that acetal function locks the C6-O6 bond antiperiplanar to the the C5-O5 bond, which orients the dipole away from the positive charge in the transient oxocarbenium ion. As a consequence, electron-withdrawing effect is maximized and charge dipole interaction minimized, thus provoking electronic and electrostatic destabilization of the transient oxocarbenium ion (50). Other conformation-constraining protecting groups have been developed such as carbonate and oxazolidinone (51) or cyclic silyl groups (52).
1. Background and Purpose

Lately a new concept has emerged called hydrogen bond-mediated aglycon delivery (HAD) in which a remote protecting group (picolinyl or picoloyl) forms a hydrogen bond with the glycosyl acceptor thus directing the nucleophilic attack from the same face with respect to the protecting group (Scheme 1, D) (53). This approach is reminiscent of the intramolecular glycosylation (or intramolecular aglycon delivery) that has been developed in the last decade (45a, 46).

**Scheme 1** Examples of stereoselective glycosylations. A) Neighbouring group participation promoting 1,2-trans glycosides. B) Neighbouring group participation promoting 1,2-cis glycosides. C) Constraining 4,6-O-benzylidene mannoside promoting 1,2-cis glycosides. D) Hydrogen bond-mediated aglycon delivery promoting syn glycosides in respect to 3, 4 or 6-picolinyl/picoloyl.

Efforts to understand the mechanism underlying the stereocontrol of the anomeric center have helped to shed light on the various factors that influence the stereoselectivity. One of the most critical parameters is the effect of the protecting groups that can increase or decrease the reactivity of the glycosyl donor, participate to the formation of the glycosidic bond, induce face discrimination and influence conformation. However, a clear picture of the mechanistic principles remain elusive, as Green and Ley mentioned it “much of the evidence used to substantiate proposed inter-glycosyl coupling mechanisms is anecdotal or circumstantial” (47a). A deeper understanding in the distinction between $S_\text{n}1$ and $S_\text{n}2$ pathway, in the elements that govern conformational preference of the transition state or that induce face discrimination of the incoming nucleophile would allow the prediction of reactivity and lead to considerable breakthroughs.
Chapter 3 of this thesis is dedicated to the study of 2-fluorosugars glycosylation. Fluorine directs excellent β-stereoselectivity in the galactopyranosyl system. A systematic study was realized to give insight into the effect of the fluorine, and supported the suggestion that fluorine induces strong conformational preference in the oxocarbenium ion leading to β-stereoselectivity. Application of this methodology was assessed on the synthesis of common disaccharide building block analogues containing the 2-fluorogalactopyranosyl β-linked motif. Finally, synthesis of a fluorinated analogue of the glycosphingolipid β-galactose ceramide illustrated the utility of this methodology.
1. Background and Purpose

1.3. Inositol phosphates

1.3.1. Biological role

Inositol phosphates are small molecules ubiquitously found in eukaryotic cells where they play a number of key biological roles. They can be found in the form of inositol phosphates (InsPs), the higher-energy diphosphoinositol phosphates (PP_x-InsPs), and the membrane-bound phosphatidylinositol phosphates (Ptd-InsPs) (Figure 3). The various forms of inositol phosphates are all metabolically interconnected via a large network of kinases and phosphatases (54).

There are 63 theoretically possible phosphorylated isomers of InsPs (54a). Many of them have been observed, but only few have been linked to a clear biological function. However, we know that InsPs are crucial players in a number of key cellular processes such as calcium homeostasis, signaling, and gene regulation. For instance, Ins(1,4,5)P_3 (Scheme 1, D) has been widely studied and its role as second messenger in calcium release mechanism is well understood (4). The numerous biological functions of InsPs have been reviewed elsewhere and will not be discussed in detail here (5, 54a, 55).

Despite their obvious importance, the specific role of InsPs has been elucidated only in a few cases and many questions remains.

The PP_x-InsPs, are fully phosphorylated inositol with one or two pyrophosphate groups and are thus highly energetic, with a similar phosphorylation potential to ATP (56). PP_1InsP_5 and PP_2InsP_4 have been identified only recently, and have since been found to act in apoptosis, vesicle trafficking, stress response of cells, regulation of telomere length, DNA damage repair, insulin signaling, and innate immune response (57).

Finally, the Ptd-InsPs contain one glycerophospholipid chain attached to position 1 of inositol. A total of 8 isomers have been described, with 0 to 3 phosphate groups in position 3, 4 and or 5. The plasma membrane-bound Ptd-InsPs play a key role in the regulation of membrane trafficking, cell signaling and cell structural maintaining by recruiting soluble proteins to the membrane through interaction with the pleckstrin homology (PH) domain found in over 250 proteins (58). However, only a fraction of these putative protein partners have been shown to bind strongly to Ptd-InsPs.
1. Background and Purpose

Despite their crucial roles in cell biology, a comprehensive picture of InsPs’ functions is still lacking, and a better understanding of the interactions of InsPs on a molecular-level is necessary. This is due in part to the challenge of isolating pure InsPs, the large number of possible inositol phosphate structures and the daunting number of interacting partners. In addition, highly phosphorylated inositols have an unusually high charge density that affects strongly their physicochemical properties and solubility, a fact that has led to a number of artifacts in the biological literature (59). Furthermore, the high charge density renders most InsPs impermeable to the cellular membrane, which complicates their study in cells.

The chemical synthesis of InsPs has been pivotal in deciphering their role and enabling the study of key interaction partners. The synthesis not only allows access to well defined and pure structures, but also enables the preparation of unnatural analogues that can be used as invaluable chemical tools in cell biology studies. The myriads of analogues and their use have been reviewed recently and will not be discussed in details here (5). Noteworthy examples include metabolically stable analogues of the phosphate groups (54b, 60), and the incorporation of probes on the glycerol backbone of PtdInsP such as biotin for affinity labeling, fluorescent dyes for optical detection, and photoaffinity tags for cross linking interacting proteins (61). In addition, caged and cell-permeable inositol derivatives designed to hydrolyze once in the cytosol have allowed the intracellular study of InsPs (62). Recently, Ptd-InsPs-specific antibodies have been developed using synthetic material and expand the toolbox available for their study (63).

The structural study of PP$_2$-InsPs has been particularly problematic due to their instability, high charge and low cellular concentrations resulting from rapid turnover. Chemical synthesis of the different PP$_2$-InsPs regioisomers contributed a lot in their structural assignment (64). The more heavily phosphorylated chiral 1,5- and 3,5-PP$_2$-InsP$_4$ have only been synthesized recently and should prove pivotal in the elucidation of their role (65).

Beyond chemical tools, some InsPs analogues have interesting therapeutic potential as recently demonstrated by the synthesis of red blood cell-permeable IP$_6$ analogues that have application in the
treatment of diseases involving hypoxia due to their allosteric regulation of oxygen release by hemoglobin (66).

A large number of InsPs, including all Ptd-InsPs, are chiral thus complicating significantly their synthesis. Chiral precursors such as glucosyl (67), conduritol (68), and quebrachitol (69) have been used as starting material but usually require a large number of chemical steps. Thus the meso myo-inositol precursor remains the main starting material for InsPs synthesis. The challenge of discriminating the inositol’s 6 nearly equivalent hydroxyl groups has been addressed by a number of strategies, giving access to different partially protected key intermediates such as ketals or orthoformates (5, 69a, 70). Optically pure InsPs have been obtained via chiral resolution of the racemic inositol intermediates using chiral auxiliary like camphor acetal (71), O-acetylmandelic acid (72), or menthylacyl (70b) groups. Although these methods allow access to a myriad of InsPs and derivatives, the routes are generally long due to protecting group manipulations. Of note, the phosphate protecting groups’ relative sensitivity toward hydrolysis and migration, coupled to the tedious purification of the highly charged InsPs often result in low overall yields.

In the light of these difficulties, it is obvious that more efficient methods are required to perform phosphorylation on myo-inositol in a regio- and stereoselective fashion. Elegant new strategies based on desymmetrization of myo-inositol by phosphorylation have recently been developed and stand to improve dramatically the synthesis and allow the preparation of more complex analogues. These strategies will be discussed here, along with potential future directions in chiral phosphorylation methods, especially in the context of the biologically relevant InsPs.

1.3.2. New paradigms for the Chiral Synthesis of Inositol Phosphates


1.3.2.1. Challenges

Myo-inositol is a meso compound with a plane of symmetry along the axis containing C2 and C5. The hydroxyl groups in position 1 and 3 as well as 4 and 6 are enantiotopic to each other. The desymmetrization of myo-inositol by phosphorylation event can be achieved either via a chiral resolution with chiral phosphate protecting group or an asymmetric phosphorylation.

The phosphorylation of alcohols is usually performed with organophosphorus reagents of oxidation state +5 (P(V)) (e.g. chlorophosphates, pyrophosphates) or +3 (P(III)) (e.g. phosphochloridites, phosphoramidites, and phosphites) followed by oxidation.
1. Background and Purpose

In the case of chiral resolution, a chiral phosphate group needs to be present so that the reaction results in two separable diastereoisomers.

Asymmetric phosphorylation to desymmetrize meso inositol derivative is even more challenging but nonetheless a powerful tool. Enantioselective desymmetrization of meso inositol is complex due to the presence of several reactive centers. In addition to that, the distance of the reactive centers of the 1,3-diol motifs of meso inositol derivatives (1,3 or 4,6 positions) requires an efficient chiral induction to break the symmetry plane.

1.3.2.2. Enantioselective synthesis of inositol phosphate

*Enantioselective phosphorylation synthesis*

Inspired from nature, Miller *et al.* have successfully designed the first non-enzymatic catalytic enantioselective phosphorylation (73). Pentapeptides mimics of histidine-specific kinases (14 and 15) containing a modified histidine residue were used to catalyze the asymmetric phosphorylation of inositol 13 to afford 1P-Ins and 3P-Ins in high optical purity (enantiomeric excess (ee) > 98%) (Scheme 2, A).

![Scheme 2 A](image)

**Scheme 2 A** Enantioselective phosphorylation. a) (PhO)₃POCl, Et₂N, Peptide 2.5 mol%. B) Catalytic phosphorylation.
1. Background and Purpose

Of the 3 possible hydroxyl groups in inositol 13 only the two more reactive enantiotopic hydroxyl groups in position 1 and 3 react. The catalytic cycle starts with the formation of the activated phosphoryl imidazolium ion 16, which provides a chiral environment for the stereospecific phosphorylation and concomitant regeneration of the peptide (Figure 2, B). The catalyst 14 was selected from a screening of a 39-membered peptide library constructed randomly to maximize diversity. An extended library of 136 peptides followed by a focused library of 42 members yielded the sequence of catalyst 15. It is noteworthy to point out the enantiodivergence of the catalysts, both have the same absolute configuration and contain a β-turn secondary structure but each of them yields a distinct enantiomer. In analogy to enzymes, the stereochemical information relies on the catalyst’s ability to adopt a specific three-dimensional conformation that results in enantiomer-specific rate acceleration (73a). This enantioselective catalytic phosphorylation allowed the access to a wide range of highly optically pure InsPs derivatives (74) as well as Ptd-InsPs (63, 75) in a relatively concise and efficient route (Scheme 3).

Modification of benzyl protecting groups to p-methoxybenzyl groups and allyl groups on the substrate does not affect the stereochemical outcome of the asymmetric phosphorylation thus allowing orthogonal manipulation of the protecting groups to give rapid access to numerous enantiomerically pure inositol polyphosphates such as InsP₂, InsP₃, InsP₄ (Scheme 3, A) (75c). Alternatively, deoxygenated analogues 19, useful molecules to scrutinize interactions with proteins, were readily accessible via Barton-McCombie deoxygenation reaction (Scheme 3, B) (74d). In addition, phosphitylation of inositol 20 with glycerol phosphoramidite enlarged the scope to the enantioselective synthesis of Ptd-InsPs (61e, 75a). Moreover, introduction of Lewis acid-labile final protecting group in this synthesis strategy allowed the introduction of the natural arachidonate lipid which contains unsaturation that are not compatible with the usual final deprotection strategies (e.g. hydrogenation) (Scheme 3, C) (63, 75a). Further manipulation of the phosphate group expanded even more the usefulness of this methodology. Introduction of a wide variety of probes on the glycerol moiety (e.g. fluorescent dye, photoaffinity tag, etc), that are useful instruments for biological investigations, could be obtained by development of a Mitsunobu-type reaction on a partially hydrolyzed inositol 22 (Scheme 3, D) (61e, 75b). Meanwhile, modification of inositol phosphate 17 to obtain inositol chlorophosphate 22 offers a new substrate for the catalytic asymmetric phosphorylation to deliver di-inositol-1,3-phosphate 24 that is involved in temperature regulation of several hyperthermophilic organism (Scheme 3, E) (74a).
Unequivocally, the histidine peptide-based catalyst provides a remarkable tool in asymmetric phosphorylation to rapidly deliver both inositol 1-InsP$_1$ and 3-InsP$_1$ in high optical purity and provides relatively concise routes to other enantiopure inositol phosphate derivatives. Nevertheless, the methodology suffers from drawbacks such as protecting group on the chlorophosphate agent, which is limited to phenyl phosphate. Phosphorylation attempt with the more useful dibenzyl phosphate chloridate under the peptide-catalyzed conditions led to nucleophilic substitution on the benzylic carbon instead of the phosphorus (76). This limitation can be overcome by a cumbersome but high-yielding three steps process: TBS protection of the free hydroxy groups, transesterification of the phenyl phosphate groups to the benzyl with benzyl alcohol under basic conditions and TBS deprotection (75a). In addition, the scope of this reaction is limited to position 1 and 3 and does not proceed enantioselectively for the phosphorylation of the more challenging enantiotopic position 4 and 6 (77).

*Enantioselective phosphitylation synthesis*

In the widely used phosphoramidite chemistry, an activator such as tetrazole is required to promote the phosphoryl transfer. Tetrazole protonates the nitrogen of the P-N bond thus enhancing the electrophilicity of the phosphorus which is subsequently attacked by the tetrazolide anion to form the intermediate tetrazolyl phosphate 26 (Scheme 4, A) (78). The latter is then prone to undergo alcoholysis. Usually an excess of tetrazole is used because it ultimately forms an ion pair with the protonated amine released from the phosphoramidite reagent. Hayakawa (79) and more recently Sculimbrene (80) have developed a catalytic version of this P$_{III}$ phosphitylation where an amine scavenger such as 10 Å molecular sieves or phenylisocyanate is added to the mixture in order to regenerate the tetrazole catalyst. This enabled Miller to design a new chiral catalyst for the phosphorylation by replacing the histidine of his previous peptide catalyst by a tetrazolylalanine moiety (Scheme 4, B) (81).
1. Background and Purpose

A) Tetrazole promoted phosphitylation

B) Tetrazolylalanine-based peptide catalytic phosphitylation

Scheme 4 A) Tetrazole promoted phosphitylation. B) Tetrazolylalanine-based peptide catalytic phosphitylation. A small library of peptide terminating with Boc-L-tetrazolylalanine has been screened for the challenging differentiation of enantiotopic hydroxy groups 4 and 6 of the meso inositol 30. Depending on the peptide sequence, phosphitylation could be observed preferentially on position 4 or 6 with an enantiomer ratio of at least 70:30 and formation of bisphosphorylated product 33 was observed.

However, this asymmetric phosphitylation revealed to be more useful in the context of kinetic resolution on the racemic mixture of 4/6 monophosphorylated inositol 32 (Scheme 5, A). Indeed, phosphorylation of one enantiomer leads to the bisphosphorylated compound 33 with the high relative rate of 34.5. Enantioselective synthesis of inositol 6 phosphate can thus be achieved in high optical purity (> 98 enantiomeric ratio (e.r.)) via 2 sequential asymmetric phosphitylation (Scheme 5, B). A first phosphorylation with peptide 27 gives 32 in 71% yield in e.r. of 85:15. The minor enantiomer undergoes a second phosphorylation under kinetic resolution and 32 was obtained in e.r. of 98:2. Thus D-my-ino-sitol-6-phosphate could be prepared in 3 steps from in a total yield of 52.4% and an optical purity of 98.1%.
1. Background and Purpose

A) Kinetic resolution

\[
\begin{array}{c}
\text{PMBO} & \text{OH} & \text{OPMB} \\
\text{HO'} & \text{OPMB} & \text{OPMB} \\
\text{(t)-32} & \text{a, b} & \text{32} + \text{33} \\
\end{array}
\]

\[
k_{rel} = 34.5
\]

B) Asymmetric synthesis of 6-InsP₁

\[
\begin{array}{c}
\text{PMBO} & \text{OH} & \text{OPMB} \\
\text{HO'} & \text{OPMB} & \text{OPMB} \\
\text{30} & \text{a, b} & \text{32} \text{ er 85:15} \\
\end{array}
\]

\[
\begin{array}{c}
\text{PMBO} & \text{OH} & \text{OPMB} \\
\text{HO'} & \text{OPMB} & \text{OPMB} \\
\text{32} \text{ er 98.2} & \text{c} & \text{6-InsP₁} \text{ er 98:2} \\
\end{array}
\]

Scheme 5 A) Kinetic resolution. B) Asymmetric synthesis of 6-InsP₁. a) (BnO)₂PNET₂, Peptide 27 5 mol%, 10 Å M.S.; b) H₂O₂; c) NH₃, Na, -78 °C; d) Dowex 50WX8-500, cyclohexylamine.

1.3.2.3. Desymmetrisation via C₂-chiral phosphoramidite

Jessen et al. have merged the classical methods used in desymmetrisation of myo-inositol, meaning the resolution by chiral auxiliary, and phosphitylation to give birth to a desymmetrizing phosphitylation via a chiral phosphoramidite (65, 82). The chiral phosphoramidite 34 was designed so that it is C₂-symmetric (Scheme 6, A), therefore the chirality is not on the phosphorus atom where takes place the nucleophilic displacement but rather on the chiral substituents avoiding any racemization problems. The chiral auxiliary is easily obtained in few steps from mandelic acid, and contains a β-cyanoethyl moiety, which is a base-labile phosphate protecting group commonly used in nucleic acid chemistry.

Desymmetrisation of numerous meso inositol could be achieved and delivered chiral inositol phosphate in good to excellent optical purity (Scheme 6). Phosphitylation with 34 and subsequent oxidation of various meso inositol derivatives gave a mixture of diastereoisomers separable by crystallization or flash column chromatography. Meso compound 35 owning two enantiotopic hydroxy groups in positions 1 and 3 was subjected to monophosphitylation with the chiral phosphoramidite 34 affording a 1:1 mixture of two diastereoisomers (Scheme 6, B). In the case R = PMB, the two isomers were separated by crystallization and both could be purified giving excellent diastereomeric ratio of 98:2 and > 99:1 for 36a and 37a respectively. As for diastereoisomers 36b and 37b, R = (FmO)₂P(O), they were separated by repeated flash chromatography and were obtained in good chiral purity of 90 and 95% respectively. Monophosphitylation of enantiotopic hydroxy group in position 4 and 6 in meso compound 38 afforded a 1.0:0.8 mixture of diastereoisomers 39 and 40, which were separated by flash chromatography and obtained in excellent diastereomeric ratios.
1. Background and Purpose

above 99:1 (Scheme 6, C). One drawback of this method is the excess of inositol substrate needed to avoid bisphosphitylation. Based on phosphoramidite, 3 equivalents of protected inositol are required to obtain monophosphitylation; however starting material can be recovered.

Application of this \( C_2 \)-symmetric phosphoramidite to the challenging synthesis of all chiral PP-InsP\(_5\) isomers and the first synthesis of chiral PP\(_2\)-InsP\(_4\) underscores the great potential of this new methodology (Scheme 7). Synthesis of PP\(_x\)-InsP\(_y\) requires a strategy with two different sets of phosphate protecting groups orthogonal to each other. Expecting that the newly designed chiral auxiliary would behave similarly to its close relative \( \beta \)-cyanoethyl, this chiral phosphotriester was used to generate the P-anhydride bond. Once the inositol intermediates have been desymmetrized with the chiral phosphate at the position corresponding to the future phosphoanhydride bond, the rest of the protecting groups were cleaved and the resulting free hydroxy groups were phosphorylated with o-xylylene phosphoramidite and oxidized to form the hexaphosphate such as 41. Orthogonal deprotection of the chiral auxiliary and subsequent phosphitylation/oxidation led to the formation of the protected PP-InsP\(_5\), which is ultimately deprotected by hydrogenolysis. Synthesis of the PP\(_2\)-InsP\(_4\) differs slightly in the way that the P-anhydride bond in position 5 is protected by the bis-fluorenyl (Fm) phosphate ester, which is later on deprotected in parallel with the chiral auxiliary.
1. Background and Purpose

This strategy led Jessen et al. to develop a new and efficient methodology to form P-anhydride bond using P\textsuperscript{III} chemistry in a one pot sequence: clean chiral auxiliary cleavage and P-anhydride bond formation yielded the protected anhydride 43 in about 90% within one hour (Scheme 7, B). The mild removal of the chiral auxiliary and Fm proceeds through their replacement under basic conditions with the more labile TMS groups, which were then methanolized in presence of TFA to give the monoprotonated intermediate 47 (Scheme 7, B). Phosphitylation with dibenzylphosphoramidite and subsequent oxidation forms the P-anhydride bond 43. In addition to being very efficient and streamlined, the newly formed P-anhydride is not protected on the \(\alpha\)-phosphate rendering the pyrophosphate more stable toward hydrolysis, which contributed to deliver PP\textsubscript{x}-InsPs in good purity. Furthermore, this new methodology using P\textsuperscript{III} gives the advantage that sulfur or selenium can easily be used in the oxidation step, allowing the synthesis of new thio phosphate or selenophosphate analogues.

\begin{scheme}
\textbf{A) Synthesis of PP\textsubscript{x}-InsPs}

\begin{center}
\begin{tikzpicture}
\node at (0,0) {41};
\node at (1.5,0) {42};
\node at (3,0) {43};
\node at (4.5,0) {44};
\node at (6,0) {45};
\node at (7.5,0) {46};
\end{tikzpicture}
\end{center}

\textbf{B) Chiral auxiliary deprotection}

\begin{center}
\begin{tikzpicture}
\node at (0,0) {41};
\node at (1.5,0) {42};
\node at (3,0) {43};
\node at (4.5,0) {44};
\node at (6,0) {45};
\node at (7.5,0) {46};
\end{tikzpicture}
\end{center}

\textbf{Scheme 7 A) Synthesis of PP\textsubscript{x}-InsPs. a) 1. DBU, BSTFA, 2. TFA in MeOH; b) 1. \(\text{BnO}_2\) \(P-N\)(\text{Pr})\textsubscript{2}, tetrazole, 2. mCPBA; c) \(H_2\) (80 bar), PtO\textsubscript{2} or Pd (black). B) Chiral auxiliary deprotection.}

1.3.3. Future perspectives in asymmetric phosphorylation

Asymmetric peptide-based catalyst and desymmetrization with \(C_2\)-chiral phosphoramidite 34 are the only two chiral phosphorylation methodologies that are available not only for inositol phosphate but in general. Considering the large demand in synthesis of chiral InsPs derivatives and the importance of other chiral molecules containing phosphate groups (83), there is space and need for new desymmetrizing phosphorylation methods to arise in this area. While this field remains
underexploited, we would like to highlight the potential of existing methods to be applied to chiral phosphorylation.

1.3.3.1. Asymmetric phosphorylation with phosphorylating agent containing chiral auxiliary

A suitable asymmetric phosphorylating reagent should fulfill several criteria, namely strong stereoselective induction, easy displacement of the leaving group and separable products for chiral enrichment.

Jones et al. have developed phosphorylation method based on phosphoramidates: phosphoryl oxazolidinone (84). Chiral oxazolidinones are known to be successful in asymmetric acylation of alcohol (85). However Jones pointed out the difficulty to induce efficient stereoselectivity in phosphorylation reaction, which stems from the difference of mechanism involved in the two reactions (Figure 4) (86). Nucleophilic attack on the carbonyl group proceeds via Bürgi-Dunitz angle whereas at the phosphorus center, nucleophile comes in apical position, opposite to the leaving group. This change in the incoming nucleophile trajectory means that in phosphorylation, the stereochemical information of the auxiliary is further apart from the nucleophile, thus decreasing its ability to discriminate one of its faces. Therefore the design of new chiral auxiliaries should contain extensive ligands that could favor interaction with one enantiotopic side of the substrate. Furthermore the chiral auxiliary should be tuned to be a good leaving group, and interesting works on phosphoramidates chemistry revealed that thiazolidines are valuable chiral leaving groups (87). Therefore both of these elements would contribute to lower the transition state energy of one enantiotopic side. Finally, if the introduced phosphate group is chiral, phosphorylation would result in two diasteroisomers instead of enantiomers, hence chiral enrichment would be facilitated.

![Figure 4](image)

**Figure 4** A) Acylation with chiral oxazolidinone. B) Phosphorylation with chiral oxazolidinone. C) Phosphorylation Newman projection.
1.3.3.2. Asymmetric phosphorylation with chiral catalyst

Stereoselective desymmetrization of diols via chiral catalysis reaction has lately attracted interest and powerful synthetic tools have been developed especially in the acylation realm (88) but also few other transformations such as sulfonylation (89) and silylation (90). Based on these achievements, different strategies to stereoselectively desymmetrize inositol by catalytic phosphorylation reaction can be considered: nucleophilic organocatalysis including the use of scaffold (Figure 5, A and B) and metal catalyst (Figure 5, C).

![Catalytic asymmetric phosphorylation approaches](image)

**Figure 5** Catalytic asymmetric phosphorylation approaches **A)** Nucleophilic organocatalyst. **B)** Scaffold catalyst. **C)** Metal catalyst.

**Nucleophilic organocatalyst**

An array of chiral nucleophilic organocatalysts have been designed and successfully applied in the asymmetric desymmetrization of diols for stereoselective acyl transfer. Miller’s peptide-based catalysts are the perfect illustration that highlights the use of chiral nucleophilic catalyst in both reactions phosphorylation and phosphitylation to stereoselectively desymmetrize inositol (see section 1.3.2.2). The chiral nucleophilic organocatalysts are based on chiral derivatization of strong nucleophile moieties such as imidazole, 4-aminopyridine, amidine, vicinal diamine (cinchona alkaloid). To enable the stereoselective desymmetrization, the catalyst should possess a chiral chain giving phosphoryl intermediate (Figure 5, A) the ability to interact with the substrate in order to orient it in an energetically favored and conformationally well-defined transition state, thus accelerating the reaction rate of one enantiotopic hydroxy group.

**Scaffold catalyst**

A less common but nonetheless powerful method is the use of the so called “scaffold catalyst” (91). The distinct feature of this concept, inspired from enzyme (e.g. glycosyltransferases, enzyme
1. Background and Purpose

proceeding via catalytic triad such as protease) mode of action, lies in the formation of a reversible covalent bond between the catalyst and the substrate (Figure 5, B). As a consequence the reaction will be intramolecular, leading to an entropic gain corresponding to an enhancement in the reaction rate. It is particularly interesting in the context of diol desymmetrization because we have two functional hydroxyl groups, while one can form the covalent bond with the catalyst scaffold, the other can undergo the transformation. One recent example of such catalyst applied to the challenging desymmetrization of glycerol by silylation underscores the potential of this method (91b).

**Metal Catalyst**

Alternatively, stereoselective desymmetrization of diols has also been performed with chiral metal complexes (Figure 5, C) (88b). The mechanism relies on the coordination of the metal catalyst with the substrate rendering the hydroxyl group more reactive toward transformation. The chiral ligands give access to two diastereotopic intermediates and provide a chiral environment for the substrate to undergo selective reaction. This approach could be especially of interest in the desymmetrization of orthoformate inositol derivatives in which the two axial hydroxyl groups are prone to Lewis acid complexation. Interestingly, Sculimbrene et al. have lately developed a desymmetrization of 1,3-diols by phosphorylation based on titanium alkoxide catalyst (Scheme 8(Scheme 8 **Selective monophosphorylation of diols via catalytic titanium phosphorylation with pyrophosphates.**) (92).

One can easily envision 1,3- or 4,6-diols of inositol derivatives as substrate for selective monophosphorylation. Development of a chiral version of the titanium alkoxide catalyst represents a great opportunity to develop a new tool in the asymmetric phosphorylation of inositol.

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**Scheme 8** Selective monophosphorylation of diols via catalytic titanium phosphorylation with pyrophosphates.

1.3.3.3. Conclusion

The two approaches of desymmetrization by phosphorylation made an incredible breakthrough in the synthesis of chiral InsPs. They offer a high optical purity and considerably streamlined the synthesis. The asymmetric catalytic phosphorylation/phosphitylation designed by Miller et al. gave rapid access to highly pure enantiomers inositol monophosphates 1-InsP<sub>1</sub>, 3-InsP<sub>1</sub> and 6-InsP<sub>1</sub>. Over the years, a tremendous potential has been demonstrated in complex syntheses of chiral inositol
1. Background and Purpose

polyphosphates, phosphatidylinositols and analogues. Despite all of that, this approach has some constraints such as the access to the peptide catalysts and the limitation in the phosphorylating reagent choice even if this can be circumvent as discussed above. The more recent approach developed by Jessen et al. based on desymmetrization using a chiral C₂-symmetric phosphoramidite complements the available tools in chiral inositol phosphate syntheses. Like other chiral resolution, this method suffers from the fact that only 50% of one diastereoisomer can be obtained, on the other hand it can be interesting to obtain the two diastereoisomers in one step. The chiral phosphoramidite allows the desymmetrization of enantiotopic position 1/3 and 4/6 and offers an orthogonal protecting group basic labile and stable to acidic conditions. This new property showed its powerful potential in the challenging synthesis of the densely phosphorylated inositols PPₓ-InsPs. The contrast between the available methods and the large demand to prepare chiral InsPs underlines the need to develop new phosphorylation methods that allows chiral synthesis. Chiral auxiliaries, chiral nucleophilic and metal catalysts are all approaches that had success in asymmetric acylation and others transformations. Many elements discussed above show that these approaches should be promising to bring new methods in desymmetrization phosphorylation.
2. The synthesis of inositol hybrids: towards a potential drug against Clostridium difficile Infection

2. The synthesis of inositol hybrids: towards a potential drug against

*Clostridium difficile* Infection

2.1. Introduction

2.1.1. *Clostridium difficile* Infection

*Clostridium difficile* infection (CDI) is a bacterial infection of the intestine that can lead to serious consequences and is potentially fatal. Symptoms range from mild to severe diarrhea, to pseudomembranous colitis and in more severe cases to life-threatening toxic megacolon (93).

CDI is usually associated with hospitalized patients that have been under antibiotic treatments. *Clostridium difficile* is a Gram-positive bacterium that forms spores that are resistant to most disinfectant cleanser routinely used. These spores can survive in hostile environment up to two years, and are easily spread out through contact or air. These spores are transmitted via oral-fecal route, thus hospitals and other care facilities represent favorable environment for their propagation, making CDI the most important causes of infectious nosocomial diarrhea (94). *Clostridium difficile* can be present in the gut flora of healthy people but is kept under control by the microbiota. Antibiotic treatment disrupts the gut flora and allows *C. difficile* to overgrow and to express the virulent factors causing disease (95). CDI is the primary cause of antibiotic-associated colitis and represents 15 to 25% of the nosocomial antibiotic-associated diarrhea (96).

In 2013, the center for disease control and prevention (CDC) has reported that yearly, 250 000 people become infected in the United States and around 14 000 deaths are attributed to CDI (97). Epidemiology studies in the United States and in Europe have shown an explosion in the frequency, it has risen by 3 times within the last 15 years, and the rate of recurrence (98). The most affected patients are the elderly, however recent reports present an increasing number of CDI within the younger population (99). These alarming observations are the consequence of the emergence of hypervirulent strains, making CDI an immediate public health treat (100).

To date only antibiotic treatments are available on the market: metronidazole, vancomycin and fidaxomicin. Metronidazole is usually prescribed as a first line therapy for nonsevere CDI and vancomycin is more effective in the treatment of more severe case or recurrent infection (101). Metronidazole and vancomycin’s efficacies are far from satisfying and a recent systematic review have estimated treatment failure rate at 22.4% and 14.1% and a recurrence rate after treatment of 27.1% and 24.0% respectively (102). Fidaxomicin has recently been approved by the US Food and
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Drug Administration (FDA) against CDI. In contrast to its counterparts that are both broad-spectrum antibiotics, fidaxomicin is more specific to C. difficile and seems to not only reduce the risk of recurrence but also the risk of reinfection with different strains (103). Fecal Microbiota Transplantation (FMT) has recently emerged as a promising therapy that aims to restore the healthy gut flora. It has proven remarkably effective at eradicating CDI in patients where antibiotic treatments failed (104). Despite its success, the FDA has not yet established a clear position concerning FMT because of ethical reasons (105). Indeed, the standardization of this type of therapy will be challenging. Other therapies currently being explored such as alternative antibiotics, passive immunization, vaccines, or microbial therapies are reviewed elsewhere (106).

2.1.2. Clostridium difficile pathogenesis

Pathogenesis of CDI is mainly caused by two toxins secreted by Clostridium difficile: toxin A (TcdA) and toxin B (TcdB). The importance of TcdB in causing disease has been widely approved, however, discussions to clarify the importance of TcdA are still ongoing (107).

TcdA and TcdB belong to the large clostridial toxins (LCTs) family which is commonly described as “AB toxins” (108). They are composed of two main domains, the B moiety is involved in the binding to the target cell and the A moiety possesses the toxic enzymatic activity. In LCT the A moiety is a glucosyltransferase that inactivates the Rho GTPase by glucosylation resulting in perturbation of the downstream cellular activities that ultimately leads to cell deaths. TcdA and TcdB possess 68% of homology with TcdA being larger than TcdB (308 and 270 kDa, respectively), thus presenting similar structure and mode of action (109). The structure can be represented by four functional domains: a receptor binding domain (RBD), a translocation domain, a cysteine protease domain (CPD) and an enzymatic domain (Figure 6, A) (110). The RBD is composed of repeat units and enables TcdA and TcdB, which are secreted in the lumen, to interact with the epithelial cells and induce toxin endocytosis (Figure 6, C) (111). The endosome acidifying pH leads to a conformational change of the toxin resulting in insertion of the hydrophobic translocation domain in the endosomal membrane, pore formation and translocation of the CPD and enzymatic domain to the cytosol (110b). Intracellular InsP6 then binds to the CPD and induces toxin auto-cleavage and release of the enzymatic glucosyltransferase “warhead” which causes pathogenesis (108).
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Figure 6 Toxins structure and mechanism. A) Structural organisation of TcdA and TcdB functional domain. B) 3D structure of TcdA revealed by negative stain electron microscopy (109). Receptor binding domain (green), translocation domain (yellow), cysteine protease domain (blue), glucosyltransferase (red). C) Molecular mechanism of action of TcdA and TcdB (34). 1. The toxin binds to the enterocyte cell surface receptors via the receptor binding domain; 2. Internalisation of the toxin via endocytosis; 3. Endosomal acidic pH leads to toxin conformational change and translocation; 4. InsP₆ binds to the cysteine protease domain and induces autocleavage releasing the enzymatic domain into the cytosol; 5. Glucosyltransferase glucosylates Rho GTPase which inhibits downstream cellular processes. Figure 6, B and C were taken from reference (34) with permission.

The release of the enzymatic domain has been shown to occur through InsP₆-induced allosteric activation of CPD autoprocessing (112). InsP₆ binding to CPD provides stability and induces a conformational change that organizes the active site to bind the substrate and promote proteolysis via catalytic triad or oxyanion hole (113). InsP₆, which is highly negatively charged due to the 6 phosphate groups, binds CPD in a positively charged pocket. X-ray crystallographic structure of CPD – InsP₆ shows that 7 lysines, 1 arginine and 1 tyrosine are in direct contact with InsP₆ for TcdA and the TcdB binding site is highly conserved at the exception of 2 direct contact lysines replaced by 2 arginines. The InsP₆-CPD binding is thus mainly characterized by strong electrostatic interactions (112-113).
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2.1.3. Project presentation

The increasing threat of CDI and the lack of effective treatments prompted us to design an innovative therapeutic strategy. Inspired by the toxins mechanism of action, we aimed at inducing preemptive toxin B auto-processing in the lumen thus preventing cell penetration of the toxin warhead. However, the naturally occurring allosteric activator, \( \text{InsP}_6 \), is also an excellent cations chelator due to the high density of phosphate groups (59). Therefore, presence of high calcium concentration in the extracellular space leads to nonspecific binding and prevent the allosteric activity of \( \text{InsP}_6 \) (114). Consequently, we hypothesized that IP6 analogues could be designed so that affinity toward calcium is minimized while allosteric activity on the toxin remains.

In order to verify this hypothesis, preliminary studies have been made by M. E. Ivarsson (115). Cleavage assays to evaluate auto-proteolysis have been conducted on a recombinant CPD in the absence and presence of luminal calcium concentration (10 mM). As expected, in the absence of calcium, \( \text{InsP}_6 \) induced cleavage in the low nanomolar range (half maximal effective concentration \( \text{EC}_{50} \) of 25 nM) while high calcium concentration dramatically decreased its potency \( \text{EC}_{50} \) 601 µM). Inositol hexakisulfate (\( \text{InsS}_6 \)) is an analogue of \( \text{InsP}_6 \), in which phosphate groups are substituted with sulfate being also a negatively charged group. Although cleavage assay in the absence of calcium showed that \( \text{InsS}_6 \) induces autoproteolysis with lower efficacy \( \text{EC}_{50} \) 590 µM), its activity is not compromised by high calcium concentrations \( \text{EC}_{50} \) 783 µM). On one hand \( \text{InsP}_6 \) has a strong affinity with CPD and on the other hand \( \text{InsS}_6 \) has lower affinity with \( \text{Ca}^{2+} \). Thus \( \text{InsP}_6 \) analogues containing phosphate and sulfate groups were designed with the expectation to identify a lead that possesses both characteristics: strong binding affinity with CPD and poor interaction with \( \text{Ca}^{2+} \).
In this chapter the synthesis of inositol phosphate/sulfate hybrids that were unknown will be presented in order to test the hypothesis that they will possess allosteric activity in the presence of calcium. The inositol phosphate/sulfate hybrids were designed so that they do not contain vicinal phosphates to avoid calcium chelation and are not chiral to avoid synthetic complication at the early stage of the project. The biochemical results of the prepared analogues will be briefly presented along with the design of a scale-up synthesis for the best candidate. Finally, synthesis of other bisphosphate inositol hybrids will be presented along with the development of a new chiral phosphoramidite agent allowing phosphorylation and chiral resolution.

Figure 8 Hybrid phosphate/sulfate InsP$_6$ analogues.
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2.2. Unified and streamlined synthesis of *meso* inositol phosphate/sulfate hybrids

2.2.1. Strategy

A unified and streamlined synthesis has been designed for the preparation of inositol hybrids containing from one to three phosphate groups, the remaining alcohols being sulfated. These inositol hybrids were designed so that they are not chiral, hence avoiding synthetic complication for the establishment of a new method. Inositol is used as precursor because it is the common core of the three analogues and has the advantage to present a stereochemistry already established in addition to have a low cost. Thus the main challenges to be addressed will be the regioselective functionalization of the inositol core and the high negative charge density owing to the two functional groups, phosphate and sulfate, which complicates purification of the molecules.

The common strategy is depicted in the retrosynthesis (Scheme 9). The sulfate groups are introduced at the latest stage of the synthesis to avoid complications linked to charged molecules. Phosphate groups are selectively introduced as organophosphorous on the key intermediates that have been appropriately protected. Inositol orthoformate is an interesting intermediate that is symmetric and can be used to derivatize the inositol precursor to further introduce selectively orthogonal protecting groups. Of note, the selection of protecting groups is important since they have to be orthogonal to each other: phosphate protecting group $R_1^1$ should be orthogonal to hydroxy protecting groups $R_2^1$, and $R_2^2$ to $R_3^2$. In addition to that $R_2^2$ removal conditions have to be tolerated by phosphate groups that are sensitive to bases. Moreover removal of phosphate protecting group will be restricted to hydrogenolysis, because it is known to be usually clean and highly efficient.

Scheme 9 Retrosynthesis of *meso* inositol phosphate / sulfate hybrids
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2.2.2. Synthesis

2.2.2.1. Preparation of the key intermediates

Inositol orthoformate 48 is an interesting intermediate that has been widely used owing to the various opportunities that it offers in term of regioselectivity (Scheme 10). Hydroxy groups in position 1, 3 and 5 are protected in a single step, and the remaining free hydroxy groups present different reactivities. Hydroxy groups in position 4 and 6 are oriented in axial position while the hydroxy group at the C2 is equatorial. The axial hydroxy groups benefit from the hydrogen bond and the chelation effect with metal alkali that respectively lowers the pKa of the hydroxy groups and stabilizes the deprotonated intermediate 48a (Scheme 10, A). Thus O-alkylation in presence of NaH has been achieved in remarkable high regioselectivity (116). The equatorial hydroxy group is sterically less hindered and more nucleophilic compared to the axial hydroxy that have a lone pair involved in a hydrogen bond (Scheme 10, A). The protected hydroxy groups in position 1, 3 and 5 can be simultaneously deprotected by hydrolysis of the orthoester or, alternatively, one hydroxy group can be selectively released via selective reduction (Scheme 10, B). Hence, trimethyl aluminium (AlMe₃) liberates selectively hydroxy group in position 1 or 3 to afford intermediate 51, due to the preferential coordination of the aluminium to the oxygen in position 1 or 3 that is supported by an additional coordination of the oxygen in position 2 (48b). In contrast, the use of a more hindered aluminium hydride such as diisobutylaluminium hydride (DIBAL-H) will favored coordination of the sterically less hindered oxygen in position 5 (48c), hence releasing the C5 hydroxy group to yield intermediate 52 (70a, 117).

Scheme 10 myo-Inositol orthoformate regioselective opportunities.
Myo-inositol orthoformate was easily synthesized in multi-gram quantities from inositol (118). Access to the **PSPSPS** key intermediate **30** was rapidly achieved from the orthoformate in a single PMB protection of the positions 2, 4 and 6 (Scheme 11, orthogonal protection). Ortho ether hydrolysis in mild acidic condition \( \text{HCl}_\text{aq} 2 \text{m} \) afforded **30** that contains free hydroxy groups at the positions 1, 3 and 5, ready to undergo subsequent phosphorylation.

The **SSSSPS** key intermediate **55** was obtained by 1- and 3-O-PMB protection of the intermediate **30**. With the expectation to obtain selectivity on the position 1 and 3 due to the known lower reactivity of the hydroxy group in position 5 (63, 73a), alkylation was performed with 2 eq. of PMBCl in presence of NaH. Unfortunately, the reaction proceeded with poor selectivity resulting in a mixture of 4 compounds: expected 5-OH **55**, the racemic (±)-1/3-OH resulting from the unwanted bis-alkylation on position 1/3- and 5-, the fully O-PMB inositol, and starting material **30**. However, the expected compound **55** was the major product and could be purified by flash column chromatography and obtained in a yield of 21%.

Finally, the **SSSPSP** key intermediate **58** was prepared in one step from the orthoester by selective protection of the equatorial hydroxy group in position 2 with the hindered TBDMS group in presence of lutidine (119).

### 2.2.2.2. Phosphorylation and orthogonal deprotection

The phosphorylation of inositol intermediates can be problematic due to their steric crowding. Phosphoramidite reagents being more reactive and sterically less hindered than P\(^\text{V} \) reagents have proved to be efficient phosphorylating reagents (120), and among them the \(\text{o-xylylene} \) phosphoramidite has shown promising results (121). Thus phosphitylation of **30**, **55** and **58** with \(\text{O-xylylene} \) phosphoramidite in presence of \(\text{1H-tetrazole} \) and subsequent oxidation provides the phosphorylated intermediates **53**, **56** and **59** in good to excellent yield (Scheme 11, phosphorylation).

In order to deprotect the remaining hydroxy groups of the phosphorylated intermediates, R\(^2 \) group has to be removed orthogonally to the R\(^1 \) phosphate protecting group (Scheme 9).

O-PMB group and xylyl phosphate protecting group are both acid labile, however, xylyl phosphate seemed to be slightly more resistant according to literature investigations (122). Thus, treatment of phosphorylated intermediate **53** and **56** with TFA\(_\text{aq} \) could selectively hydrolyze PMB groups. The poor solubility of these intermediates in organic solvent and their relative instability prevented any purification by normal means such as chromatography. However, they could be purified by trituration in diethylether giving clean deprotected xylyl phosphate intermediate in yield above 90%.

Finding the right conditions to hydrolyze the orthoformate of **59** while keeping the xylyl phosphate intact revealed to be more challenging, especially since it is important to have a clean reaction due to
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the limited product purification options. Cleavage of phosphorylated inositol orthoformates is not common. Literature reports the opening of a 4-phosphorylated orthoformate, however the phosphate group was first deprotected (72, 123). Another paper reports the opening of a 2-phosphorylated-4,6-dibenzyl orthoformate bearing two decyl chains on the phosphate and results in a product that could be purified by silica chromatography (124). Only recently, a 4-phosphorylated orthoformate was opened with catalytic amount of \( \text{p-toluene sulfonic acid} \), however the phosphate protecting group was basic labile and resistant to acid (82a). The previous conditions \( \text{HCl}_\text{aq} \ 2 \text{ M} \) used to cleave the PMB protected orthoformate, hydrolyzed TBDMS group without problems, however, the orthoester was hydrolyzed in only 30% of conversion. Other reaction conditions such as \( \text{TFA}_\text{aq} \) or \( \text{H}_2\text{SO}_4\text{aq} \) caused degradation of the xylyl phosphate group. Thus a short screening of the \( \text{HCl}_\text{aq} \) reaction conditions including temperature, quantity, molarity and time was performed. The best results afforded 70% of conversion using 10 eq. of \( \text{HCl}_\text{aq} \ 2 \text{ M} \) for 5 h at room temperature. The intermediate could easily be purified from its starting material by suspension in dichloromethane. Any further conversion led automatically to degradation of the phosphate group, which was then difficult to purify. Unfortunately, conversion was not reproducible, thus repetition of the reaction gave as best result 55% conversion and an isolated yield of 42%.

2.2.2.3. Sulfation

Once the remaining hydroxy groups of the phosphorylated intermediates have been liberated, they could undergo sulfation (Scheme 11, sulfation). Complexes of sulfur trioxide with amines are common reagents used for sulfation of organic molecules, they are more user friendly and versatile than the sulfuric acid or the free sulfur trioxide (125). Hence following procedures used for per-\( \text{O-} \)sulfation of polyols (126), PSPSPS intermediate was treated with an excess of sulfur trioxide triethylamine complex (\( \text{SO}_3\cdot\text{NEt}_3 \)) (5 eq. per OH) at a temperature of 55 °C and gave after 20 h the sulfated product 54, which was purified by reverse phase column to obtain a yield of 61%.

Sulfation of SSSSPS intermediate 57 under the same conditions worked almost as well with the exception that traces of a partially sulfated compound were present. Purification by small pre-packed reverse phase chromatography columns was not efficient at eliminating the by-product. In order to improve the separation efficiency, the triethylammonium counter ion was exchanged to tetrabutylammonium to render the molecule less polar and hopefully obtain better efficiency. Improvement in the separation on reverse phase chromatography was observed, but yet not sufficient. Hence reverse phase separation was performed on preparative high pressure liquid chromatography (HPLC), and separation was observed. Unfortunately, hydrolysis of the expected compound during purification was observed, increasing the amount of by-product and leading to
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very low yield of clean expected compound. Thus, it appeared that the sulfated intermediate 60 is not stable in presence of water rendering its purification complicated. Therefore it was necessary to find reaction conditions that lead to clean product. Krylov et al. have reported an efficient acid-promoted per-O-sulfation with addition of triflic acid which favors decomplexation of the sulfur trioxide complex (127). Thus sulfation of SSSSPS intermediate 57 was repeated with addition of triflic acid (1 eq. / OH) to the reaction mixture, and after 16 h at room temperature full conversion was obtained and the crude was used without further purification in the next step.

Sulfation of the SSSPSP intermediate 61 without addition of triflic acid was even less complete, resulting in a mixture of several partially sulfated products. However sulfation in presence of triflic acid yielded full conversion and the crude could also be used without further purification in the next step.

2.2.2.4. Hydrogenolysis and final purification

Removal of phosphate protecting groups to afford the final phosphate / sulfate hybrids was carried out by hydrogenolysis catalyzed by Pd/C under an atmosphere of hydrogen and was neutralized with ammonium hydroxide to form the ammonium salt (Scheme 11, hydrogenolysis).

PSPSPS obtained by hydrogenolysis of the stable intermediate 54 could be purified by reverse phase column and afforded clean product in 50% yield.

Purification of the SSPPSP intermediate hydrogenolysis consisted mainly of removing the excess of sulfate salt from the sulfation reaction which had not been purified. It was found that size exclusion chromatography on a pre-packed PD-10 column for preliminary purification followed by size exclusion chromatography using sephadex G-10 could afford clean product in 90% yield over two steps.

Purification of SSSPSP following the same procedure as SSPPSP yielded clean product in 86% yield over two steps. However, the reaction was plagued with reproducibility issues, and in most cases apparition of hydrolyzed by-product could not be avoided. The impurity was present in the purified product in about 10 mol % and could not be further removed by size exclusion chromatography. An alternative approach to purify molecules containing charges is ion-exchange chromatography. This method has been used in inositol phosphates identification (128) and purification (129). Therefore, a third purification by anion-exchange on Q Sepharose fast flow was performed. Elution with aqueous triethylammonium bicarbonate (TEAB) 1 M with a gradient of NaCl from 0 to 0.75 M was effective in removing the contaminant from SSSPSP. Although TEAB is a volatile salt and could be removed by
lyophilization, the NaCl remained and required a new size exclusion chromatography to eliminate it. Hence clean SSSPSP could be afforded albeit with a lower yield of 45% (over two steps).

Scheme 11 Streamlined and unified synthesis of the meso phosphate/sulfate inositols. a) 1. NaH, PMBCl, DMF; 2. HCl aq, MeOH; b) NaH, PMBCl; DMF c) TBDMSCl, lutidine, DMF; d) 1. O-xyylene phosphoramidite, 1H-tetrazole, CH2Cl2; 2. mCPBA; e) SO3. NEt3, DMF; f) SO3. NEt3, TfOH, DMF; g) H2 (1 atm), Pd/C, H2O; h) H2 (1 atm), Pd/C, MeOH.

A common strategy to synthesize meso inositol phosphate/sulfate hybrids has been established and allowed the synthesis of PSPSPS, SSSPSP and SSSSSS. Orthoformate inositol allowed the derivatisation of the inositol giving access to the 3 key intermediates that could be further phosphorylated and sulfated. Difficulties were encountered after installation of the phosphate groups. The inherent instability of phosphorylated intermediates, polarity and charge density restricted the purification options. Therefore, it was important to find optimal reaction conditions to perform clean reactions, so that purifications were facilitated. Nevertheless, the three analogues could be synthesized in a relative concise route, including relatively high yielding transformations at the exception of the orthoformate hydrolysis of the SSSPSP intermediate 59, and PSPSPS (IP3S3), SSSSSS (IP1S5) and SSSPSP (IP2S4) could be delivered in high purity for biological evaluation.

2.2.3. Biological evaluation

The evaluation of the derivatives briefly described in this section was performed by M. E. Ivarsson. In a first study, affinity of the InsP₆ analogues with calcium ions was investigated. InsP₆ analogues were incubated with calcium ions and the quantity of free calcium was determined using murexide as
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colorimetric indicator (Figure 9, A). As expected, InsP₆ sample has the lowest free calcium concentration which increased with the other compounds as phosphates are replaced with sulfates. Therefore, the inositol containing fewer phosphate groups have weaker affinity with calcium ions.

In order to evaluate the ability of the InsP₆ analogues to trigger TcdB autoproteolysis in the lumen, extent of cleavage was monitored on the holotoxin in presence of calcium ions (Figure 9, B). IP₂S₄ and IP₁S₅ showed a stronger activity than the others analogues (extent of cleavage below 10%). IP₂S₄ exhibited a distinct superiority in term of efficacy, being at least two times superior to IP₁S₅.

Figure 9 A) Determination of InsP₆ analogues affinity with calcium ions. Free calcium quantification. 0.5 mM of analogues, CaCl₂ and murexide were mixed, incubated and centrifuged. Absorbance of the supernatant at 474 nm and 544 nm was used to determine free calcium concentration. Mean ± s.d., n = 3; Asterisk indicates statistical difference compared with InsP₆ (P < 0.05). B) Evaluation of InsP₆ analogues in triggering TcdB autocleavage in presence of calcium. Extent of cleavage was determined by densitometry and normalized to the positive and negative controls. Error bars shows s.d.; Asterisk indicates statistical difference compared to IP₂S₄ (P < 0.05); n = 3. This figure has been made by M. E. Ivarsson (115).

The efficacy of IP₂S₄ as treatment against TcdB has been further explored in a studies using mice that were infected by C. difficile strain (NAP1) expressing only TcdB, and not TcdA. Mice developed moderate to severe colitis with elevated activity of the inflammation marker myeloperoxidase (MPO) and histological features that are characteristic of CDI. One group was treated with IP₂S₄, a second group with IP₆ and a third group with myo-inositol as a negative control. The level of MPO activity was significantly reduced in the mice treated with IP₂S₄ compared to the ones treated with myo-inositol (Figure 10.A). Histological examination of excised colon of mice treated with myo-inositol revealed abnormalities in the mucosa architecture with overlying exudates that are the consequence of an acute chronic inflammation (Figure 10.B). Mucosal histology of the mice treated with IP₆ presented a healthier profile and even better when treated with IP₂S₄. Although MPO activity in the control group shows variability, these first results suggest that IP₂S₄ attenuates CDI pathology and further studies with higher doses and other animal models will be made to further evaluate IP₂S₄.

The in vivo evaluation was performed in collaboration with Dr. Jun Lu, Prof. Elena Verdu, and Prof. Premysl Bercik of McMaster University.
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Figure 10 In vivo SSSPSP activity. A) Colonic MPO activity. Germ-free NIH Swiss mice were inoculated with 200 µL of a 1:10 dilution in saline of fecal matter from a patient with recurrent CDI. InsP₆, SSSSPSP or inositol (negative control) were administered as doses of 12.3 µmol/kg (corresponding to 10 mg/kg SSSPSP) in a blinded fashion given in 5 mL drinking water starting 6 h after inoculation and for three nights thereafter, as well as per intragastric gavage once a day for four days starting the day after infection. The animals were sacrificed at the end of day five post-infection and the colon excised for histological evaluation and myeloperoxidase activity measurement. Boxes represent interquartile ranges, dividing lines in boxes median values, and whiskers maximal and minimal values (n(inositol) = 8, n(InsP₆) = 7, n(SSSPSP) = 9). Asterisk indicates statistical difference compared to inositol according to two-tailed Mann-Whitney test (P < 0.05). B) Histological sections of excised colons. Inositol-treated mice (negative control) displayed overt colonic structural changes characterized by mucosal ulceration and overlying exudate, marked acute and chronic inflammatory infiltrate and submucosal edema. This figure has been made by M. E. Ivarsson (115) and reused with permission.
2. Design of a scale-up synthesis of SSSPSP

As shown above, IP2S4 (SSSPSP) was identified as a lead compound, hence it was primordial to develop a synthetic route that enabled the production of material in higher quantity and high purity in order to perform further in vivo studies. An important part of the work in this section has been realized with the collaboration of S. Huwiler and S. Salzmann during their master project.

SSSPSP synthesis is already concise, 5 steps from myo-inositol orthoformate, however, the overall yield fluctuates from 18% to the very low yield of 0.4% (Scheme 12). Even though TBDMS protection is achieved in only 50% yield, it is not the priority since it is an early stage of the synthesis and none of the starting material is expensive. The main critical step is the hydrolysis of the orthoformate in presence of the protected phosphate groups which gives compound 61 in a low and non-reproducible yield (10 to 45%). In addition, the sulfation and hydrogenolysis steps are also critical and non-reproducible as the final product is obtained in yields ranging from 10 to 85%.

![Scheme 12 Synthesis of SSSPSP](image)

Scheme 12 Synthesis of SSSPSP a) TBDMSCl (1 eq.), 2,6-lutidine (2.5 eq.), DMF, rt, 24 h, 50%; b) 1H-tetrazole (8 eq.), o-xylylene-N,N-diethylphosphoramidite (4 eq.), CH$_2$Cl$_2$, rt, 20 h; 2. mCPBA (12 eq.), 0 °C -> rt, 1 h, 93%; c) HCl$_{aq}$ 2 M (10 eq.), MeOH, rt. 4 h, 10-45%; d) SO$_3$·NET$_3$ (20 eq.), TFOH (4 eq.), DMF dry, 0 °C -> rt, 16 h; e) H$_2$, Pd/C, MeOH dry, rt, 1.5 h, 10-85% over two steps.

2.3.1. Orthoformate hydrolysis optimization

One main issue of this hydrolysis is the limited and non-reproducible conversion which is between 20 and 50%. As mention is section 2.2.2.2, this result has been obtained after screening the HCl$_{aq}$ 2 M reaction conditions including temperature, quantity, molarity and time. In an effort to improve the reaction, HCl$_{aq}$ 2 M was changed to HCl concentrated (HCl$_{cc}$) following a procedure used for orthoester hydrolysis of a deprotected 4-phosphate orthoformate inositol by Song et al (124) and a new screening was performed. Hydrolysis conversion and by-products formation were monitored by $^1$H NMR and successful reaction conditions could be established leading to full conversion with less impurities (Figure 11). Hence the optimized reaction conditions consisted in two additions of HCl$_{cc}$ (10 eq for 3.5 h and then 5 eq. for 0.5 h).
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The high polarity of the bisphosphate compound 59, its poor solubility in organic solvent, and its sensitivity to hydrolysis considerably limited the purification options. The formed impurities are presumed to be inositol derivatives in which phosphate protecting group had been degraded, hence the impurities possess similar polarity to the desired product 61. Various purification methods were attempted including trituration with different solvents, crystallization or chromatography-based purification such as reverse phase (C18) and size exclusion (sephadex LH-20), which has been used for inositol purification (130). Unfortunately, none of these methods could afford the high purity expected.

Therefore, although optimized reaction conditions that afforded full conversion were identified, none of the purification methods could succeed to eliminate the traces of impurities. It was thus preferable to perform the orthoformate hydrolysis with the previous conditions using HClaq 2 M, where the only impurity is the more hydrophobic starting material that is easily removed in organic solvents in which the desired product precipitates. Suspension of the crude in a mixture acetonitrile / ethanol 30:1 instead of dichloromethane improved the purification, affording clean bisphosphate 61 in 30% yield.

![Figure 11](image-url) ¹H NMR spectra of hydrolysis crude a) 2 M HClaq, 10 eq., 0.06 M, rt, 5 h. b) HClcc, 7 eq., 0.02 M, 35 °C, 4 h. c) 1. HClcc, 10 eq., 0.06 M, rt, 3.5 h; 2. HClcc, 5 eq., rt, 0.5 h. This figure was prepared by S. Huwiler and reused with permission.
2.3.2. Sulfation/hydrogenolysis optimization

As mentioned in section 2.2.2.3, the instability of the sulfated intermediate 60 is troublesome. Despite minimal manipulation, apparition of by-product (around 10 mol%) could not be avoided in the final product, requiring tedious purification including size exclusion chromatography to remove excess of sulfate salt, followed by strong ion exchange purification to eliminate the by-product, and finally a new size exclusion purification to desalt the pure fractions.

A one-pot sulfation/hydrogenolysis reaction was attempted in order to avoid the formation of this side product. Thus sulfation was performed as mentioned above, and after neutralization, hydrogenolysis was directly started in the same vessel. Under these conditions, less or no degradation was observed depending on the reactions. Thus, while the direct hydrogenolysis improved the purity of the final SSSPSP, it could not reliably eliminate the impurity completely.

2.3.3. Investigation of a more stable acid-labile phosphate protecting group

As attempts to optimize the reaction conditions could not fix the main problems concerning hydrolysis nor sulfation/hydrogenolysis steps, we turned our attention to see whether a change in phosphate protecting group could solve these problems. Hence xylyl group was replaced by dibenzyl as it is compatible with the synthesis strategy and the reagent is commercially available (Scheme 13).

Phosphorylation of the TBDMS protected compound was performed with tetrabenzylpyrophosphate, and gave 62 in 34%. Phosphorylation with dibenzyl-\(N,N\)-diethylphosphoramidite could not afford pure material because of impurity in the phosphorylating agent that was impossible to remove. However, orthoformate hydrolysis of the new intermediate 62 with \(\text{HCl}_{\text{aq}}\) 2 M afforded clean hydrolyzed product 63 with a gratifying yield of 85%. This improvement is due to the better stability of the dibenzyl compared to the xylyl group that allows to reach a higher conversion of 95% by heating the reaction at 40 °C for 5 h without observing degradation.
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Although the change in phosphate group improved the orthoformate hydrolysis step, the phosphorylation reaction is not economically viable on a large scale because of the mediocre yield and the higher cost of the tetrabenzylpyrophosphate (357 against 91 CHF/g).

2.3.4. New synthesis strategy

Neither reaction conditions optimization, nor change of phosphate protecting group provided satisfying results to support a scaled-up synthesis. Therefore a new route had to be devised in order to avoid the problems described above. We began to contemplate the idea of performing the sulfation reaction on the completely deprotected \( 4,6\text{-InsP}_2 \) that is more stable than partially protected intermediate 61 (Scheme 14). Moreover, this would eliminate the need the problematic final hydrogenolysis reaction. However, it was anticipated that this sulfation would be challenging since \( 4,6\text{-InsP}_2 \) is completely deprotected, and the negative charges of the phosphate groups diminish the hydroxy group reactivity. Indeed, the per-O-sulfation was already challenging on uncharged inositols, as shown above. Undeterred, we first optimized the complete deprotection reaction.

![Scheme 14 New route for SSSPSP synthesis. Reagents and conditions: a) TMSBr (50 eq.), rt, 4h, 79%; b)SO\(_3\)NEt\(_3\) (20 eq.), TfOH (4 eq.), rt, 16 h, 50%.

2.3.4.1. TMSBr Hydrolysis

Deprotection of orthoester, TBDMS and phosphate protecting groups on small scale (10 mg) was performed in dichloromethane with two additions of TMSBr (22 eq. for 2 h, and then 56 eq. for 5 h). However on bigger scale (about 100 mg) the reaction did not go to completion. It appeared that addition of methanol was necessary to enable orthoformate hydrolysis and optimization of the reaction conditions resulted in a single addition of TMSBr (56 eq.) in a mixture methanol / dichloromethane. Suspension of the crude reaction mixture in acetone and then in acetonitrile afforded the fully unprotected \( 4,6\text{-InsP}_2 \) in 90 to 100% yield. Repetition of the procedure on 500 mg and then 1 g of starting material afforded same purity and same yield.
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2.3.4.2. Sulfation

Previously, sulfation reactions were performed with an excess of sulfur trioxide (5 eq. / OH) in presence of triflic acid (1 eq. / OH) in dry DMF. We attempted to use the same conditions to perform sulfation of 4,6-InsP₂ but failed to obtain the desired SSSPSP. This result was not surprising since the fully deprotected intermediate is expected to be relatively non reactive. However, observation of partial sulfation was encouraging and prompted us to further explore this reaction. Increasing the reaction temperature improved conversion but was not sufficient. To our satisfaction, increasing the amount of triflic acid from 4 eq. to 20 eq. was successful, and full conversion was achieved. This remarkable advance enabled to perform per-O-sulfation of the fully deprotected compound and afforded the desired SSSPSP in 49% yield after size exclusion chromatography.

This procedure worked well on small scale (around 5 mg of starting material), but had to be scaled up to provide larger amounts of product for in vivo experiments. The purification on small scale was performed with size exclusion chromatography using Sephadex G-10. However, the size of columns available and the limited separation efficiency impeded the purification on a larger scale. We thus attempted to limit the large quantities of salts formed in the sulfation reaction by reducing the quantity of sulfur trioxide used. It was found that a total of 12 eq. of sulfur trioxide triethylamine complex could be used (down from 20 eq.) and full conversion was obtained after reaction optimization with minimum amount of by-product formed. Scale-up of the optimized reaction on 50 mg, 70 mg and 100 mg afforded similar results but purification was still problematic.

The purification itself was also optimized. On a small scale, most of the DMF was removed under high vacuum after neutralization of the triflic acid with Et₃N and a preliminary purification was performed on a short pre-pack sephadex column (G-25) followed by a second size exclusion on a longer self-pack sephadex (G-10). Upon scale-up to 100 mg, evaporation of the DMF was sluggish, leaving large amount of solvents in the crude reaction mixture. It also became clear that shorter time between neutralization of the reaction mixture and purification was desirable, leading to lower amounts of by-product. Therefore, it was decided to load directly the mixture onto the G-10 sephadex column, without prior evaporation of the DMF. Reaction ran on 30 mg of starting material achieved good purity product (> 95%) in a yield of 51% using this protocol, but was unfortunately not reproducible. On this scale, the product was usually obtained in a purity of 90%. It is important to note that efficiency of the sephadex column, which were washed and re-used could erode with time. The by-product that appears is presumed to arise from hydrolysis of one sulfate, and is therefore very similar to the SSSPSP, rendering their separation difficult. The fact that sephadex G-10 size exclusion chromatography could diminish to some extent this impurity let us think that it could be possible to
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eliminate it with a more efficient installation, such as the use of high performance commercial columns on HPLC. Alternatively, strong anion chromatography proved to be efficient to eliminate the impurity and deliver material in high purity, but is also challenging to perform on a larger scale.

**Scheme 15**

SSPSP optimized synthesis a) TBDMSCI (1 eq.), 2,6-lutidine (2.5 eq.), DMF, rt, 24 h; b) 1H-tetrazole (8 eq.), α-xylylene-N,N-diethylphosphoramidite (4 eq.), CH₂Cl₂, rt, 20 h; 2. mCPBA (10 eq.), 0 °C -> rt, 1 h; c) TMSBr (56 eq.), MeOH/CH₂Cl₂ 30%, rt, 5 h, d) SO₃-NEt₃ (12 eq.), TfOH (24 eq.), DMF dry, 45 °C, 20 h.

Thus, while a satisfactory purification protocol for larger scale synthesis remained elusive, we could identify a shorter and more elegant synthesis route to inositol phosphate/sulfate hydrids that resolved many of the problems encountered before. The key finding was that the per-O-sulfation could be performed on the deactivated bisphosphate 4,6-InsP₂. **SSPSP** can now be synthesized in four steps starting from myo-inositol orthoformate and optimization resulted in an overall yield of 23% (Scheme 15), albeit only on a small scale.
2.4. Synthesis of bisphosphorylated inositol analogues

The analogue SSSPSP containing two phosphate groups has been identified as the most effective candidate among the first series prepared. We then became interested to probe whether the position of the phosphate groups had an influence on allosteric activity. We thus aimed to evaluate other bisphosphate isomers that are chiral compounds (Figure 12).

![Figure 12](image-url) Structure of the IP$_{2}S_{4}$ analogues.

The synthesis of these new IP$_{2}S_{4}$ analogues is based on the optimized route presented in the previous chapter, that is sulfation of fully deprotected diphosphate inositols. Section 1.3.2 describes the recently described paradigm shifts for the chiral synthesis of InsPs. Asymmetric phosphorylation mediated by peptide catalysts and desymmetrization by a chiral phosphoramidite proved to be valuable methods. However, application of these methods does not fit our synthesis strategy. The asymmetric phosphorylation of Miller et al. is more beneficial in the case of a single target inositol phosphate and would require lengthy protecting group manipulations for each isomer. We were more inspired by the chiral phosphoramidite developed by Jessen and decided to embark on the development of a new chiral phosphoramidite that would fit our chiral IP$_{2}S_{4}$ isomers and benefit chiral synthesis of InsPs in general.

2.4.1. Racemic synthesis

Prior to initiate the challenging chiral synthesis, we explored the synthesis of the new derivatives as racemic mixtures. Furthermore, synthetic priorities can be set based on preliminary biological evaluation of the racemic IP$_{2}S_{4}$.
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2.4.1.1. Preparation of the key intermediates

In a first instance, myo-inositol has to be appropriately derivatized to afford key intermediates that will then undergo phosphorylation (Scheme 16).

The (±)-SPSPSS key intermediate 65 was rapidly accessed in one step from myo-inositol orthoformate by chemoselective \( p \)-methoxybenzylation (123). Hence, treatment of the orthoester with PMBCl in presence of NaH afforded the racemic (±)-65 in 75% yield.

The (±)-PSSSSPS key intermediate 68 was also prepared from myo-inositol orthoformate in 4 high yielding steps. The hydroxyl groups in position 2-, 4- and 6- were protected in one step with benzyl groups in quantitative yield. The orthoformate 66 was then selectively opened on the position 3/1 with trimethyl aluminium following the procedure established by Gilbert et al. (117) and, subsequently benzylated. Finally, hydrolysis released the 1/3 and 5 hydroxy groups to afford (±)-PSSSSPS key intermediate 68 with an overall yield of 64% over the 4 steps.

In contrast to the others, (±)-PSSPSS key intermediate 70 was not prepared from myo-inositol orthoformate, but from (±)-2,3;5,6-di-O-isopropylidene-myo-inositol 70, another widely used
2. The synthesis of inositol hybrids: towards a potential drug against Clostridium difficile Infection

precursor (131). Treatment of myo-inositol with acetone ketal catalyzed by acid afforded a mixture of regioisomers. Following the convenient procedure from Khersonsky et al. (132), the desired product could be easily separated from the mixture after benzylation. Finally, basic deprotection furnished the (±)-PSSPSS key intermediate 70.

2.4.1.2. Phosphorylation and sulfation

Phosphorylation of each IP$_2$S$_4$ intermediates was uneventful and afforded the phosphorylated intermediates in good to moderate yields (Scheme 17).

The phosphorylated intermediates orthoformate (±)-71 and acetal (±)-75 were then fully deprotected by hydrolysis with TMSBr, as previously reported (cf. section 2.3.4.1), and could be purified by precipitation to isolate pure compounds in good yields of 85 and 98%, respectively. Benzylated intermediate (±)-73 was deprotected by hydrogenolysis and like its counterparts was purified by precipitation to afford bisphosphate (±)-74 in 89% yield.

Gratifyingly, two of the fully deprotected intermediates (±)-72 and (±)-76 underwent sulfation with sulfur trioxide triethylamine complex and triflic acid and purification by size exclusion
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chromatography afforded pure (±)-SPSPSS and (±)-PSSPSS in 51 and 38% yield respectively. Unfortunately, the bisphosphate (±)-74 required heating to obtain full conversion, and (±)-PSSSSPS was only obtained in a yield of 16% and was contaminated with a significant by-product. Analysis by $^{31}$P NMR spectroscopy showed signals resonating at the low chemical shift of -15.4 and -16.3 ppm, which usually correspond to phosphorus involved in P-anhydride bond (see examples in the following references (82a, 133)). Although no chemical structure could be clearly established at this point, it was suspected that the excess of triflic acid, together with heating for 20 h could promote the formation of this by-product, hence, decreasing triflic acid amount, time and/or temperature could be helpful in reducing the impurity.

![Figure 13 $^{31}$P NMR spectra of the (±)-PSSSSPS containing the by-product.](image)

The improved synthesis strategy proved to be effective in the preparation of the others IP$_2$S$_4$ isomers as well, despite, the issues encountered in the sulfation of the (±)-PSSSSPS. For comparison, Chung et al. have also established a common strategy to synthesize the nine possible bisphosphate isomers (134a). In this strategy, the complete deprotection to afford the bisphosphate compounds is performed in a one-pot high-yielding procedure. However, although the synthesis is not detailed, it appears clear that the preparation of intermediates prior to phosphorylation involves several synthetic steps from the common precursor, making in general the synthesis longer than what is described here.

2.4.2. Biological evaluation of the (±)-IP$_2$S$_4$

The evaluation of the derivatives (±)-SPSPSS and (±)-PSSPSS briefly described in this section was performed by N. Romantini under the supervision of M. E. Ivarsson.
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Affinity of the new IP$_2$S$_4$ analogues with calcium ions was investigated and compared to SSSPSP to verify if the position of phosphate groups on the inositol could have an influence (Figure 14, A). The results show that although they might have slightly less affinity with calcium ions, there is no significant difference from SSSPSP.

Then the extent of cleavage of the holotoxin with the derivatives was monitored in presence of calcium ions (Figure 14, B). The (±)-SPSPSS proved to be significantly less active than the SSSPSP whereas the (±)-PSSPSS displays a slightly better activity, albeit without statistical difference from SSSPSP. These results firstly demonstrate that the binding interaction nature with the CPD is not only a matter of charge density but requires a more detailed structural analysis. The structure of the InsP$_6$-CPD TcdB complex shows that phosphate on position 2 and 3 point out of the binding pocket hence should be less involved in the binding than phosphate in position 1, 4, 5 and 6 which are driven inside (Figure 7, B on page 26) (112). The result obtained with the less active (±)-SPSPSS lends support to this observation since the two enantiomers contain one phosphate group in the “less involved” position 2 whereas SSSPSP presents the two phosphate groups in the “more involved” positions 4 and 6. Following the same rationale, the result obtained for the (±)-PSSPSS proved to be encouraging in revealing a compound as potent as SSSPSP. Indeed, one of the enantiomer displays one phosphate group in the “less involved” position 3 while the other enantiomer has the two phosphates in the “more involved” position 1 and 4. Since the activity resulting from the two enantiomers demonstrated a similar efficacy to the SSSPSP, it would not be unreasonable to expect that the activity of the optically pure PSSPSS would have a higher activity. It is therefore essential to
carry out the chiral synthesis of the IP$_2$S$_4$ analogues in order to ascertain the formal activity and to a complete picture of the structure-activity relationship.

### 2.4.3. Chiral synthesis

#### 2.4.3.1. Design and synthesis of chiral phosphoramidite

We designed a new chiral phosphoramidite reagent for the chiral resolution of our inositol intermediates. We wanted an acid-labile chiral auxiliary but that was sufficiently stable to enable purification of the phosphorylated inositol without degradation. A C$_2$ symmetry is required to avoid a chiral center at the phosphorus atom that could result in further diastereomeric mixtures. The presence of a benzyl phosphate ester bond is desired to obtain similar reactivity to the xyylene phosphoramidite, but also to provide hydrogenolysis as an alternative deprotection strategy. Ease of the phosphorylating agent preparation is a non negligible aspect and literature reports that preparation of cyclic phosphoramidites is easier and gives cleaner reaction mixture than acyclic counterparts (121). Finally, concerned by the stability of the resulting phosphate triester, a search of the literature directed us towards 7-membered rings. Although only few reports discuss the stability of phosphate triesters, one study compared the stability between 5-, 6- and 7-membered ring phosphotriesters toward acidic hydrolysis (135). While the 5-membered ring was remarkably labile, the 6-membered ring displayed good stability and the 7-membered ring was strikingly stable. With all these criteria in mind, we settled on the C$_2$-symmetric 7-membered ring phosphoramidite 80 (Scheme 18).

**Scheme 18** Synthesis of C$_2$ chiral phosphoramidite. a) rongalite (1.3 eq.), DMF dry, rt, 22 h, 79%; b) 1. (S)-(-)-α,α-diphenylprolinol (0.25 eq.), B(OMe)$_3$ (0.25 eq.), THF dry, rt, 1 h; 2. BH$_3$.SMe$_2$ (2.1 eq.), 78 (1 eq.), rt, 2.5 h, 50%, dr > 98%; c) (Et$_2$N)$_3$P in toluene dry (1 M, 1.03 eq.), CHCl$_3$ dry, 110 °C, 40 min, 85%.

The development of the synthesis has been done with the collaboration of S. Huwiler during his master thesis. The chiral diol 79 could rapidly be obtained in two steps (Scheme 18). Following a procedure from Jarvis (136), coupling of bromoacetophenone promoted by the reducing agent rongalite afforded the diphenylbutadiene 78. Asymmetric reduction of the dione has been effectively carried out by Kemppainen et al. *via* chiral oxazaborolidine catalyst (137). Following a procedure from Masui (138), oxazaborolidine was generated *in situ* from chiral prolinol and trimethylborate
and after addition of borane, \((R,R)\)-diphenylbutanediol 79 was obtained in diastereomeric ratio of \((R,R)/(R,S)\) 86:14, which could be further enriched by crystallisation in diisopropyl ether to reach a high purity with a diastereomeric ratio above 98:2.

Phosphoramidite 80 was prepared following Arbuzov’s procedure used for the preparation of o-xylylene phosphoramidite (139) (Scheme 18). Hence, diol 79 was mixed with phosphorus triamide in equimolar amount, but \(^{31}\text{P}\) NMR analysis revealed that the oxidized form of phosphoramidite 80 was obtained. Indeed, \(^{31}\text{P}\) NMR chemical shift of phosphoramidites are above 100 ppm while the chemical shift of the obtained species was 6.7 ppm, which corresponds to the oxidized phosphorus. Repetition of the reaction with degassed solution afforded the expected phosphoramidite 80, which was confirmed by \(^{31}\text{P}\) NMR measurement showing a signal at 125.5 ppm. Attempts to purify the phosphoramidite 80 by distillation failed due to a presumably high boiling point. This is not surprising given that the smaller o-xylylene phosphoramidite has a boiling point reported to be 95-96 °C at a pressure of 0.1 mbar (139). Nonetheless, we could generate the phosphoramidite 80 in situ prior to the phosphorylation because the reaction was very clean.

### 2.4.3.2. Chiral resolution via phosphitylation

Phosphitylation of the racemic key intermediates was performed using the in situ generated phosphoramidite 80 in presence of tetrazole. Excess of tetrazole was used to compensate the diethylamine released from the phosphoramidite formation. Subsequent oxidation afforded the phosphorylated diastereomeric mixtures with full conversion.
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Scheme 19 Chiral resolution via phosphorylation. a) 1. (R,R)-diphenylbutadiol 79 (5.5 eq.), (Et₂N)₃P in toluene dry (1 M, 5.5 eq.), CHCl₃ dry; 2. 1H-tetrazole (23 eq.), CHCl₃, rt, 20 h; 3. mCPBA (10 eq.), full conversion from crude NMR. FC : flash column chromatography, cryst : crystallisation. Recovered yield based on the diastereomer itself which represents 50% of the racemic starting material. [a] Configurations could not be determined at this time.

2,4 and 2,6 protected bisphosphate diasteromers 81 could be separated by normal flash column chromatography with diastereomeric ratio above 99:1. The absolute configuration could not be assigned at this time of the synthesis. Removal of the protecting groups to access the fully deprotected enantiomers 2,4- and 2,6-bisphosphate inositols enabled to determine the assignment by measuring the optical rotation that are known (134b). Attempts to separate the diasteromers 1,5 and 3,5 by flash chromatography failed. However, diastereomer 1,5 82a could be separated from the diastereomeric mixture by crystallisation and obtained in diastereomeric ratio above 99:1. Crystals could be analyzed by X-Ray to determine the absolute configuration. The 3,5 diastereomer 82b was purified by crystallization but was only obtained in a diastereomeric ratio of 85:15. The 1,4 and 3,6 protected bisphosphate diasteromers could also be separated by crystallization delivering diastereomer 1,4 83a in a diastereomeric ratio above 99:1. Analysis of crystals by X-Ray could unambiguously assign the absolute configuration (Figure 15). 3,6 diastereoisomer 83b was purified by recrystallisation and obtained with a diastereomeric ratio above 99:1. Thus, all but one of the isomers prepare could be resolved with excellent diastereomeric ratios.
In conclusion, a new chiral phosphoramidite 80 could be easily and rapidly generated in situ from chiral diphenylbutanediol 79 and phosphorus triamide. This method allowed successful phosphitylation of the racemic intermediates to afford diastereomeric mixtures in good yields, which could then be separated by crystallization or column chromatography albeit in low yield. However, in comparison to classic chiral resolution, it remains an efficient method as phosphate groups are simultaneously installed. With the optically pure bisphosphates in hand, access to the chiral InsP$_2$ natural products is only a deprotection away. The synthesis of the chiral IP$_2$S$_4$ derivatives should then proceed as for the racemic synthesis.
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2.5. Conclusion and Outlook

A common synthetic strategy was established to access meso inositol phosphate / sulfate hybrids PSPSPS, SSSPSP and SSSSSPS. myo-Inositol orthoformate was derivatized with carefully chosen protecting groups in a minimum of manipulation steps. Performance of clean reactions could circumvent the relative instability of phosphorylated intermediates and the challenging purifications inherent to the polarity and charge density of inositol phosphates. These were all challenges that were successfully overcome to furnish PSPSPS, SSSPSP and SSSSSPS in high purity for biological evaluation.

Biological and biochemical studies realized by M. E. Ivarsson identified SSSPSP as a good candidate for triggering TcdB autoprocessing in the presence of calcium. It was further supported by preliminary in vivo studies on mice realized in collaboration with the group of Premysl Bercik and Elena Verdu at McMaster University, which indicated attenuation of inflammation when infected mice were treated with SSSPSP.

The need to provide SSSPSP in high quantity and high purity for further in vivo evaluation spurred the optimization of the synthesis. A key finding was that the per-O-sulfation could be performed successfully on the fully deprotected inositol phosphate. This new route eliminates the need to perform the sensitive and problematic orthogonal deprotection in presence of phosphate protecting groups. Hence, the new optimized route delivers the SSSPSP in 4 synthetic steps from myo-inositol orthoformate in an overall yield of 23%. The only drawback of this route is the purification on large scale, although strong anion chromatography was successful of small scale. Indeed, a commercial supplier commissioned to prepare the SSSPSP on a multi-gram scale has mirrored the overall synthetic route, except for the final purification.

Finally the ambitious chiral synthesis of various IP$_2$S$_4$ isomers was attempted in the view to probe the structure-activity-relationship. The viability of the new synthetic route was tested on the racemic synthesis, which proved to be successful, with the exception of the sulfation of the (±)-PSSSPS intermediate that requires improvement. A new chiral phosphorylating agent allowing phosphorylation and chiral resolution of three different racemic substrates was developed. Phosphorylation and chiral resolution with the new chiral agent on the different intermediates accessed the optically pure compounds in high purity at the exception of the SSSPSPS intermediate. This approach comes to complement the toolbox of chiral phosphorylation strategies that could be beneficial to the synthesis of well-defined InsPs. The first results of the structure activity relationship of the racemic analogues indicate that interesting results should be expected with the chiral IP$_2$S$_4$. 

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3. Fluorine-directed β-glycosylation

3.1. Introduction and Background

3.1.1. Fluorosugars

Fluorosugars have recently gained considerable interest and a growing number of fluorinated carbohydrates are developed. The low van der Waals radius of the fluorine, the inert and highly polarized C-F bond are features that render fluorosugars excellent bio-isosteres of 2-deoxy and 2-hydroxy sugars with enhanced pharmacokinetic profile. They have been used in various applications such as tools to probe enzyme mechanism, as therapeutic drugs or for diagnosis.

In the interest to gain insight into carbohydrate-processing enzymes, Withers has introduced fluorosugars which revealed to be valuable tools to probe retaining glycosidases by trapping the glycosyl-enzyme intermediate (140). Substitution of 2-hydroxy group by fluorine destabilizes the transient oxonium ion, resulting in accumulation of the covalent glycosyl enzyme intermediate (Figure 16, A) (141). Davies et al. have also shown the utility of fluorosugars, with the synthesis of fluorinated trisaccharide probe to study adhesion in Toxoplasmosis (Figure 16, B) (142) or the radiolabelled proteins with 18F-sugars for providing positron emission tomography (PET) radiotracers (143).

Development of synthetic carbohydrate-based vaccines has recently emerged (c.f. section 1.2.1), however they suffer from low metabolic stability which affects their efficiency. Hoffmann-Röder et al. have developed fluorinated analogues of the Thomsen-Fridenreich antigen, found in 90% of carcinoma (144), to incorporate them in the glycopeptides antigen for enhancing immunogenicity and metabolic stability (Figure 16, C) (145). Preliminary encouraging results show that the fluorinated antigens bind to mouse antisera with equal potency to the natural MUC1 glycopeptide (146).
3. Fluorine-directed β-glycosylation

![Diagram of 2-fluorogalactose containing sugars](image)

**Figure 16** Illustration of 2-fluorogalactose containing sugars. A) 2-Fluorogalactose, glycosidase probe (141). B) 2-Fluorogalactose containing trisaccharides, ligand for TgMUC1 from *Toxoplasma gondii* (142). C) Fluorinated Thomsen Fridenreich antigen Carbohydrate-based vaccine Hoffmann-Röder et al.

### 3.1.2. C-F bond, a powerful conformational tool

Fluorine is the most electronegative element in the periodic table (χ ≈ 4) (147), rendering the C-F bond highly polarized. The electron density is concentrated around the fluorine atom, consequently fluorine bears a partial negative charge and carbon bears a partial positive charge. Hence a significant electrostatic attraction rises between F<sup>-</sup> and C<sup>+</sup>, which gives an ionic character to the C-F bond. This peculiarity is an argument to explain the unusual strength of the bond which is the strongest bond in organic chemistry (Table 1) (148). Although fluorine possesses 3 lone pairs, they are held tightly, which makes fluorine quite unreactive, for example it is a poor hydrogen bonding acceptor (149). To summarize, the C-F bond is strong, unreactive and highly polarized which provide remarkable electrostatic and electronic features (148, 150).

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*Table 1* Properties of fluorine and the common elements hydrogen and oxygen (148, 150).

### 3.1.2.1. Electrostatic interactions

One consequence of this highly polarized C-F bond is its ability to interplay with its environment via electrostatic interactions: dipole-dipole or charge-dipole interaction.
3. Fluorine-directed β-glycosylation

![Diagram](image_url)

**Figure 17** A) Dipole-dipole interactions; top: protein-ligand interaction (C-F-C=O) (151); bottom: α-fluoroamide dipole-dipole interaction (C-F-C=O) favoring the trans conformer (152). B) Charge-dipole interactions; top: 3-fluoropiperidinium derivative from Snyder, axial conformer stabilized through (C-F-N⁺) (153); bottom: 3-fluoroazetidinium from O'Hagan, axial conformer stabilized trough (C-F-N⁺) (154).

The partial negative charge of the C-F bond can interact with partial positive charge center of other molecules. For example, it has been demonstrated that fluorinated drugs interacted with protein through C-F-C=O interaction (Figure 17, A, top) (151). Stronger dipole-dipole interactions are observed intramolecularly, and represent an important conformational factor. For example, the α-fluoroamide favors the trans conformation, in which the C-F dipole is opposed to the amide dipole (Figure 17, A, bottom) (152). This preference decreases as the dipole moment of the carbonyl group diminishes (amide > ester > ketone > aldehyde) (148). An even more powerful interaction is the charge–dipole interaction. A striking example from Lankin, Snyder et al. with the 3-fluoropiperidinium derivatives that largely favor the axial orientation of the fluorine which is described as a charge-dipole interaction (CF⁻N⁺) (Figure 17, B, top) (153, 155). These results were further supported by O’Hagan findings with the 3-fluoroazetidinium (Figure 17, B, bottom) (154).

### 3.1.2.2. Hyperconjugative Interactions

The other consequence of the highly polarized C-F bond is the low-lying σ* C-F antibonding orbital. This vacant orbital can accept electron from electronic rich system such as σ bond (e.g. σ C-H), π bonds or oxygen/nitrogen lone pair to stabilize a conformer.
3. Fluorine-directed β-glycosylation

This hyperconjugation effect is well illustrated in the 1,2-difluoroethane molecule which favors the gauche conformer rather than the intuitive anti conformer found in the 1,2-dichloroethane, hence accommodating two stabilizing hyperconjugations ($\sigma_{C-H} \rightarrow \sigma^*_{C-F}$) (Figure 18, A) (156). Another pertinent example is the anomeric effect in the 2-fluoropyran (Figure 18, B) (157). Conformation presenting fluorine in the axial orientation is favored, so that the electronic lone pair of the oxygen can be delocalized in the $\sigma^*_{C-F}$ which is consistent with the anomeric effect observed in the 2-methoxypyran.

3.1.3. Fluorine effect in stereoselective glycosylation

The intrinsic property of fluorine atom to modulate molecular conformation was exploited as stereoselective tool in glycosidic reaction.

Preliminary works in the laboratory realized by C. Bucher have shown that introduction of fluorine at the C2 position of a glucosyl donor greatly enhances β-stereoselectivity (Scheme 20) (158). When C2 is substituted with fluorine, glycosylation reaction is performed with excellent β-diastereoselectivity ($\beta/\alpha$ 57:1) whereas 2-OBn and 2-deoxy display moderate β-selectivity ($\beta/\alpha$ 7:1 and 6:1, respectively).

This stereoselectivity is proposed to result from the fluorine effect that favors one particular oxocarbenium ion conformation by through space electrostatic interaction. In the $^3$H$_4$ half-chair conformation, the axial orientation of the C-F bond and OBN at C3, C4 and C5 positions point toward the electrophilic center, hence stabilize the positive charge. While in the $^4$H$_3$ half-chair conformation, all substituents are in equatorial position pointing away from the electrophilic center. When C2 position is substituted with OBN or H, the stereoinduction is decreased, showing that fluorine is decisive in orchestring the transfer of chirality (Scheme 20).
3. Fluorine-directed β-glycosylation

Scheme 20 Effect of C2-F of a glucosyl donor on stereoselectivity. Reaction conditions: TMSOTf (0.1 eq.), iPrOH (1.2 eq.), CH₂Cl₂, -78°C, 2 h. These results were obtained by C. Bucher (158).

These remarkable results, prompt us to explore the potency of this methodology to galactopyranosyl system. Together with glucose, they are part of the most abundant motifs in mammalian and bacterial monosaccharides. Expansion of this work, would allow the access to 2-fluoro-glycostructures which can be valuable tools in glycobiology, diagnosis and therapeutic.
3. Fluorine-directed β-glycosylation

3.2. Stereocontrolled 2-fluorogalactosylation

3.2.1. C2-Fluorine direct β-galactosylation

Stereoselectivity of glycosidic reactions of the 2-fluoro-galactopyranosyl system was investigated. A systematic study was realized with 1) variation of substituents at the C2 position with F, H or OR groups and 2) variation of the remote protecting groups to evaluate their influence in inducing stereoselectivity.

3.2.1.1. Preparation of the galactopyranosyl substrates

Synthesis of the 2-fluorogalactopyranoses

The fluorinated galactopyranosyl systems were prepared from the commercially available tri-O-acetyl-D-galactal 84 (Scheme 21). The synthesis started with a change to the desired protecting group, hence acetyl groups were removed under basic conditions and the crude was directly treated with benzyl bromide or methyl iodide following standard procedure to afford tri-O-benzyl-D-galactal 85 and tri-O-methyl-D-galactal 86 in 79 and 65% yield over two steps, respectively. Fluorination were carried out following the procedure of Ortner (159), using Selectfluor® (Scheme 21) as electrophilic fluorinating agent in acetone/H₂O and provided 2-fluorogalactose 87 and 88 in 71% and 37% yield respectively. In the case of R = Ac, the reaction was not selective affording an unseparable mixture of galacto- and talo- configured lactols 89 in 51% yield. However installation of a temporary acyl group at the anomeric alcohol with acetic anhydride enabled separation of the diastereomers by flash column chromatography affording the desired galacto- 90 in 78% yield (160). The more labile
3. Fluorine-directed β-glycosylation

Anomeric acetyl group was removed, following a procedure from Zhang and Vasella (161), under mild basic conditions with ammonia at low temperature to furnish the galactose 91 in a yield of 70%.

**Synthesis of the 2-deoxygalactopyranoses**

Scheme 22 Synthesis of 2-deoxygalactopyranosyl substrates. a) Ph₃P·HBr (0.05 eq.), H₂O (1.5 eq.), THF, rt, 5 h, 74% for 92 and 14% for 93; b) Amberlite® IR120, LiBr (3.1 eq.), H₂O (18 eq.), CH₃CN, rt, 3 h, 58%.

Synthesis of the 2-deoxygalactopyranosyl substrates protected with ether groups were accessed following the procedure developed by Mioskowski and Falck (162). Treatment of the suitably protected galactal with triphenyl phosphine hydrobromide in presence of H₂O afforded the benzyl protected 2-deoxygalactopyranosyl 92 in 74% yield and the methyl protected 93 in 14% yield. Regarding the acyl protected 2-deoxygalactose was prepared following a procedure from Sabesan (163), using a resin Amberlite® IR120 and LiBr in presence of H₂O to afford the hydrated product 94 in 58% yield.

**Synthesis of the 2-OR galactopyranoses**

Scheme 23 Synthesis of 2-O-benzyl-galactopyranose 97. a) 1. BF₃·OEt₂ (0.3 eq.), allyl alcohol, rt, 4 h; 2. NaH (7 eq.), BnBr (6 eq.), DMF, rt, 16 h, 37% over 2 steps; b) 1. tBuOK, DMF, 70 °C, 20 min; 2. HClₐq (6 M), ice, 37% over 2 steps.

Substrates substituted at the C2 position with OR group were prepared from the commercially available D-(+)-galactose 95. The benzylated galactopyranose 97 was prepared following a procedure from Zhang and Vasella (161) (Scheme 23). The synthesis started with protection of the anomeric position with allyl group using the Fisher glycosylation. Subsequent benzylation under basic condition with benzyl bromide furnished 96 in 37% yield over 2 steps. Removal of the allyl group, proceeding via isomerization of the double bond by heating under basic condition gave the 1-propenyl galactopyranose, which was then hydrolyzed under acidic condition to afford the 2-O-benzyl-galactopyranose 97 in 37% yield over two steps.
3. Fluorine-directed β-glycosylation

Synthesis of the methyl protected substrate 100 (Scheme 24) started with peralkylation of the methyl α-D-galactopyranoside in basic aqueous solution with methyl iodide following a method from Wang et al. (164) and yielded 98 in 45%. Selective deprotection of the anomeric position was promoted with aqueous solution of triflic acid and glacial acetic acid with heating to furnish methylated galactopyranose 100 in a yield of 34% (165).

For the preparation of the acyl galactopyranose 101, galactose 95 was acetylated with acetic anhydride catalyzed with DMAP to give the fully acetylated compound 99 in 98% yield (166). Anomeric position was then selectively deprotected, like previously under mild basic condition to afford the desired acetyl galactopyranose 101 in a yield of 77% (161).

3.2.1.2. Glycosylation reaction

With the desired galactosyl substrates in hand, stereoselectivity of the glycosidic reactions could be investigated (Table 2). Among the various glycosylation methods, the widely used Schmidt type was chosen (162). Trichloroacetimidate glycosyl donors are easily prepared from the lactols, and display very good leaving group property. Owing to the steric hindrance and the strong electron withdrawing of the trichloromethyl group, formation of the transient oxocarbenium ion is facilitated. Hence lactols were converted to galactosyl trichloroacetimidate under base-catalyzed addition of trichloroacetonitrile. Glycosylation were then performed upon acid-catalyzed activation with trimethyl silyl triflate (TMSOTf) and isopropanol as glycosyl acceptor. Reactions were performed in dichloromethane to minimize the risk of solvent - oxocarbenium ion interaction that would influence stereoselectivities.
3. Fluorine-directed β-glycosylation

Table 2 Investigating the influence of the C2 substituent and the electronic nature of the remote protecting groups on stereoselectivity. Reagents and conditions: a) Cl\(_3\)CCN (10 eq.), DBU (0.1 eq.), CH\(_2\)Cl\(_2\), 0 °C -> rt, 2 h; b) IPrOH (1.2 eq.), TMSOTf (0.1 eq.), CH\(_2\)Cl\(_2\), -78 °C, 2 h. [a] Reactions were performed at -50 °C. [b] No reactions were observed at -78, -50 and -30 °C, therefore reactions were performed at 0 °C. [c] Yield after the two-step preactivation/glycosylation sequence. [d] Analysis of the 2-deoxy proved problematic; no yield determined. [e] Ratios were determined by \(^1\)H and/or \(^{19}\)F NMR spectroscopy of the crude reaction mixture.

To our delight, 2-fluoro-benzyl-galactopyranosyl substrate 87 not only performed glycosylation with β-stereoselectivity but with remarkably high level of diastereoselectivity (2-F-galacto- β/α 150:1 versus 2-F-gluco- β/α 57:1, entry 1 and Scheme 20, respectively). Noteworthy are the lower stereoselectivity with 2-OBn donor (β/α 7.5:1) and the loss of stereoselectivity with the 2-deoxy substrate (β/α 1.5:1) showing that fluorine is crucial in controlling stereoselectivity. The methyl protected glycosyl donors display a similar stereoselective propensity, albeit at a lower level (entry 2, X = F, OMe, H, β/α 22:1, 3.9:1 and 1:1.4, respectively). The reaction required higher temperature and was performed at -50 °C instead of -78 °C. For comparison, glycosylation of 2-fluoro-benzyl substrate 87 was repeated at -50 °C and gave the product in a ratio β/α 40:1. Finally when Bn groups are substituted with the electron-withdrawing Ac groups, stereoselectivity with the 2-fluoro donor is completely eroded (entry 3, X = F β/α 1:1). It was also observed a lower reactivity of the acyl protected donors which required increasing the temperature up to 0 °C. Importantly, glycosylation of substrate 87 at this elevated temperature still displayed β selectivity (β/α 6:1). The high β selectivity with substrate bearing OAc at the C2 position (entry 3, X = OAc, β/α > 20:1) is the consequence of the anchimeric assistance.

<table>
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<tr>
<th>Entry</th>
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<th>X</th>
<th>Product</th>
<th>Yield [%][c]</th>
<th>β/α[e]</th>
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<td>F</td>
<td>OMe</td>
<td>111</td>
<td>72</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>H</td>
<td>113</td>
<td>69</td>
<td>1.5 :1</td>
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<td>58</td>
<td>22 : 1</td>
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<td></td>
<td></td>
<td>H</td>
<td>115</td>
<td>53</td>
<td>3.9 :1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H</td>
<td>116</td>
<td>n.d. [d]</td>
<td>1 : 1.4</td>
</tr>
<tr>
<td>3[b]</td>
<td>F</td>
<td>OAc</td>
<td>117</td>
<td>45</td>
<td>1 : 1</td>
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<td></td>
<td>H</td>
<td>118</td>
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<td></td>
<td></td>
<td>H</td>
<td>119</td>
<td>63</td>
<td>1 : 3</td>
</tr>
</tbody>
</table>
3. Fluorine-directed β-glycosylation

3.2.1.3. Discussion

Prior to start any discussion on the oxocarbenium ion, it is important to clarify the mechanism pathway $S_N1$ versus $S_N2$ (Scheme 25). Glycosylation with the α-trichloroacetimidate afford β-glycoside with excellent selectivity ($\beta/\alpha$ 150:1). The β-trichloroacetimidate also furnishes β-glycoside albeit with moderate selectivity ($\beta/\alpha$ 2.5:1). Both anomeric configurations of the 2-fluoro donors converge to the β-glycoside, which is reminiscent of a $S_N1$ pathway, although a partial $S_N2$ mechanism cannot be totally excluded.

Scheme 25 β-Stereoconvergence of the trichoroacetimitade donors. Reagents and conditions: iPrOH (1.2 eq.), TMSOTf (0.1 eq.), $\text{CH}_2\text{Cl}_2$, -78 °C, 2h.

The stereoselectivities obtained with the galactopyranosyl substrates are consistent with the previous findings with the glucos- system, and come to support the rationale of inductive conformational preference of the transient oxocarbenium ion (158). The nucleophile can approach each half-chair intermediate via two trajectories, but one will result in a chair conformation whereas the other one in a skew boat conformation which is thermodynamically disfavored (Fürst-Plattner rule) (167). The facial approach leading to the more stable chair like product will always predominate. Thus the $^3\text{H}_4$ half chair intermediate will lead to the β-glycoside whereas the $^4\text{H}_3$ half chair to the α-glycoside. The oxocarbenium ion containing benzyl groups in positions 3, 4 and 5 and fluorine at the C2 would favor the $^4\text{H}_3$ half chair conformation in which the axial F, and OBn in positions C3 and C6 can stabilize the positive charge by through space electrostatic interaction (Scheme 26, top, left). Whereas in the $^3\text{H}_4$ half chair conformation, only the OBn in position C4 could provide electrostatic interaction which is far less electronegative than fluorine. In the absence of fluorine, stabilization effect by through space electrostatic interaction in the two half chair conformations is almost equal (Scheme 26, bottom, left). There is electrostatic stabilisation effect of two OBn groups for the $^3\text{H}_4$ half chair conformation versus one in the $^4\text{H}_3$ half chair conformation. The acyl protected oxocarbenium ion substituted with fluorine shows competitive effect for both half chair conformations (Scheme 26, top, right). In the $^3\text{H}_4$ half chair, the axial fluorine stabilizes the positive charge but the two axial acyl groups are destabilized whereas in the the $^4\text{H}_3$ half chair the acyl groups are oriented in the more stable equatorial position. This competitive effect can be further confirmed with the 2-deoxy oxocarbenium ion which cannot be anymore stabilized by electrostatic interaction and will favor the more stable $^4\text{H}_3$ half chair conformation orienting most of the
substituents in equatorial position (Scheme 26, bottom, right). In conclusion, the C2-F seems to strongly stabilize the positive charge of the oxocarbenium ion by through space electrostatic interaction, inducing a conformational preference. Nonetheless this effect has to be supported by the protecting groups to concur to the same conformational preference, otherwise conformers will exist in similar population. The analysis of the conformational preference according to the through space electrostatic stabilization effect is highly correlated with the observed stereoselectivity. Indeed, when a conformation is favored, selectivity is observed while in the opposite case the selectivity is eroded.

It can also be envisaged that the nucleophile attacks the oxocarbenium ion following the Anh-Eisenstein 1,2 induction model (168). In the $^3$H$_4$ half chair intermediate, the gauche conformation of the fluorine, allow alignment of the σ*$_{C-F}$ with the nascent σ$_{C-Nu}$ stabilizing the transition state.

**Scheme 26** Possible half-chair conformations of the transient oxocarbenium ion.

### 3.2.2. Effect of glycosyl acceptor steric hindrance on stereoselectivity

Glycosyl acceptors have an influence on the stereoselectivity outcome of glycosydic reactions. Therefore, the effect of the steric hindrance of glycosyl acceptor was investigated on the on glycosylation with the 2-fluoro-galactopyranosyl donor 87. In order to appreciate the effect that the fluorine has on the stereoselectivity, glycosylation with 2-deoxy substrate 92 was also probed.
Table 3 Probing the steric influence of the glycosyl acceptor on stereoselectivity. Reagents and conditions: a) Cl3CCN (10 eq.), DBU (0.1 eq.), CH2Cl2, 0 °C -> rt, 2 h; b) iPrOH (1.2 eq.), TMSOTf (0.1 eq.), CH2Cl2, -78 °C, 2 h. [a] Yield after the two-step preactivation/glycosylation sequence. [b] Ratios were determined by 1H and/or 19F NMR spectroscopy of the crude reaction mixture. [c] not determined because anomeric proton of b-glycoside was obscured by the benzyl signals; purification by column chromatography afforded a β/α ratio 1:1.1.

The initial glycosylation of ethanol with 2-fluoro donor furnished the desired β-glycoside with astounding selectivity while reaction with the 2-deoxy showed almost no selectivity (entry 1, X = F, H, β/α 300:1 and 1.5:1, respectively). Glycosylation of the others primary alcohols, including allyl alcohol and benzyl alcohol, were also gratified of excellent β selectivities when performed with the 2-fluorogalactopyranosyl substrate (entries 4 and 6, X = F, β/α 120:1 for both). Same reactions with the 2-deoxy analogue resulted in no selectivity (entries 4 and 6, X = H, β/α 1:1, β/α 1:1.1, respectively). Similar tendency is observed with bulkier acceptor, such as isopropanol previously seen (entry 2, X = F, H, β/α 150:1, β/α 1.5:1, respectively). Even the sterically crowded tert-butanol gave the β-product with lower but still good selectivity (entry 3, β/α 15:1 versus 1.3:1 for X = F and X = H, respectively). Finally glycosylation using phenol exhibited an exclusive α selectivity with the 2-deoxy...
donor. Interestingly, introduction of the fluorine at the C2, not only abrogate the α-selectivity but also reversed the selectivity to furnish the β-glycoside in acceptable selectivity (entry 5, β/α 6:1 versus α only for X = F and X = H, respectively). This study showed that the steric hindrance of the glycosyl acceptor has an influence on the selectivity, which tends to decrease as the glycosyl acceptor is becoming more hindered. However, it also highlights the propensity of the 2-fluorogalactosyl donor to induce good to excellent level of β-stereoselectivity of glycosylation even with sterically crowded glycosyl acceptor.

3.2.3. 2-fluoro-β-galactosylation in synthesis of disaccharides

In the interest of evaluating the potency of the 2-fluorogalactopyranosyl donor in carbohydrate synthesis, glycosylation of monosaccharide acceptors was performed to access analogues of disaccharides building blocks in an efficient stereocontrolled manner. Monosaccharide acceptors were chosen so that the resulting disaccharides are not only analogues of commonly encountered motifs in the mammalian and bacterial “glycospace” (24), but also to probe the different branching positions (Figure 19).

**Figure 19** Structures of the glycosyl acceptors.

### 3.2.3.1. Preparation of the glycosyl acceptors

**Synthesis of the 3-hydroxygalactopyranoside 130**

```
Scheme 27 Synthesis of the 3-hydroxygalactopyranoside. Reagents and conditions: a) PhCH(OMe)₂ (1.3 eq.), CSA (0.1 eq), ACN, 82 °C, 45 min, 37%; b) BnBr (1.2 eq.), nBu₄NI (0.2 eq.), NaOH aq (1.5 eq.), CH₂Cl₂, rt, 24 h, 41%; c) Ac₂O (4 eq.), DMAP (0.1 eq.), pyridine, rt, 24 h, 44%; d) MeONa (0.4 eq.), MeOH, rt, 2h, 23%.
```
3. Fluorine-directed β-glycosylation

Synthesis of the acceptor 130 started with the commercially available 1-methylgalactopyranoside 136 which was protected with a 4,6-O-benzylidene acetal following a procedure from Thomas et al. (169) in a yield of 37%. Monobenzylation under basic biphasic conditions led to an unseparable mixture of the two isomers 138 and 139. Gratifyingly, application of the previous procedure to separate the two diastereomers by acylation of the free hydroxyl group succeeded to furnish the desired isomer 140 in 44% yield. After removal of the ester group, the galactosyl acceptor 130 could be accessed.

**Synthesis of the glucopyranosides acceptors**

![Scheme 28 Synthesis of the glucopyranosides acceptors. Reagents and conditions: a) PhCH(OMe)₂ (1.3 eq.), CSA (0.1 eq), ACN, 82 °C, 45 min, 81%; b) BnBr (1.2 eq.), nBu₄NI (0.2 eq.), NaOHaq (1.5 eq.), CH₂Cl₂, rt, 24 h, 40%; c) BnBr (1.2 eq.), NaH (3 eq), DMF, rt, 16 h, 96%; d) NaBH₃CN (5.0 eq.), I₂ (3.5 eq.), CH₂Cl₂, rt, 45 min, 38%; e) BH₃·THF (5.0 eq.), TMSOTf (0.2 eq.), CH₂Cl₂, rt, 2.5 h, 83%.](image)

The 3-hydroxyglucopyranosyl acceptor 131 was prepared in a similar way than its galacto-homolog. The difference was that the mixture resulting from the non selective benzylation could be separated by flash column chromatography to afford the desired 131 in 44% yield.

To access the 4- and 6-hydroxylglucopyranosides 134 and 135, at first, the remaining hydroxy groups of 142 were benzyalted. The resulting 4,6-O-benzyl acetal 143 could then be regioselectively opened on the position 4 or 6. Use of cyanoborohydride in presence of iodine, according to a procedure from Rao et al. (170), afforded the 4-hydroxy 134 in a yield of 38%. On the other hand, selective reduction of the position 6 was performed with a borane THF complex catalyzed with TMSOTf and acceptor 135 was obtained in a yield of 83% (171).

The acceptor 132 was available in the laboratory (172) and the 3-hydroxy-glucofuranoside 133 is commercially available.
3. Fluorine-directed β-glycosylation

3.2.3.2. Glycosylation with glycosyl acceptors

Once the requisite glycosyl acceptors prepared, the glycosylation could be performed with the 2-fluoro-galactopyranosyl donor, and with its 2-deoxy homolog for comparison.

<table>
<thead>
<tr>
<th>Entry</th>
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<td>41</td>
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<td>F</td>
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<td>H</td>
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<td></td>
<td>155</td>
<td>54</td>
<td>1.7:1</td>
</tr>
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</table>

Table 4 Application of the 2-fluorogalactosyl for β-stereoselective syntheses of disaccharides. Reagents and conditions: a) Cl3CCN (10 eq.), DBU (0.1 eq.), CH2Cl2, 0 °C → rt, 2 h; b) iPrOH (1.2 eq.), TMSOTf (0.1 eq.), CH2Cl2, -78 °C, 2h. [a] Yield after the two-step preactivation/glycosylation sequence. [b] Ratios were determined by 1H and/or 19F NMR spectroscopy of the crude reaction mixture. [c] Ratio could not be determined with certainty because anomeric proton of an eventual β-glycoside could have been obscured by the benzyl signals; purification by column chromatography afforded only the α-glycoside.

Assembly of the 2-fluorogalactopyranosyl donor 87 with the sterically hindered 3-hydroxygalactosyl acceptor 130 proceeded well to provide the disaccharide Gal-(1->3)-Gal 144 in a satisfying yield of 41% and with the expected β-selectivity (β/α 5:1, entry 1, X = F). Noteworthy, repetition with the 2-deoxy homolog led only to the α-glycoside (entry 1, X = H). Coupling with the gluco-relative acceptor afforded the same selectivity, albeit with a lower yield (β/α 5:1, 31%, entry 2, X = F). Using the less hindered 2-deoxy glucosyl acceptor resulted in an improvement of both selectivity and yield.
3. Fluorine-directed β-glycosylation

(β/α 11:1, 56%, entry 3, X = F). These results testify of the sensitivity of the glycosylation toward the steric hindrance of the acceptors. Coupling with the glucofuranose acceptor 133 also furnished the fluorinated disaccharide in respectable yield and good level of diastereoselectivity (β/α 10:1, 53%, entry 4, X = F) whereas the 2-deoxy donor was α selective. The very hindered benzylated 4-hydroxy acceptor 134 proved one more time the effect of the steric hindrance on the stereoselectivity, since the glycosylation with the 2-fluoro donor resulted in almost no selectivity (β/α 1.3:1, 43%, entry 5, X = F). On the other hand, the sterically less hindered benzylated 6-hydroxy acceptor 135, which is a primary alcohol, provided the disaccharide in excellent β selectivity (β/α 40:1, 49%, entry 6, X = F).

Introduction of the fluorine substituent at the C2 clearly overrides the substrate-based stereocontrol, changing the selectivity from α to β. These results show that this methodology represents a valuable tool to synthesize analogues of disaccharides containing Gal-β-linked motif in respectable yield and good selectivity. In the synthesis of the fluorinated Thomsen-Fridenreich antigen, from Hoffmann-Röder (Scheme 29), using the same glycosyl donor was observed similar results. Glycosylation of the benzylated 2-fluorogalactosyl trichloroacetimidate 102 with the GalNAc acceptor 156 provided the disaccharide with a good β-selectivity (β/α 10:1). Interestingly, glycosylation with the fluorinated acceptor 158 afforded only a moderate β-selectivity (β/α 3:1). These findings suggest that the glycosylation with the 2-fluoro donor is not only sensitive to the steric hindrance of the glycosyl acceptor but also to the electronic effect (145, 173).

Scheme 29 Fluorinated Thomsen-Fridenreich antigens (145, 173).

3.2.4. Synthesis of fluorinated β-galactoceramide analog

The utility of the presented methodology was showcased in the synthesis of the fluorinated galactoceramide analog, which was achieved by C. Bucher (158b, 174). Member of the glycosphingolipid family, the β-galactosylceramides have been recognized to be important player in the Natural Killer T (NKT) cells stimulation. Synthetic β-galactosylceramides and analogues have contributed to get a better understanding of the role of the glycolipids in NKT cell response (175).
The synthesis started with the glycosylation of the 2-fluorogalactosyl trichloroacetimidate 102 with the azido sphingosine 160 (176). To our delight, the reaction proceeded with good yield and excellent selectivity (80%, β/α 28:1). The crucial role of the fluorine-directed β-selectivity was one more time demonstrated, since the 2-deoxy glycosyl homolog 104 performed the reaction with opposed stereoselectivity (β/α 1:4). The use of ceramide 160 as glycosyl acceptor affected the performance of the reaction mainly due to solubility problems. Moreover, it could be possible that steric hindrance of the ceramide and possibly the electron withdrawing nature of the amide could affect the selectivity. Then the synthesis proceeded smoothly with deprotection of the azide under standard Staudinger reduction conditions (177) to give the primary amine 162. Coupling with the second lipid chain using the activated N-succinimide ester afforded the protected glyceroceramide 163 in 73% yield (178). The final deprotection in presence of the unsaturated lipid chain was performed under Birch reduction conditions (179), delivering the fluorinated galactoceramide analogue 164 in a yield of 69%. This fluorinated galactoceramide analogue could also serve as bulding block for more complex structures (e.g. trisaccharide GM3). Probably metabolically more stable than the natural, 164 could be a useful tool in elucidating role and function of sphingolipids.
3. Fluorine-directed β-glycosylation

3.3. Conclusion

The goal of this project was to introduce a fluorine atom at the C2 position of the galactosyl donor in order to direct β stereoselectivity in glycosidic reaction. A systematic study probing the effect of the fluorine at the C2 position and the electronic nature of the protecting groups was realized. The results of this study led to the conclusion that fluorine at the C2 position together with the electron donating nature of the ancillary protecting groups are required to obtain excellent level of β-selectivity. Indeed glycosylation of isopropanol with the benzyl protected 2-fluorogalactosyl donor provided β product with outstanding diastereoselectivity (β/α 150:1), whereas with the acyl protected donor selectivity was eroded (β/α 1:1). These findings support the rationale already proposed in a previous study with the 2-fluoroglucopyranosyl system made by C. Bucher. Fluorine would induce strong conformational preference of the oxocarbenium ion intermediate by through space electrostatic stabilization effect.

In the second part of the project, we focused on the applicability of the method. At first, investigation of various glycosyl acceptors showed that steric hindrance has an influence of diastereoselectivity. However, this study also confirmed that fluorine was decisive in orchestring β-selectivity. In general, moderate to good β selectivities were obtained (β/α from 5:1 to 40:1) depending on the glycosyl acceptor steric hindrance. Of note, was the spectacular inversion of selectivity, disaccharides preparation, since glycosylation with the 2-deoxy donor led exclusively to α products at one exception. Finally, utility of this methodology was successfully showcased in the synthesis of the β-galactosylceramide performed by C. Bucher. Glycosylation of the azido sphingosine with the fluoro glycosyl donor was accomplished with very good yield and excellent level of diastereoselectivity (80%, β/α 28:1).

These findings come to support the rationale already advanced in a previous study with the 2-fluoroglucopyranosyl system made by C. Bucher. Fluorine would induce strong conformational preference in the oxocarbenium ion by through space electrostatic interaction that leads to β-stereoselectivity. Furthermore, this hypothesis is supported by the elegant work of Woerpel, showing the importance of electrostatic interactions in inducing the conformational preference of oxocarbenium ion (167, 180). The Ahn-Einsenstein 1,2-induction model would also be consistent with the our results, leading to the formation of the 1,2-trans glycosides. Providing evidence of the trough space electrostatic interaction effect would be of great interest to lend support to the inductive model. Conformational study of suitable oxocarbenium mimics by NMR spectroscopy and X-ray diffraction crystallography would be ideal. However preparation of such material remains a real challenge as they are highly instable species. Nonetheless formation of the dioxocarbenium ion from
3. Fluorine-directed β-glycosylation

Woerpel (180a) and progress in tool to stabilize very reactive species (181) are encouraging elements.
4. Conclusion and Outlook

The work presented in this thesis show development of methodology to synthesize biomolecule analogues.

In the Chapter 2 was presented unified and streamlined synthesis of inositol phosphate/sulfate hybrids from myo-inositol precursor. As a detailed conclusion of the project was reported at the end of the chapter (c.f. section 2.5, p 51), here is summarized the important progresses that have been made. The main challenges related to inositol phosphate synthesis are regioselectivity, instability of phosphorylated intermediates and difficult purifications inherent to the polarity and charge density of inositol phosphates. In addition to that per-O-sulfation and chiral synthesis come to complicate the panel. Derivatization of myo-inositol in a minimum of synthetic steps with carefully chosen protecting group and performance of clean reaction were essential for circumventing with success these obstacles. A key finding was that the per-O-sulfation could be performed successfully on the fully deprotected inositol phosphate. This new route eliminates the need to manipulate the sensitive phosphorylated intermediates and the need of orthogonal protection/deprotection. These findings contributed to the design of a scale-up synthesis of SSSPSP that could be synthesized in 4 steps from myo-inositol orthoformate in a total yield of 23%. For the ambitious chiral synthesis of various IP$_2$S$_4$ isomers, a new phosphorylating agent was developed that allow phosphorylation and chiral resolution. Indeed, phosphorylation of three different racemic substrates led to chiral resolution and the 6 diastereomers were obtained in excellent purity at the exception of one. This approach comes to complement the toolbox of chiral phosphorylation strategies that could be beneficial to the synthesis of well-defined InsPs.

The goal behind the synthesis of inositol phosphate/sulfate hybrids was to find a candidate that can trigger the pre-emptive toxin B auto-processing in the lumen. The replacement of phosphate groups by sulfate groups in the SSSPSP proved to be successful in decreasing the calcium ions affinity while retaining the allosteric activity. Accomplishment of the chiral synthesis of the IP$_2$S$_4$ isomers should enable to find the best candidate trough a structure activity relationship and refine our understanding on the InsP$_6$-CPD interactions.

The next step of this project on the chemical point of view would be to enhance the pharmacokinetic profile of the drug candidate. One aspect that has to be considered is the eventual degradation of the InsP$_6$ hybrid analogue by phytase. Phytase is a type of phosphatase that is specific to InsPs. Presence of phytases in the gastrointestinal tract can come from food intake or can be produced by micro-organisms (182). Therefore it is important to introduce a non-hydrolisable phosphate group (60a). As negative charges are crucial for retaining the allosteric activity, it shortens the list of
phosphate bioisosteres. Phosphorothioate, in which the oxygen has been replaced by a sulfur, would be a first interesting one to try for its very close similarity with phosphate group. They have already proved their stability toward phytase in inositol phosphates (183). On the synthetical aspect, it would be very convenient since the synthesis strategy would not have to change. Only the oxidation step after phosphitylation would be modified to oxidize with sulfur. The question of the stability of sulfate group toward phytase can rise. However, a recent study on phytase from *Bacteroides thetaiotaomicron* (BtMinpp) provided a crystal structure of the complex InsS6-BtMinpp (184), which reinforces the hypothesis that sulfate groups should be resistant to phytases.

The *chapter 3* was dedicated to the investigation of fluorine-directed β-stereoselective glycosylation in the 2-fluorogalactopyranosyl donor. Also a detailed conclusion was presented at the end of the chapter (c.f. section 3.3, p 69). It could be shown that fluorine at the C2 position together with the electron donating nature of the remote protecting groups are driving excellent level of β-selectivity in glycosidic reaction. Application of this methodology was successfully used for the synthesis of fluorinated analogue of more complex glycostructures such as disaccharides and sphingolipid β-galactosylceramides.

Glycoproteins are a very important class of biomolecules that are important player in cellular processes and raise a considerable interest for therapeutic development which were briefly described in the *Chapter 1*. Fluorine atom possesses very interesting features such as enhancement of metabolic stability and bioavailability (151b), enhancement of binding affinity (151a), diagnosis, ... Therefore synthesis of fluorinated analogues of glycosylated amino acid building block would be a useful tool to contribute to the understanding of their function and the development of carbohydrate-based drugs.
5. Experimental Part

5.1. General Information

Reactions requiring dry conditions were performed under an atmosphere of dry nitrogen in dried glassware.

All chemicals were reagent grade and used as supplied unless stated otherwise.

Solvents for reactions (diethylether, \textit{N},\textit{N}-dimethylformamide, methylene chloride, methanol) were purchased of analytical grade from commercial suppliers and used without further purification unless stated otherwise. Solvents for extractions and chromatography were technical grade and distilled prior to use.

Ultrapure water was prepared by a Barnstead Nanopure system (Thermo Fischer Scientific, Reinach, Switzerland).

Analytical thin layer chromatography (TLC) was performed on pre-coated \textit{Merck} silica gel 60 F_{254} plates and visualized with UV light or cerium molybdate stain.

Flash column chromatography was carried out on \textit{Fluka} silica gel 60 (230-400 mesh). For reverse phase column chromatography, Sep-Pak\textsuperscript{\textregistered} Vac 6cc (1 g) C18 cartridges (Waters, Ireland) were used.

Size exclusion column chromatography was performed on PD-10 pre-packed columns (GE Healthcare Life Sciences, Buckinghamshire, UK) or on Sephadex G-10 (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Ion exchange column chromatograph was performed on Dowex\textsuperscript{\textregistered} 50WX8-400 (Sigma-Aldrich, St. Louis, USA) or on HiTrap\textsuperscript{TM} Q pre-packed columns (1 or 5 mL) (GE Healthcare Bio-Sciences AB, Uppsala Sweden).

Concentration in vacuo was performed at \(~10\) mbar and 40 °C unless stated otherwise, drying at \(~10^{-2}\) mbar at room temperature (rt).

Lyophilizations were performed on a Christ Freeze Dryer Alpha 2-4 LSC (Birsfelden, Switzerland)

\textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{19}F and \textsuperscript{31}P NMR spectra were recorded on a \textit{Bruker} AV 400 MHz spectrometer or a \textit{Bruker} AV 500 MHz spectrometer. NMR data are reported as follows: chemical shifts (δ, ppm, relative to residual solvent peaks), integration, multiplicity (s = singlet, br = broad, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (\textit{J}, Hz).

High-resolution mass spectra (HR ESI) were performed by the MS service at the Laboratory for Organic Chemistry, ETH Zurich. The MS of \textit{IP1S5}, \textit{IP2S4}, and \textit{IP3S3} were performed on an aliquot that was passed through a Dowex 50X8 400 (NH\textsubscript{4}\textsuperscript{+} form obtained by washing with NH\textsubscript{4}OH followed by water) column in order to obtain the ammonium salt.
5. Experimental Part

5.2. Synthesis of the inositol phosphate/sulfate hybrids

General procedures

Phosphorylation reactions

Procedure A: A solution of substrate (1 eq.) in dry CH₂Cl₂ (0.05 M) under an atmosphere of nitrogen was treated with tetrazole in acetonitrile 0.45 M (4 eq. for each hydroxy group) and o-xylylene-N,N-diethylphosphoramidite (2 eq. for each hydroxy group). The reaction mixture was stirred at rt for 20 h. A solution of mCPBA (75% w/w, 5 eq. for each hydroxyl group) in CH₂Cl₂ was dried over Na₂SO₄, added at -10 °C to the reaction mixture and stirred at rt for an additional 45 min. The mixture was then diluted in EtOAc, treated with solution of aqueous NaSO₃ and washed with a saturated solution of aqueous NaHCO₃ and with brine. The organic phase was dried over Na₂SO₄, filtered and evaporated to dryness. The obtained residue was purified by flash chromatography as indicated.

Procedure B: All the solvents used were dried and degassed and the glassware was flame-dried under vacuum followed by argon flush – purge.

In situ preparation of (1R,4R)-1,4-diphenylbutane-N,N-diethylphosphoramidite 80: A solution of (1R,4R)-1,4-diphenylbutandiol 79 in CHCl₃ (5.5 eq., 1M) was added to boiling toluene. The hexaethyl phosphorous triamide solution in toluene (5.5 eq., 1M) was then added dropwise over a period of 30 min and then stirred for a further 10 min. An aliquot was taken to verify the purity by ¹H and ³¹P NMR.

The solution of tetrazole in acetonitrile (ACN) (0.45M, 23 eq.) was added to the in situ prepared phosphoramidite 80, followed by addition of the solution of inositol in CHCl₃ (1 eq., 0.02M). The reaction mixture was stirred at rt for 20 h. A solution of mCPBA (75% w/w, 10 eq.) in CH₂Cl₂ was dried over Na₂SO₄, added at -10 °C to the reaction mixture and stirred at rt for an additional 45 min. The mixture was then diluted in EtOAc, treated with solution of aqueous NaSO₃ and washed with a saturated solution of aqueous NaHCO₃ and with brine. The organic phase was dried over Na₂SO₄, filtered and evaporated to dryness. The obtained residue was purified by flash chromatography as indicated.

Sulfation reactions

Procedure C: A solution of the substrate (1 eq.) in dry DMF (0.02 M) was treated with sulfur trioxide triethylamine complex (5 eq. for each hydroxy group). The reaction mixture was stirred at 50 °C for 20 h and concentrated to dryness. The obtained residue was treated as indicated.
5. Experimental Part

**Procedure D:** A solution of the substrate (1 eq.) in dry DMF (0.03 M) was treated with sulfur trioxide triethylamine complex (5 eq. for each hydroxy group). After 10 min, the reaction mixture was cooled at -10 °C, treated with trifluoromethanesulfonic acid (1.1 eq. for each hydroxy group) and stirred to 4 °C for 16 h. The reaction mixture was neutralized by the addition of Et$_3$N and concentrated to dryness at rt *in vacuo* and then under high vacuum. The mixture was used in the next step without further purification.

**Procedure E:** The substrate (1 eq.) was co-evaporated with toluene (3x) and dried under high vacuum for 1 h. Dry DMF (0.03 M) was added and the reaction mixture was treated with sulfur trioxide triethylamine complex (5 eq. for each hydroxy group) and TfOH (5 eq. for each hydroxy group) and stirred at rt for 20 h. The reaction mixture was neutralized by the addition of Et$_3$N. The reaction mixture was neutralized by the addition of Et$_3$N and concentrated to dryness at rt *in vacuo* and then under high vacuum. The obtained residue was purified by size exclusion chromatography, preliminary on pre-packed column (Sephadex G-25) followed by gravity column (Sephadex G-10).

**Hydrogenation reactions**

**Procedure F:** To a solution of substrate (1 eq.) in MeOH/H$_2$O (1:3, 4 mM) under nitrogen atmosphere was added Pd on activated carbon 10% (w/w) (0.3 eq.). The reaction mixture was evacuated and flushed with hydrogen gas, the operation was repeated 3 times and the reaction mixture was stirred vigorously at rt for 2 h. The reaction mixture was flushed with nitrogen, neutralized with concentrated NH$_4$OH (few drops) to basic pH, filtered through a pad of celite and concentrated to dryness. Purification by size exclusion chromatography preliminary on pre-packed column (Sephadex G-25) followed by flash column (Sephadex G-10).

**Ion exchange to afford the Na$^+$ salt**

**Procedure G:** Dowex H$^+$ was equilibrated to Na$^+$ by passing a solution of NaOH 2 M and washed with nanopure H$_2$O. The solution of inositol phosphate/sulfate hybrids was loaded on the column to afford the Na$^+$ salt.

**Ion exchange to afford the NH$_4^+$ salt**

**Procedure H:** Dowex H$^+$ was equilibrated to NH$_4^+$ by passing a solution of NH$_4$OH$_{aq}$ 25% and washed with nanopure H$_2$O. The solution of inositol phosphate/sulfate hybrids was loaded on the column to afford the NH$_4^+$ salt.
5. Experimental Part

**Purification by strong anion exchange** (Q Sepharose Fast Flow)

**Procedure I:** The product was loaded on strong anion exchange chromatography column (Q Sepharose Fast Flow) and was eluted with a solution of triethylammonium bicarbonate 1 M and NaCl gradient (gradually from 0 → 0.5 M). The pure fractions were then desalted on size exclusion chromatography sephadex G-10.

**Full deprotection by TMSBr hydrolysis**

**Procedure J:** Protected inositol phosphate (1 eq.) in MeOH/DCM 30% (0.05 M) was treated with TMSBr (56 eq.) and stirred for 5 h. The reaction mixture was degased with N₂ and the HBr was neutralized with 1 M NaOH solution. After 1-2 h it was concentrated to dryness. The crude was trituated with acetone and then with ACN to afford clean bisphophate.

**Determination of yield in final products**

The yield of the final product inositol phosphate/sulfate hybrids were determined by ¹H NMR using dioxane as internal reference.

**Synthesis of PSPSPS**

**Synthesis of 2,4,6-tri-O-(4-methoxybenzyl)-myo-inositol 30**

2,4,6-tri-O-(4-methoxybenzyl)-myo-inositol 30 was synthesized as described in the literature.(185) Purification by flash chromatography (SiO₂, EtOAc/hexane 50%) afforded 30 as white solid (600 mg, 1.11 mmol, 55%).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.30 (4H, d, J 8.7 Hz, Ph), 7.26 (2H, d, J 8.4 Hz, Ph), 6.91 (2H, d, J 8.4 Hz, Ph), 6.89 (4H, d, J 8.7 Hz, Ph), 4.78 (4H, s, CH₂-Ph), 4.75 (2H, s, CH₂-Ph), 3.98 (1H, t, J 2.7 Hz, H-C2), 3.83 (3H, s, OCH₃), 3.81 (6H, s, OCH₃), 3.62 (2H, t, J 9.3 Hz, H-C4/6), 3.46-3.56 (3H, m, H-C1/3, H-C5), 2.44 (1H, bd, OH), 2.32 (2H, bd, OH). In accordance with literature.(185)
5. Experimental Part

Synthesis of 2,4,6-tri-O-(4-methoxybenzyl)-1,3,5-tri-O-(o-xylylenephospho)myo-inositol 53

![Chemical Structure](image)

Synthesized from 30 according to procedure A; Eluent (SiO$_2$, CH$_2$Cl$_2$/MeOH gradually from 0 to 4%, three times); 53 was obtained as a white solid (0.901 g, 0.98 mmol, 98%).

$^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) 7.38-7.35 (6H, m, Ph), 7.30-7.33 (6H, m, Ph), 7.22-7.24 (2H, m, Ph), 7.14-7.19 (4H, m, Ph), 6.89 (2H, d, J 8.5 Hz, Ph), 6.73 (4H, d, J 8.5 Hz, Ph), 5.26 (1H, t, $^3$J$_{HP}$ 13.6, $^2$J$_{HH}$ 13.7 Hz, C$_{6}$H$_5$Ph), 5.21 (2H, dd, $^3$J$_{HP}$ 16.6, $^2$J$_{HH}$ 13.7 Hz, C$_{6}$H$_5$Ph), 5.18 (1H, d, J 13.7 Hz, C$_{6}$H$_5$Ph), 5.13 (1H, d, J 13.7 Hz, C$_{6}$H$_5$Ph), 4.93-5.10 (8H, m, C$_{6}$H$_5$Ph), 4.88-4.91 (4H, m, C$_{6}$H$_5$Ph), 4.65-4.69 (3H, m, C$_{6}$H$_5$Ph), 4.59 (1H, q, J 9.2 Hz, C$_{6}$H$_5$Ph), 4.40 (2H, ddd, J 2.4, 8.1, 9.5 Hz, C$_{6}$H$_5$Ph), 4.12 (2H, t, J 9.5 Hz, C$_{6}$H$_5$Ph), 3.81 (3H, s, CH$_3$), 3.74 (6H, s, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm) 159.3 (iPh), 159.1 (2x iPh), 135.4 (2x iPh), 135.3 (2x iPh), 135.2 (2x iPh), 135.1 (2x iPh), 130.8 (iPh), 130.3 (2x iPh), 129.7 (4xPh), 129.6 (2xPh), 129.2 (2xPh), 129.13 (2xPh), 129.12 (2xPh), 129.0 (2xPh), 128.9 (2xPh), 128.6 (2xPh), 113.74 (2xPh), 113.57 (4xPh), 80.6 (d, $^1$J$_{CP}$ 6.0 Hz, C$_5$), 78.1 (2C, dd, $^3$J$_{CP}$ 6.9, 3.2 Hz, C$_4$/$C_6$), 77.6 (C$_2$), 77.1 (2C, C1/3, under chloroform peak), 76.0 (C$_{6}$H$_5$Ph), 74.9 (2xCH$_2$-Ph), 68.74 (d, $^2$J$_{CP}$ 6.3 Hz, 2xCH$_2$-Ph), 68.65 (d, $^2$J$_{CP}$ 6.6 Hz, 2xCH$_2$-Ph), 68.3 (d, $^2$J$_{CP}$ 5.6 Hz, 2xCH$_2$-Ph), 55.4 (CH$_3$), 55.3 (2xCH$_3$); $^{31}$P NMR (160 MHz, $^1$H-decoupled, CDCl$_3$) δ (ppm) 1.10 (P-C$_5$), -1.32 (P-C$_1$/$C_3$); [m/z] (ESI) (M+H)$^+$ C$_{54}$H$_{58}$O$_{18}$P$_3$ calculated 1087.2831, found 1087.2821.

Synthesis of 1,3,5-tri-(O-xylylenephospho)-myo-inositol 53b

![Chemical Structure](image)

To a solution of 2,4,6-tri-O-(4-methoxybenzyl)-5-(O-xylylenephospho)-myo-inositol 53 (97 mg, 89 µmol) in CH$_2$Cl$_2$ (1 mL) was slowly added premixed trifluoroacetic acid and H$_2$O (ratio TFA/H$_2$O 5:1, 6mL). The reaction turned dark pink and was stirred for 25 min. The reaction mixture was diluted with toluene and co-evaporated in vacuo 3 times. The residue was triturated in a mixture hexane-dichloromethane, dried under high vacuum to afford product 53b as a white solid that was used immediately without further purification in the next step to avoid compound decomposition.

$^1$H NMR (400 MHz, MeOD) δ (ppm) 7.44 (8H, s, Ph), 7.42 (4H, s, Ph), 5.71 (2H, dd, $^2$J$_{HH}$ 13.5, $^3$J$_{HP}$ 8.9 Hz, C$_{6}$H$_5$H$_5$Ph), 5.62 (2H, dd, $^2$J$_{HH}$ 13.5, $^3$J$_{HP}$ 9.8 Hz, C$_{6}$H$_5$H$_5$Ph), 5.58 (2H, dd, $^2$J$_{HH}$ 13.3, $^3$J$_{HP}$ 9.3 Hz, C$_{6}$H$_5$H$_5$Ph), 5.13 (2H, dd, $^2$J$_{HH}$ 22.7, $^3$J$_{HP}$ 13.4 Hz, C$_{6}$H$_5$H$_5$Ph), 5.11 (2H, dd, $^3$J$_{HP}$ 22.4, $^2$J$_{HH}$ 13.5 Hz, C$_{6}$H$_5$H$_5$-
5. Experimental Part

Ph), 5.07 (2H, dd, $^3J_{HP}$ 23.4, $^2J_{HH}$ 13.4 Hz, CH$_3$H$_2$-Ph), 4.66 (1H, t, $J$ 2.6 Hz, H-C2), 4.44 (2H, ddd, $J$ 2.6 Hz, $^3J_{HP}$ 7.6, $J$ 10.5 Hz, H-C1/3), 4.35 (1H, q, $J$ 9.4, $^3J_{HP}$ 7.6 Hz, H-C5), 4.22 (2H, t, $J$ 9.4 Hz, H-C4/6).

Synthesis of 1,3,5-tri-(O-xylenephospho)-myo-inositol 2,4,6-tri-O-sulfate 54

Synthesized from 53b according to procedure C; the residue was purified by reverse phase column chromatography (Sep-Pak, C18, methanol/water gradually 0 -> 40%) afforded the product 54 as white solid (32 mg, 33 µmol, 61%).

$^1$H NMR (400 MHz, MeOD) δ (ppm) 7.40-7.45 (4H, m, Ph), 7.33-7.39 (4H, m, Ph), 7.24-7.28 (2H, m, Ph), 7.19-7.21 (2H, m, Ph), 5.66 (2H, dd, $^2J_{HH}$ 13.4, $^3J_{HP}$ 8.0 Hz, CH$_3$H$_2$-Ph), 5.63 (2H, dd, $^2J_{HH}$ 13.6, $^3J_{HP}$ 9.7 Hz, CH$_3$H$_2$-Ph), 5.47 (2H, dd, $^2J_{HH}$ 13.2, $^3J_{HP}$ 10.2 Hz, CH$_3$H$_2$-Ph), 5.41 (2H, t, $J$ 2.8 Hz, H-C4/6), 5.34-5.39 (2H, m, H-C1/3), 5.18 (1H, dt, $^3J_{HH}$ 10.9, $J$ 2.7 Hz, H-C5), 5.06-5.11 (1H, m, H-C4/6), 5.06-5.11 (1H, m, H-C2), 5.07 (2H, dd, $^3J_{HP}$ 22.2, $^2J_{HH}$ 13.7 Hz, CH$_3$H$_2$-Ph), 5.02 (2H, dd, $^3J_{HP}$ 24.1, $^2J_{HH}$ 13.4 Hz, CH$_3$H$_2$-Ph), 4.35 (1H, q, $J$ 9.4, $^3J_{HP}$ 7.6 Hz, H-C5), 4.22 (2H, t, $J$ 9.4 Hz, H-C4/6), 4.22 (2H, t, $J$ 9.4 Hz, H-C4/6).

$^{13}$C NMR (125 MHz, MeOD) δ (ppm) 137.5 (2x iPh), 137.4 (2x iPh), 137.1 (2x iPh), 130.6 (4xPh), 130.5 (2xPh), 130.33 (2xPh), 130.30 (4xPh), 75.8 (d, $^3J_{CP}$ 4.3 Hz, C4/6), 75.6 (d, $^3J_{CP}$ 6.0 Hz, C1/3), 73.7 (d, $^3J_{CP}$ 6.0 Hz, C5), 70.6 (d, $^3J_{CP}$ 7.3 Hz, 2xCH$_3$H$_2$-Ph), 70.5 (d, $^3J_{CP}$ 6.5 Hz, 2xCH$_3$H$_2$-Ph), 70.3 (d, $^3J_{CP}$ 7.4 Hz, 2xCH$_3$H$_2$-Ph), 70.0-70.2 (C2 determined by HSQC, not apparent on $^{13}$C NMR spectra); $^{31}$P NMR (162 MHz, $^1$H-decoupled, MeOD/CDCl$_3$) δ (ppm) -3.7 (P-C1/3), -4.8 (P-C5).

Synthesis of 1,3,5-tri-(O-phosphate)-myo-inositol 2,4,6-tri-O-sulfate PSPSPS

Synthesized from 54 according to procedure F; Purification different than the one indicated in the procedure C. The residue was purified by reverse phase column chromatography (Sep-Pak, C18, water) and afforded PSPSPS as a white solid (ammonium salt, 16 mg, as PSPSPS·2Et$_3$NH+$^+$·6NH$_4^+$ (by $^1$H NMR) MW 963.84, 17 µmol, 50%).

$^1$H NMR (400 MHz, D$_2$O) δ (ppm) 4.98 (1H, bd, H-C2), 4.90 (2H, bd, H-C4/6), 4.47-4.60 (3H, m, C4/6), 71.3 (d, $^3J_{CP}$ 5.1 Hz, C1/3), 46.7 (CH$_3$CH$_2$NH), 8.2 (CH$_3$CH$_2$NH), (1); 78
5. Experimental Part

$^{31}$P NMR (162 MHz, $^1$H-decoupled, D$_2$O) δ (ppm) -0.39 (2P, s, P-C1/3), -0.81 (1P, s, P-C5); [m/z (ESI) [IP$_3$S$_3$·7H$^+$·3NH$_4^+$] C$_{6}$H$_{25}$N$_3$O$_2$P$_3$S$_3$ calculated 711.9197, found 711.9199].

(1) C2 and C5 were neither visible on the $^{13}$C NMR nor on the HSQC spectra.

**Synthesis of SSSSPS**

**Synthesis of 1,2,3,4,6-penta-O-(4-methoxybenzyl)-myo-inositol 55**

![Structure of 1,2,3,4,6-penta-O-(4-methoxybenzyl)-myo-inositol 55]

To a solution of 2,4,6-tri-O-(4-methoxybenzyl)-myo-inositol 30 (530 mg, 0.98 mmol, 1 eq.) in DMF (10 ml, 0.1 M) at 0 °C was added portionwise NaH (60% in oil w/w; 86 mg, 2.16 mmol, 2.2 eq.). The reaction mixture was then treated with PMBCl (0.29 mL, 2.16 mmol, 2.2 eq.) and heated at 50 °C for 3h. The mixture was quenched with MeOH and concentrated in vacuo. The crude was diluted with EtOAc and washed with H$_2$O and then brine. The organic phase was dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, EtOAc/hexane 30 -> 40%) gave a mixture of the two monohydroxylated isomers. The mixture was separated by a second flash column chromatography (SiO$_2$, EtOAc/hexane 30%) to afford 55 as a white solid (170 mg, 0.22 mmol, 22%).

$^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) 7.32 (2H, d, J 8.7 Hz, Ph), 7.27 (4H, d, J 8.3 Hz, Ph), 7.24 (4H, d, J 8.7 Hz, Ph), 6.83-6.89 (10H, m, Ph), 4.84 (2H, d, J 10.8 Hz, CH$_2$-Ph), 4.79 (2H, s, CH$_2$-Ph), 4.70 (2H, d, J 10.8 Hz, CH$_2$-Ph), 4.56 (2H, d, J 11.3 Hz, CH$_2$-Ph), 4.52 (2H, d, J 11.3 Hz, CH$_2$-Ph), 3.99 (1H, t, J 2.2 Hz, H-C2), 3.87 (2H, t, J 9.5 Hz, H-C4/6), 3.82 (6H, s, CH$_3$), 3.81 (3H, s, CH$_3$), 3.80 (6H, s, CH$_3$), 3.45 (1H, t, J 9.2 Hz, H-C5), 3.28 (1H, dd, J 9.7, 2.2 Hz, H-C1/3), 2.40 (1H, br, OH); $^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm) 159.33 (2x$i$Ph), 159.31 (2x$i$Ph), 159.2 (iPh), 131.33 (iPh), 131.30 (2x$i$Ph), 130.7 (2x$i$Ph), 129.8 (4xPh), 129.5 (2xPh), 129.3 (4xPh), 113.97 (4xPh), 113.91 (4xPh), 113.7 (2xPh), 81.0 (C4/6), 80.7 (C1/3), 75.3 (C5), 75.1 (2xCH$_2$-Ph), 74.3 (C2), 73.9 (CH$_2$-Ph), 72.4 (2xCH$_2$-Ph), 55.43 (2xCH$_3$), 55.42 (3xCH$_3$); [m/z (ESI) (M+Na)$^+$ C$_{46}$H$_{52}$NaO$_{11}$ calculated 803.3402, found 803.3387].

**Synthesis of 1,2,3,4,6-penta-O-(4-methoxybenzyl)-5-(O-xylylenephospho)-myo-inositol 56**

![Structure of 1,2,3,4,6-penta-O-(4-methoxybenzyl)-5-(O-xylylenephospho)-myo-inositol 56]
5. Experimental Part

Synthesized from 55 according to Procedure A; Eluent: EtOAc/hexane gradually from 30 -> 40%; A mixture of product 56 and phosphate, a reagent by-product, was obtained. Trituration in Et₂O afforded the pure product 56 as a white solid (110 mg, 0.11 mmol, 71%).

^1H NMR (400 MHz, CDCl₃) δ (ppm) 7.31-7.37 (6H, m, Ph), 7.29 (2H, dd, J 5.6, 3.3 Hz, Ph), 7.19 (4H, d, J 8.6 Hz Ph), 7.13 (2H, dd, J 5.6, 3.3 Hz, Ph), 6.81-6.87 (10H, m, Ph), 5.22 (2H, dd, JHP 14.9, JHH 13.9 Hz, CH₃H₆-Ph), 5.01 (2H, dd, JHP 17.5, JHH 13.9 Hz, CH₃H₆-Ph), 4.79 (2H, s, CH₂-Ph), 4.78 (4H, s, CH₂-Ph), 4.47-4.55 (1H, m, H-C5), 4.52 (2H, d, J11.3 Hz, CH₂-Ph), 4.47 (2H, d, J 11.3 Hz, CH₂-Ph), 4.01 (2H, t, J 9.6 Hz, H-C4/6), 3.93 (1H, t, J 1.9 Hz, H-C2), 3.81 (6H, s, CH₃), 3.80 (3H, s, CH₃), 3.80 (6H, s, CH₃), 3.30 (2H, dd, J9.8, 1.9 Hz, H-C1/3); ^31P NMR (160 MHz, 1H-decoupled, CDCl₃) δ (ppm) -2.04 (P-C5); m/z (ESI) (M+Na)⁺ C₅₄H₅₉NaO₁₄P calculated 985.3535, found 985.3534.

Synthesis of 5-(O-xylylenephospho)-myo-inositol 56b

To a solution of 1,2,3,4,6-penta-O-(4-methoxybenzyl)-5-(O-xylylenephospho)-myo-inositol 56 (45 mg, 47 µmol) in CH₂Cl₂ (1 mL) was slowly added premixed trifluoroacetic acid and H₂O (ratio TFA/H₂O 5:1, 6 mL). The reaction turned dark pink and was stirred for 25 min. The reaction mixture was diluted with toluene and co-evaporated 3 times. The crude was suspended in diethylether and dried under high vacuum to afford product 56b as a white solid that was used without further purification in the next step to avoid compound decomposition.

^1H NMR (400 MHz, MeOD) δ (ppm) 7.42 (4H, s, Ph), 5.63 (2H, dd, J13.5, JHP 9.3 Hz, CH₃H₆-Ph), 5.07 (2H, dd, J13.5, JHP 9.3 Hz, CH₃H₆-Ph), 4.11 (1H, td, J 9.5, JHP 7.6 Hz, H-C5), 3.99 (1H, t, J 2.8 Hz, H-C2), 3.88 (2H, t, J 9.5 Hz, H-C4/6), 3.44 (2H, dd, J 9.5, 2.8 Hz, H-C1/3); ^31P NMR (160 MHz, 1H-decoupled, MeOD) δ (ppm) -2.04 (P-C5); m/z (ESI) (M+Na)⁺ C₅₄H₅₉NaO₁₄P calculated 985.3535, found 985.3534.
5. Experimental Part

**Synthesis of 5-(O-xylenephospho)-myo-inositol 1,2,3,4,6-penta-O-sulfate 57**

![Chemical Structure](image)

Synthesized from 56b according to procedure D; the residue was used without further purification in the next step to avoid compound decomposition.

**1H NMR** (400 MHz, MeOD) δ (ppm) 7.42 (4H, s, Ph), 5.95 (2H, d, 2_J_HH 13.6, 2_J_HP 6.1 Hz, CH_6_H_6-Ph), 5.54 (2H, br, H-C4/6), 5.26 (1H, d, 2_J_HH 10.7 Hz, H-C2), 5.13 (2H, br, H-C1/3), 4.95 (2H, dd, 2_J_HH 25.4, 3_J_HH 13.6 Hz, CH_3_H_6-Ph), 4.87 (1H, t, J 3.7 Hz, H-C5); **13C NMR** 500 MHz (125 MHz, MeOD) δ (ppm) 137.0 (2x_iPh), 129.4 (2xPh), 129.1 (2xPh), 74.2-74.3 (4C, m, C1/3-C4/6), 72.1 (C2), 69.9 (2C, d, 2_J_CP 8.2 Hz, CH_3-H_6-Ph), 69.1 (2C, d, 2_J_CP 8.2 Hz, C5); **31P NMR** (160 MHz, 1H-decoupled, MeOD) δ (ppm) -6.23 (P-C5).

**Synthesis of 5-(O-phosphate)-myo-inositol 1,2,3,4,6-penta-O-sulfate SSSSPS**

![Chemical Structure](image)

Synthesized from 57 according to procedure F; SSSPS was obtained as a white solid (ammonium salt, 10.29 µmol, 40% over 2 steps, the concentration of the final product was determined by 1H NMR with dioxane as internal reference).

**1H NMR** (400 MHz, D_2O) δ (ppm) 5.31 (1H, br, H-C2), 4.85 (2H, br, 4.68 (2H, br), 4.42-4.50 (1H, m, H-C5), 3.22 (42H, q, J 7.3 Hz, CH_3CH_2NH), 1.30 (63H, t, J 7.3 Hz, CH_3CH_2NH); **13C NMR** 500 MHz (125 MHz, D_2O) δ (ppm) 76.0 (br, C4/6), 73.7 (s, C1/3), 46.7 (CH_3CH_2NH), 46.7 (CH_3CH_2NH), 8.3 (CH_3CH_2NH), (1); **31P NMR** (162 MHz, 1H-decoupled, D_2O) δ (ppm) -0.60 (P-C5); [m/z] (ESI) [IP_5S_5^3-3H^+·5NH_4]· C_6H_29N_5O_2P_2S_5 calculated 745.9538, found 745.9541.

(1) C2 and C5 were neither visible on the 13C NMR nor on the HSQC spectra.

**Synthesis of SSSPSP**

**Synthesis of 2-O-(tert-butyldimethylsilyl)-1,3,5-orthoformate-myoinositol 58**

![Chemical Structure](image)

81
5. Experimental Part

2-O-tert-Butyldimethylsilyl compound 58 was synthesized as described in the literature.(186) Purification by flash chromatography (SiO₂, EtOAc/hexane 30%) afforded 58 as white crystals (332 mg, 1.09 mmol, 50%).

\[ ^1H\ NMR\ (400\ MHz, CDCl_3)\ \delta\ (ppm)\ 5.51\ (1H, d, ^5J\ 1.4\ Hz, H-C7),\ 4.59\ (2H, dt, J\ 7.6, 3.9\ Hz, H-C4/6),\ 4.25-4.29\ (2H, m, H-C2, H-C5),\ 4.16\ (2H, dt, J\ 4.6, 1.7\ Hz, H-C1/3),\ 3.17\ (2H, d, J\ 7.6\ Hz, OH),\ 0.95\ (9H, s, (CH₃)₃C-Si),\ 0.16\ (6H, s, CH₃-Si).\]
In accordance with the literature.(186)

Synthesis of 2-O-(tert-butyldimethylsilyl)-4,6-di-(O-xylylenephospho)-1,3,5-orthoformate myo-inositol 59

Synthesized from 58 according to procedure A; Eluent: EtOAc/hexane 60 -> 70%; 59 was obtained as white solid (103 mg, 0.15 mmol, 93%).

\[ ^1H\ NMR\ (400\ MHz, CDCl_3)\ \delta\ (ppm)\ 7.34-7.39\ (4H, m, H-Ph),\ 7.23-7.26\ (2H, m, H-Ph),\ 7.21-7.18\ (2H, m, H-Ph),\ 5.52\ (1H, d, ^5J\ 1.3\ Hz, H-C7),\ 5.24\ (2H, dt, ^3J_{HP}\ 8.3, J\ 3.8\ Hz, H-C4/6),\ 4.59-4.62\ (1H, m, H-C5),\ 4.32-4.34\ (2H, m, H-C1/3),\ 0.94\ (9H, s, (CH₃)₃C-Si),\ 0.15\ (6H, s, CH₃-Si);\ ^13C\ NMR\ (125\ MHz, CDCl_3)\ \delta\ (ppm)\ 135.3\ (2xiPh),\ 135.2\ (2xPh),\ 129.59\ (2xPh),\ 129.55\ (2xPh),\ 129.3\ (4xPh),\ 102.8\ (C7),\ 72.7\ (2C, d, ^3J_{CP}\ 4.4\ Hz, C1/3),\ 71.5\ (2C, d, ^3J_{CP}\ 5.3\ Hz, C4/6),\ 68.9\ (2C, d, ^3J_{CP}\ 6.4\ Hz, 2xCH₂-Ph),\ 68.8\ (2C, d, ^3J_{CP}\ 6.3\ Hz, 2xCH₂-Ph),\ 68.0\ (t, ^3J_{CP}\ 5.6\ Hz, C5),\ 60.2\ (C2),\ 26.0\ (3x(CH₃)₃C-Si),\ 18.5\ ((CH₃)₃C-Si),\ ^31P\ NMR\ (162\ MHz, ^1H-decoupled, CDCl_3)\ \delta\ (ppm)\ -1.81\ (P-C4/6);\ [m/z\ (ESI)\ (M+H)^+]\ C_{19}H_{39}O_{12}P₂Si\ calculated\ 669.1681,\ found\ 669.1682].

Synthesis of 4,6-(di-O-xylylenephospho)-myo-inositol 61

A suspension of 59 (20 mg, 30 µmol, 1 eq.) in methanol (550 µL, 55 mM) was treated with a solution of HClaq 2 M (150 µL, 300 µmol, 10 eq.) and stirred for 4 h to afford a conversion of 55%. The reaction mixture was concentrated to dryness. Trituration in CH₂Cl₂ afforded 61 as white solid in 13 – 45% yield that was used without further purification in the next step to avoid compound decomposition.

\[ ^1H\ NMR\ (400\ MHz, CD₂OD/CDCl_3\ 5:1)\ \delta\ (ppm)\ 7.40\ (8H, s, Ph),\ 5.63\ (2H, dd, ^2J_{HH}\ 13.5, ^3J_{HP}\ 9.4\ Hz, CH₃H₂-Ph),\ 5.60\ (2H, dd, ^2J_{HH}\ 13.6, ^3J_{HP}\ 9.6\ Hz, CH₃H₂-Ph),\ 5.08\ (2H, dd, ^3J_{HP}\ 22.7, ^2J_{HH}\ 13.5\ Hz, CH₃H₂-Ph)\]
5. Experimental Part

Ph), 5.07 (2H, dd, $^3J_{HH}$ 13.6 Hz, CH$_2$H$_5$-Ph), 4.58 (2H, q, $^3J_{HH}$ 9.0, $^3J_{HP}$ 9.0 Hz, H-C4/6), 4.06 (1H, t, $^3J_{HH}$ 2.5 Hz, H-C2), 3.81 (1H, t, $^3J_{HH}$ 9.2 Hz, H-C5), 3.74 (2H, dd, $^3J_{HH}$ 2.6 Hz, $^3J_{HP}$ 9.0 Hz, H-C1/3), 3.58 (1H, t, $^3J_{HH}$ 2.5 Hz, H-C2), 3.81 (1H, t, $^3J_{HH}$ 9.2 Hz, H-C5), 3.74 (2H, dd, $^3J_{HH}$ 2.6 Hz, $^3J_{HP}$ 9.0 Hz, H-C1/3); $^{13}$C NMR (125 MHz, CD$_3$OD/CDCl$_3$ 5:1) δ (ppm) 137.0 (2x $^1$Ph), 136.9 (2x $^1$Ph), 130.4 (4xPh), 130.2 (4xPh), 83.2 (2C, d, $^2J_{CP}$ 6.1 Hz, C4/6), 73.9 (C2), 73.1-73.2 (m, C5), 71.6 (2C, br, C1/3), 70.12 (2C, d, $^2J_{CP}$ 6.3 Hz, CH$_2$-Ph), 70.11 (2C, d, $^2J_{CP}$ 6.3 Hz, CH$_2$-Ph), $^{31}$P NMR (162 MHz, $^1$H-decoupled, CDCl$_3$) δ (ppm) -1.85 (P-C4/6); [m/z (ESI) (M+H)$^+$ C$_{22}$H$_{27}$O$_{12}$P$_2$ calculated 545.0972, found 545.0968].

Synthesis of 4,6-di-(O-xylenepospho)-myo-inositol 1,2,3,5-tetra-O-sulfate 60

Synthesized from 61 according to procedure D; the residue was used without further purification in the next step to avoid compound decomposition.

$^1$H NMR (400 MHz, MeOD) δ (ppm) 7.40 (8H, s, Ph), 5.88 (1H, br, H-C2), 5.77 (2H, dd, $^3J_{HH}$ 14.0, $^3J_{HP}$ 7.2 Hz, CH$_2$H$_5$-Ph), 5.62 (2H, dd, $^3J_{HH}$ 13.8, $^3J_{HP}$ 8.1 Hz, CH$_2$H$_5$-Ph), 5.03 (2H, dd, $^3J_{HP}$ 25.4, $^3J_{HH}$ 13.9 Hz, CH$_2$H$_5$-Ph), 5.01 (2H, dd, $^3J_{HH}$ 23.8, $^3J_{HP}$ 13.8 Hz, CH$_2$H$_5$-Ph), 4.92-4.99 (1H, H-C5, under H-C1/3 and H-C4/6), 4.80 (2H, q, $^3J_{HH}$ 9.6, $^3J_{HP}$ 9.6 Hz, H-C4/6), 4.62 (2H, d, $^3J_{HH}$ 11.1 Hz, H-C1/3); $^{31}$P NMR (162 MHz, $^1$H-decoupled, MeOD) δ (ppm) -4.01 (P-C4/6).

Synthesis of 4,6-di-(O-phosphate)-myo-inositol 1,2,3,5-tetra-O-sulfate SSSPSP

Synthesized from 60 according to procedure F at the exception that solvent was MeOH dry; After purification, NH$_4^+$ cation was exchanged to Na$^+$ following the procedure G. SSSPSP was obtained as a white solid (sodium salt, 15.5 mg, assuming IP$_2$S$_4$·6Na MW: 814.24, 19 µmol, yield 86%, over two steps)

Alternatively, if the purity was not satisfying, the product was purified following the Procedure I, generally the product was obtained in a yield of 33 – 49%.

$^1$H NMR (400 MHz, D$_2$O) δ (ppm) 5.47 (1H, bd, H-C2), 4.66 (2H, bd, H-C4/6), 4.58 (2H, bd, H-C1/3), 3.22 (36H, q, J 7.3 Hz, CH$_3$CH$_2$NH), 1.30 (54H, t, J 7.3 Hz, CH$_3$CH$_2$NH), H-C5 under H-C1/3 and H-C4/6; $^{13}$C NMR (125 MHz, D$_2$O) δ (ppm) 74.6 ( $^3J_{CP}$ 3.0 Hz, C1/3), 72.7 (d, $^2J_{CP}$ 4.8 Hz, C4/6), 46.7 (CH$_3$CH$_2$NH),
5. Experimental Part

8.2 (CH$_3$CH$_2$NH), (1); $^{31}$P NMR (162 MHz, $^1$H-decoupled, D$_2$O) δ (ppm) -0.84 (bd, P-C4/6); [m/z (ESI) [IP$_2$S$_4$·6H$^+$·3NH$_4$]$^+$] C$_6$H$_2$N$_3$O$_2$P$_2$S$_4$ calculated 711.9102, found 711.9100].

(1) C2 and C5 were neither visible on the $^{13}$C NMR nor on the HSQC spectra.

**Optimized synthesis of SSSPSP**

**Synthesis of 4,6-di-O-phosphate myo-inositol 64**

![Chemical structure](image)

Synthesized from 59 according to Procedure J; 64 was obtained as a white solid (539 mg, 95%).

$^1$H NMR (400 MHz, MeOD): δ (ppm) = 4.40 (q, $^3$J$_{HH}$ = 9.1 Hz, $^2$J$_{HP}$ = 9.1 Hz, 2 H, H-C4/6), 4.01 (t, $^J$ = 2.6 Hz, 1 H, H-C2), 3.63 (dd, $^J$ = 9.68, 2.76 Hz ,2 H, H-C1/3), 3.61 (t, $^J$ = 9.27 Hz, 1 H, H-C5); $^{31}$P NMR (160 MHz, $^1$H-decoupled, MeOD): δ (ppm) = 1.15 (P-C4/6); $^{13}$C NMR (150 MHz, MeOD): δ (ppm) = 81.28 (d, $^2$J$_{CP}$ = 6.1 Hz, 2 C, C4/6), 74.12 (t, $^3$J$_{CP}$ = 3.8 Hz, 1 C, C5), 73.75 (s, 1 C, C2), 72.13 (d, $^3$J$_{CP}$ = 3.2 Hz, 2 C, C1/3); [m/z (ESI) (M+H)$^+$ C$_6$H$_{15}$O$_{12}$P$_2$ required 341.0033, found 341.0037].

**Synthesis of 1,2,3,5-tetra-O-sulfonyl-4,6-di-O-phosphate myo-inositol SSSPSP**

4,6-Di-O-phosphate-myo-inositol 64 (30 mg, 90 µmol, 1 eq.) was co-evaporated with toluene (3x) and dried under high vacuum for 1 h. Dry DMF (1 mL, 0.09 M) was added and the reaction mixture was treated with sulfur trioxide triethylamine complex (197 mg, 109 µmol, 12 eq.) and TfOH (190 µL, 215 µmol, 24 eq.) and stirred at 45 °C for 20 h. The reaction mixture was neutralized by the addition of Et$_3$N (0.15 mL, 12 eq.). Immediately after neutralization, the crude was diluted in nanopure water (2 mL) and loaded on sephadex G-10 to afford SSSPSP as a white solid (46.31 µmol, 50%). Analytical data reported on page 83.

**Synthesis of (±)-SPSPSS**

**Synthesis of (±)-6-O-(p-methoxybenzyl)-myo-inositol orthoformate 65**

![Chemical structure](image)
5. Experimental Part

To a solution of myo-Inositol orthoformate (200 mg, 1.05 mmol, 1 eq.) in DMF dry (7.5 mL, 0.14 M) at 0 °C was added portionwise NaH (60% in oil w/w; 42 mg, 1.06 mmol, 1.01 eq.). The reaction mixture was then treated with PMBCl (153 µL, 1.16 mmol, 1.1 eq.) and stirred at rt for 4 h. The mixture was neutralized with MeOH and concentrated in vacuo at 60 °C. The crude was diluted with EtOAc and washed with H₂O and then with brine. The organic phase was dried over Na₂SO₄ filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, EtOAc/hexane 50%) afforded 65 as a colorless oil (244 mg, 0.78 mmol, 75%).

**1H NMR** (400 MHz, CDCl₃) δ (ppm) 7.23 (2H, d, J 8.7 Hz, Ph), 6.90 (2H, d, J 8.7 Hz, Ph), 5.43 (1H, d, J 1.3 Hz, H-C7), 4.61 (2H, d, J 11.3 Hz, CH₂-Ph), 4.57 (2H, d, J 11.3 Hz, CH₂-Ph), 4.44 (1H, dt, J 10.3, 3.9, 2.1 Hz, H-C6), 4.40 (1H, td, J 3.9, 2.0 Hz, H-C4), 4.23-4.25 (1H, m, H-C5), 4.18-4.22 (2H, m, H-C3, H-C1), 4.06 (1H, d, J 11.8 Hz, H-C2), 3.81 (3H, s, O-CH₃), 3.77 (1H, d, J 10.3 Hz, OH-C6), 3.23 (1H, d, J 11.8 Hz, OH-C2); **13C NMR** (400 MHz, CDCl₃) δ (ppm) 160.1 (iPh), 129.9 (2xPh), 127.9 (iPh), 114.3 (2xPh), 102.7 (C7), 74.8 (C1), 73.8 (C4), 72.8 (CH₂-Ph), 72.3 (C3), 67.9 (C6), 67.3 (C5), 60.7 (C2), 55.3 (O-CH₃); [m/z (ESI) (M+Na)⁺ C₁₅H₁₈NaO₇ requires 333.0945, found 333.0943].

Synthesis of (±)-6-O-(p-methoxybenzyl)-2,4-(di-O-xylylenephospho)-myo-inositol orthoformate (±)-71

Synthesized from (±)-65 according to procedure A; Eluent: EtOAc/Hexane 60%; (±)-71 was obtained as white solid (135 mg, 20.1 mmol, 53%).

**1H NMR** (400 MHz, CDCl₃) δ (ppm) 7.30-7.41 (6H, m, Ph), 7.22-7.14 (4H, m, Ph), 5.56 (1H, d, 2Jₜₚ 10.3 Hz, CH₂-Ph), 5.45 (1H, dd, 2Jₜₚ 10.3 Hz, CH₂-Ph), 5.42 (1H, dd, 2Jₜₚ 13.6, 3Jₜₚ 8.2 Hz, CH₂-Ph), 5.36 (1H, dt, 3Jₜₚ 8.4, 3Jₜₚ 3.4 Hz, H-C6), 5.18 (1H, dd, 3Jₜₚ 20.3, 2Jₜₚ 13.6 Hz, CH₂-Ph), 5.17 (1H, dd, 3Jₜₚ 20.3, 2Jₜₚ 13.6 Hz, CH₂-Ph), 5.12 (1H, d, 2Jₜₚ 7.5 Hz, H-C2), 5.08 (1H, dd, 2Jₜₚ 13.5, 3Jₜₚ 10.1 Hz, CH₂-Ph), 4.96 (1H, dd, 2Jₜₚ 13.5, 3Jₜₚ 10.3 Hz, CH₂-Ph), 4.84 (1H, dd, 2Jₜₚ 21.9, 2Jₜₚ 13.5 Hz, CH₂-Ph), 4.72 (1H, dd, 2Jₜₚ 22.0, 2Jₜₚ 13.4 Hz, CH₂-Ph), 4.67-4.70 (1H, m, H-C3), 4.61 (1H, d, 2Jₜₚ 10.3, CH₂-Ph), 4.59-4.61 (1H, m, H-C1), 4.55-4.58 (1H, m, H-C5), 4.50 (1H, d, 2Jₜₚ 10.3, CH₂-Ph), 4.46-4.48 (1H, m, H-C4), 3.65 (3H, s, O-CH₃); **13C NMR** (125 MHz, CDCl₃) δ (ppm) 159.6 (iPh), 135.4 (iPh), 135.3 (2xPh), 135.2 (iPh), 130.1 (2xPh), 129.48 (Ph), 129.47 (Ph), 129.4 (Ph), 129.30 (Ph), 129.26 (Ph), 129.23 (Ph), 129.21 (Ph), 129.1 (Ph), 128.8 (iPh), 113.9 (2xPh), 103.1 (C7), 73.3 (C4), 72.0 (CH₂-Ph), 71.1 (t, 3Jₜₚ 5.4 Hz, C1), 71.0 (d, 3Jₜₚ 5.1 Hz, C6), 70.6 (d, 3Jₜₚ 3.6 Hz, C3), 69.2 (d, 3Jₜₚ 5.6 Hz, CH₂-Ph), 68.2 (t, 3Jₜₚ 2.5 Hz, C8), 58.4 (t, 3Jₜₚ 2.5 Hz, C10).
5. Experimental Part

69.1 (d, Jcp 5.4 Hz, CH2-Ph), 68.92 (d, Jcp 5.4 Hz, CH2-Ph), 68.85 (d, Jcp 5.4 Hz, CH2-Ph), 68.2 (d, Jcp 4.1 Hz, C5), 67.2 (d, Jcp 4.5 Hz, C2), 55.2 (O-CH3); 31P NMR (160 MHz, 1H-decoupled, CDCl3) δ (ppm) -2.05 (1P), -3.68 (1P); [m/z] (ESI) (M+H)+ C31H33O13P2 requires 675.1391, found 675.1376.

Synthesis of (±)-2,4-di-O-phosphate myo-inositol (±)-72

Synthesized from (±)-71 according to procedure J; (±)-72 was obtained as white solid (5.2 mg, 15.4 µmol, 85%).

1H NMR (400 MHz, MeOD) δ (ppm) 4.70 (1H, dt, JHP 8.2, J 2.0 Hz, H-C2), 4.32 (1H, q, JHP 9.2, J 9.2 Hz, H-C6), 3.74 (1H, d, J 9.4 Hz, H-C1), 3.67 (1H, t, J 9.5 Hz, H-C4), 3.55 (1H, dt, J 9.9, 2.0 Hz, H-C3), 3.43 (1H, t, J 9.1 Hz, H-C5); 13C NMR (125 MHz, CDCl3) δ (ppm) 81.3 (d, Jcp 6.2 Hz, C2), 81.1 (d, Jcp 6.2 Hz, C6), 75.3 (d, Jcp 3.6 Hz, C5), 73.9 (C4), 71.9 (d, Jcp 3.3 Hz, C3), 71.2 (t, Jcp 3.3 Hz, C1); 31P NMR (162 MHz, 1H-decoupled, CDCl3) δ (ppm) 1.10 (1P), 0.74 (1P); [m/z] (ESI) (M+H)+ C6H16O12P2 requires 341.0033, measured 341.0034.

Synthesis of (±)-1,3,5,6-tetra-O-sulfonyl-2,4-di-O-phosphate myo-inositol (±)-SPSPSS

Synthesized from (±)-72 according to procedure E; After purification, cations was exchanged to NH4+ following Procedure H. (±)-SPSPSS was obtained as white solid (ammonium salt, 6.3 mg, 9.6 µmol, 51%).

1H NMR (500 MHz, MeOD) δ (ppm); 5.12 (1H, bd, H-C2), 4.73-4.91 (1H, hidden under water peak, seen in COSY), 4.69 (1H, bd, H-C1), 4.59 (1H, t, J 6.8 Hz), 4.50-4.60 (2H, m); 13C NMR (125 MHz, CDCl3) δ (ppm) 75.3, 74.9, 74.4 (d, Jcp 3.0 Hz, C2), 72.61 (bd, 1); 31P NMR (162 MHz, 1H-decoupled, CDCl3) δ (ppm) -0.60 (1P); -0.93 (1P); [m/z] (ESI) [IP2Sa.5H+.4NH4+]+ C38H27N4O24P2S4 requires 728.9368, found 728.9374.

(1) 3 carbons could not be observed on the 13C NMR neither on HSQC spectra.
Synthesis of (±)-PSSPS

Synthesis of (±)-2,3,4,6-tetra-O-benzyl-1,5-O-ethyldiene myo-inositol (±)-67

To a solution of (±)-2,4,6-tri-O-benzyl-1,5-O-ethyldiene myo-inositol (483 mg, 1.01 mmol, 1 eq.) in DMF dry (10.1 mL, 0.1 M) at 0 °C was added portionwise NaH (60% in oil w/w; 57 mg, 1.42 mmol, 1.4 eq.). The reaction mixture was then treated with Bn Cl (217 µL, 1.72 mmol, 1.7 eq.) and stirred for 20 h. The mixture was neutralized with MeOH and concentrated in vacuo at 60 °C. The crude was diluted with EtOAc and washed with H₂O and then with brine. The organic phase was dried over Na₂SO₄ filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, EtOAc/hexane 10%) afforded (±)-67 as a white solid (528 mg, 0.93 mmol, 92%).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.20-7.38 (20H, m, Ph), 5.38 (1H, q, J 4.8 Hz, H-C7), 4.78 (1H, d, J 11.7 Hz, CH₂-Ph), 4.77 (1H, d, J 11.9 Hz, CH₂-Ph), 4.73 (1H, d, J 11.9 Hz, CH₂-Ph), 4.68 (1H, d, J 11.4 Hz, CH₂-Ph), 4.65 (1H, d, J 11.6 Hz, CH₂-Ph), 4.54 (1H, d, J 11.7 Hz, CH₂-Ph), 4.48 (1H, d, J 11.7 Hz, CH₂-Ph), 4.42-4.45 (1H, m), 4.41 (1H, d, J 11.5 Hz, CH₂-Ph), 4.35 (1H, dd, J 3.6, 1.9 Hz), 4.30 (1H, t, J 7.9 Hz), 4.12 (1H, t, J 7.5 Hz), 4.06 (1H, d, J 8.0 Hz), 3.97 (1H, td, J 4.0, 0.9 Hz), 1.25 (1H, d, J 4.9 Hz); ¹³C NMR (400 MHz, CDCl₃) δ (ppm) 138.5 (iPh), 138.4 (iPh), 137.9 (iPh), 137.6 (iPh), 128.50 (2C, Ph), 128.47 (2C, Ph), 128.4 (2C, Ph), 128.3 (2C, Ph), 128.2 (2C, Ph), 128.1 (2C, Ph), 127.92 (Ph), 127.90 (2C, Ph), 127.58 (Ph), 127.6 (2C, Ph), 127.52 (Ph), 127.47 (Ph), 90.9 (C7), 80.9, 76.3, 73.4, 73.3, 72.3 (CH₂-Ph), 72.32 (C3), 72.26 (CH₂-Ph), 72.2 (CH₂-Ph), 71.3 (CH₂-Ph), 69.8, 68.6, 20.8 (CH₃); [m/z (ESI) (M+Na)⁺ C₃₆H₃₈NaO₆ requires 589.2561, found 589.2556].

Synthesis of (±)-2,3,4,6-tetra-O-benzyl myo-inositol (±)-68

A solution of (±)-67 (60.9 mg, 107 µmol, 1 eq.) in MeOH (6.5 mL, 0.02 M) at 0 °C was treated with HClcc (75 µL, 900 µmol, 8.4 eq.) and stirred at rt for 2.5 h. The mixture was concentrated in vacuo at 40 °C and co-evaporated with toluene. Purification by flash column chromatography (SiO₂,
EtOAc/hexane 30%) afforded (±)-68 as a white solid (52.6 mg, 97 µmol, 91%).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) δ (ppm) 7.28-7.41 (20H, m, Ph), 5.01 (1H, d, J 11.6 Hz, CH\(_2\)-Ph), 5.01 (1H, d, J 11.2 Hz, CH\(_2\)-Ph), 4.92 (1H, d, J 11.3 Hz, CH\(_2\)-Ph), 4.81 (1H, d, J 11.3 Hz, CH\(_2\)-Ph), 4.79 (1H, d, J 11.2 Hz, CH\(_2\)-Ph), 4.76 (1H, d, J 11.6 Hz, CH\(_2\)-Ph), 4.70 (2H, s, CH\(_2\)-Ph), 4.08 (1H, t, J 2.5 Hz, H-C2), 3.93 (1H, t, J 9.4 Hz, H-C4), 3.72 (1H, t, J 9.3 Hz, H-C6), 3.55 (1H, td, J 9.2, 2.0 Hz, H-C5), 3.48-3.55 (1H, m, H-C1), 3.47 (1H, dd, J 9.8, 2.4 Hz, H-C3), 2.54 (1H, d, J 2.1 Hz, OH-C6), 2.34 (1H, d, J 6.3 Hz, OH-C1);

\(^{13}\)C NMR (400 MHz, CDCl\(_3\)) δ (ppm) 138.83 (iPh), 138.82 (iPh), 138.81 (iPh), 138.23 (iPh), 128.64 (3xPh), 128.59 (2xPh), 128.5 (2xPh), 128.2 (2xPh), 128.1 (2xPh), 127.91 (3xPh), 127.90 (2xPh), 127.88 (Ph), 127.7 (2xPh), 81.8 (C6), 81.4 (C4), 81.0 (C3), 77.2 (C2), 75.6 (CH\(_2\)-Ph), 75.1 (CH\(_2\)-Ph), 74.9 (CH\(_2\)-Ph), 72.9 (CH\(_2\)-Ph), 72.3 (C1); [m/z] (ESI) (M+Na)\(^+\) C\(_{34}\)H\(_{36}\)NaO\(_6\) requires 563.2404, found 563.2405.

(±)-2,3,4,6-O-Benzyl-1,5-(di-O-xylylenephospho)-myo-inositol (±)-73

Synthesized from (±)-68 according to procedure A; Eluent: EtOAc/Hexane 50 -> 55%, product loaded in CH\(_2\)Cl\(_2\); (±)-73 was obtained with by-product from phosphorylating agent; Suspension in Et\(_2\)O afforded the pure product (±)-73 as a white solid (232 mg, 0.26 mmol, 80%).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) δ (ppm) 7.39-7.45 (4H, m, Ph), 7.25-7.36 (17H, m, Ph), 7.18-7.20 (1H, m, Ph), 7.08-7.15 (6H, m, Ph), 5.26 (1H, t, \(^{2}\)J\(_{HH}\) 13.7, \(^{3}\)J\(_{HP}\) 13.7 Hz, CH\(_2\)-Ph), 5.18 (1H, t, \(^{2}\)J\(_{HH}\) 14.0, \(^{3}\)J\(_{HP}\) 14.0 Hz, CH\(_2\)-Ph), 4.78 (1H, dd, \(^{2}\)J\(_{HP}\) 19.8 Hz, \(^{3}\)J\(_{HH}\) 13.7, CH\(_2\)-Ph), 4.62-4.67 (3H, m, 2xCH\(_2\)-Ph, H-C5), 4.58 (1H, d, J 11.6 Hz, CH\(_2\)-Ph), 4.55 (1H, t, J 2.3 Hz, H-C2), 4.32 (1H, dd, \(^{3}\)J\(_{HH}\) 9.6, \(^{3}\)J\(_{HH}\) 7.0, \(^{3}\)J\(_{HP}\) 2.5 Hz, H-C1), 4.16 (1H, t, J 9.6 Hz, H-C6), 4.09 (1H, t, J 9.6 Hz, H-C4), 3.52 (1H, dd, J 9.8, 2.2 Hz, H-C3); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) δ (ppm) 138.9 (iPh), 138.7 (iPh), 138.1 (iPh), 137.9 (iPh) 135.5 (iPh), 135.4 (iPh), 135.23 (iPh), 135.17 (iPh), 129.3 (Ph), 129.13 (Ph), 129.09 (Ph), 129.0 (Ph), 128.9 (Ph), 128.8 (Ph), 128.64 (Ph), 128.58 (Ph), 128.5 (2xPh), 128.4 (2xPh), 128.29 (2xPh), 128.25 (2xPh), 128.1 (2xPh), 128.0 (2xPh), 127.9 (2xPh), 127.8 (Ph), 127.74 (2xPh), 127.66 (Ph), 127.6 (Ph), 129.5 (Ph), 81.5 (dd, \(^{3}\)J\(_{CP}\) 6.6, \(^{4}\)J\(_{CP}\) 2.0 Hz, C5), 79.9 (C3), 79.2 (d, \(^{3}\)J\(_{CP}\) 2.3 Hz, C4), 78.7 (dd, \(^{3}\)J\(_{CP}\) 8.0, 3.7 Hz, C6), 77.7 (d, \(^{3}\)J\(_{CP}\) 4.8 Hz, C1), 76.3 (C2), 75.64 (CH\(_2\)-Ph), 75.58 (CH\(_2\)-Ph), 75.4 (CH\(_2\)-Ph), 73.0 (CH\(_2\)-Ph), 69.0 (d, \(^{3}\)J\(_{CP}\) 6.6 Hz, CH\(_2\)-Ph), 68.7 (d, \(^{3}\)J\(_{CP}\) 6.6 Hz, CH\(_2\)-Ph), 68.2 (d, \(^{3}\)J\(_{CP}\) 5.4 Hz, CH\(_2\)-Ph), 68.1
5. Experimental Part

(d, $^{2}J_{CP}$ 5.4 Hz, CH$_2$-Ph); $^{31}$P NMR (160 MHz, $^1$H-decoupled, CDCl$_3$) δ (ppm) -1.50(1P), -2.17 (1P); [m/z (ESI) (M+H)$^+$ C$_{50}$H$_{51}$O$_{12}$P$_2$ requires 905.2850, found 905.2842].

Synthesis of (±)-1,5-diphosphate myo-inositol (±)-74

To a solution of (±)-73 (15.0 mg, 16.6 µmol, 1 eq.) dissolved in a minimum amount of CH$_2$Cl$_2$ was added methanol (9 mL, 1.8 mM) and Pd on activated carbon 10 % (w/w) (point of spatula). The reaction mixture was evacuated and flushed with hydrogen gas (operation repeated several times during the reaction time) and was vigorously stirred at rt for 1.5 h. The reaction mixture was flushed with nitrogen, filtered through a pad of celite and concentrated to dryness. Suspension in acetone, then acetonitrile and chloroform afforded clean product (±)-74 as a white solid (5.0 mg, 14.7 µmol, 89 %)

$^{1}$H NMR (400 MHz, MeOD) δ (ppm) 4.05 (1H, t, $^{3}J_{HP}$ 8.5, $^{2}J_{HP}$ 8.0 Hz, H-C2), 4.05 (1H, td, $^{3}J_{HP}$ 8.5, $^{2}J_{HP}$ 8.5, 2.3 Hz, H-C1), 3.99 (1H, t, $^{3}J_{HP}$ 8.0 Hz, H-C6), 3.93 (1H, q, $^{3}J_{HP}$ 8.0 Hz, H-C5), 3.81 (1H, t, $^{2}J_{CP}$ 9.0 Hz, H-C4), 3.44 (1H, dt, $^{2}J_{CP}$ 9.7, 2.3 Hz, H-C3); $^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm) 82.6 (d, $^{2}J_{CP}$ 6.4 Hz, C5), 78.6 (d, $^{2}J_{CP}$ 5.0 Hz, C1), 73.1 (bs, C4), 72.4 (C2), 72.3 (C3), 72.0 (bs, C6); $^{31}$P NMR (162 MHz, $^1$H-decoupled, CDCl$_3$) δ (ppm) 1.21 (1P), 0.39 (1P); [m/z (ESI) (M+H)$^+$ C$_{6}$H$_{15}$O$_{12}$P$_2$ requires 341.0033, measured 341.0034].

Synthesis of (±)-1,3,5,6-tetra-O-sulfonyl-2,4-di-O-phosphate myo-inositol (±)-PSSSPS

Synthesized from (±)-74 according to procedure E, except TfOH (6 eq for each hydroxyl group) and reaction mixture heated at 45 °C; (±)-PSSSPS After purification, cations was exchanged to NH$_4^+$ following Procedure H. was obtained as white solid (ammonium salt, 2.4 µmol, 16%, contaminated with a by-product 38% molar).

$^{1}$H NMR (500 MHz, D$_2$O) δ (ppm) 5.20 (1H, s, H-C2), 5.01-5.14 (1H, m), 4.89 (1H, hidden under water peak, seen in COSY), 4.67 (1H, hidden under water peak, seen in COSY), 4.37 (2H, s); $^{13}$C NMR (125
5. Experimental Part

MHz, D$_2$O) δ (ppm) 76.4 (bd), 76.0 (C2, seen only in HSQC), 73.7, 71.1 (d, J$_{CP}$ 5.5 Hz), (1); $^{31}$P NMR (162 MHz, $^1$H-decoupled, D$_2$O) δ (ppm) -0.53 (1P); -0.97 (1P); [m/z (ESI) (IP$_2$S$_4$4.5NH$_4$.4H)$^+$ C$_6$H$_{30}$N$_5$O$_{24}$P$_2$ S$_4$ requires 745.9633, measured 745.9634].

(1) 2 carbons could not be observed on the $^{13}$C NMR neither on HSQC spectra.

**Synthesis of (±)-PSSPSS**

Synthesis of (±)-2,3:5,6-di-isoproylidene-1,4-(di-O-xylalenephospho) myo-inositol (±)-75

![Synthesis of (±)-PSSPSS](image)

Synthesized from (±)-2,3:5,6-di-isoproylidene myo-inositol (±)-70 according to Procedure A, except purification. Purification by suspension in Et$_2$O afforded the pure product (±)-75 as a white solid (365 mg, 0.59 mmol, 76%).

$^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) 7.26-7.41 (10H, m, Ph), 5.47 (1H, dd, 2$^J$ 13.6, 3$^J_{HP}$ 4.6 Hz, CH$_2$-Ph), 5.44 (1H, dd, 2$^J$ 13.7, 3$^J_{HP}$ 4.8 Hz, CH$_2$-Ph), 5.32 (1H, dd, 2$^J$ 13.6, 3$^J_{HP}$ 5.3 Hz, CH$_2$-Ph), 5.29 (1H, dd, 2$^J$ 13.5, 3$^J_{HP}$ 5.4 Hz, CH$_2$-Ph), 5.19 (1H, dd, 3$^J_{HP}$ 19.2, 2$^J$ 13.6 Hz, CH$_2$-Ph), 5.16 (1H, dd, 3$^J_{HP}$ 19.4, 2$^J$ 13.5 Hz, CH$_2$-Ph), 5.05 (1H, dd, 3$^J_{HP}$ 22.1, 2$^J$ 13.6 Hz, CH$_2$-Ph), 5.03 (1H, dd, 3$^J_{HP}$ 22.7, 2$^J$ 13.7 Hz, CH$_2$-Ph), 4.87 (1H, dd, J 10.4, 3$^J_{HP}$ 7.3, J 4.2 Hz, H-C3), 4.73 (1H, t, J 4.5 Hz, H-C2), 4.69-4.77 (1H, m, H-C6), 4.29 (1H, dd, J 6.6, 4.6 Hz, H-C1), 4.16 (1H, t, J 10.0 Hz, H-C4), 3.56 (1H, dd, J 10.4, 9.8 Hz, H-C5), 1.63 (3H, s, iPr), 1.51 (3H, s, iPr), 1.48 (3H, s, iPr), 1.44 (3H, s, iPr); $^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm) 135.6 (iPh), 135.5 (iPh), 135.43 (iPh), 135.40 (iPh), 129.52 (Ph), 129.48 (Ph), 129.4 (Ph), 129.3 (Ph), 129.2 (2xPh), 129.04 (Ph), 128.96 (Ph), 113.6 (C(CH$_3$)$_2$), 111.25 (C(CH$_3$)$_2$), 80.1 (d, 3$^J_{CP}$ 3.6 Hz, C1), 79.8 (d, 2$^J_{CP}$ 5.2 Hz, C3), 76.7-76.8 (m, C2, C5), 75.4 (d, 2$^J_{CP}$ 7.7 Hz, C4), 74.1 (d, 2$^J_{CP}$ 4.9 Hz, C3), 69.4 (d, 2$^J_{CP}$ 7.3 Hz, CH$_2$-Ph), 69.1 (d, 3$^J_{CP}$ 7.0 Hz, CH$_2$-Ph), 68.9 (d, 3$^J_{CP}$ 7.1 Hz, CH$_2$-Ph), 68.8 (d, 3$^J_{CP}$ 7.0 Hz, CH$_2$-Ph), 28.1 (CH$_3$), 27.12 (CH$_3$), 27.08 (CH$_3$), 26.13 (CH$_3$); $^{31}$P NMR (160 MHz, $^1$H-decoupled, CDCl$_3$) δ (ppm) -1.89 (1P), -3.15 (1P); [m/z (ESI) (M+H)$^+$ C$_{28}$H$_{35}$O$_{12}$P$_2$ requires 625.1598, found 625.1593].
5. Experimental Part

Synthesis of (±)-1,4-diphosphate myo-inositol (±)-76

Synthesized from (±)-75 according to procedure J, except amount of TMSBr (21 eq.); (±)-76 was obtained as white solid (8.2 mg, 24.4 µmol, 98%).

$^1$H NMR (400 MHz, MeOD) δ (ppm) 4.35 (1H, q, J 9.2 Hz, H-C6), 4.22 (1H, t, J 2.6 Hz, H-C2), 4.04 (1H, ddd, J 9.8, 3$^J_{HP}$ 8.5, J 2.6 Hz, H-C3/1), 3.86 (1H, t, J 9.5 Hz, H-C6/4), 3.60 (1H, dd, J 9.6, 2.6 Hz, H-C1/3), 3.42 (1H, t, J 9.2 Hz, H-C5);

$^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm) 81.3 (d, 2$^J_{CP}$ 6.1 Hz, C6/4), 78.8 (d, 2$^J_{CP}$ 5.7 Hz, C3/1), 75.0 (d, 2$^J_{CP}$ 2.2 Hz, C5), 72.6-72.7 (2C, m, C2, C4/6), 72.0 (d, 3$^J_{CP}$ 3.2 Hz, H-C1/3);

$^{31}$P NMR (160 MHz, $^1$H-decoupled, CDCl$_3$) δ (ppm) 1.25 (1P), -0.07 (1P); [m/z (ESI) (M+H)$^+$ C$_6$H$_{15}$O$_{12}$P$_2$ requires 341.0033, found 341.0037].

Synthesis of (±)-2,3,5,6-tetra-O-sulfonyl-2,4-di-O-phosphate myo-inositol (±)-PSSPSS

Synthesized from (±)-76 according to procedure E; After purification, cations was exchanged to NH$_4^+$ following Procedure H. (±)-SPSPSS was obtained as white solid (acidic form, 6.1 mg, 9.3 µmol, 38%).

$^1$H NMR (400 MHz, D$_2$O) δ (ppm) 5.28 (1H, bd, H-C2), 4.73-4.80 (1H, H-C4, hidden under water peak, seen in COSY), 4.59-4.67 (1H, t, H-C5, H-C6), 4.47 (1H, t, 3$^J_{HH}$ 9.2, 3$^J_{HP}$ 9.2 Hz, H-C3);

$^{13}$C NMR (125 MHz, D$_2$O) δ (ppm) 76.9 (C6, seen only in HSQC), 76.3 (d, 3$^J_{CP}$ 4.3 Hz, C4), 74.4 (bd, C5), 72.8 (d, 3$^J_{CP}$ 5.0 Hz, C1), 71.3 (d, 3$^J_{CP}$ 4.9 Hz, H-C3);

$^{31}$P NMR (162 MHz, $^1$H-decoupled, D$_2$O) δ (ppm) -1.08-0.41 (2P, bd, P-C3, P-C6); [m/z (ESI) (M+NH$_4^+$) C$_6$H$_{15}$O$_{12}$P$_2$ requires 728.9368, found 728.9369].

Chiral synthesis of SPSPSS and SPSSSP

Synthesis of the 6-O-(p-methoxybenzyl)-2,4-(di-O-(1R,4R)-1,4-diphenylbutanephospho) myo-inositol orthoformate 81a and 6-O-(p-methoxybenzyl)-2,4-(di-O-(1R,4R)-1,4-diphenylbutanephospho) myo-inositol orthoformate 81b

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Synthesized from (±)-65 according to Procedure B; Eluent: EtOAc/hexane 30 -> 50%; afforded 81a[a] was obtained in ratio 10:1, and was repurified by FC (SiO₂, EtOAc/toluene 10 -> 20%) to obtain pure 81a[a] as a white solid (d.r. > 99:1, 24%[b]). 81b[a] was obtained in a ratio 14:1, and was repurified by FC (SiO₂, EtOAc/toluene 10 -> 20%) to obtain pure 81b[a] as a white solid (d.r. > 99:1, 16%[b]).

[a] Configuration could not be determined at this time but is under work.

[b] Yield based on the diastereomer itself, which represent 50% of the racemic starting material.

81a[a]: [α]D23° +29.0 (c 0.50, CHCl₃); 1H NMR (400 MHz, CDCl₃) δ (ppm) 7.42-7.47 (4H, m, Ph), 6.67 (2H, d, J 8.6 Hz, Ph), 6.67 (2H, d, J 8.6 Hz, Ph), 5.66 (1H, dd, J 9.4, 7.4 Hz, CH-Ph), 5.45-5.54 (2H, m, CH-Ph), 5.43 (1H, d, 3JHH 1.1 Hz, H-C7), 5.28 (1H, dd, J 9.4, 7.4 Hz, CH-Ph), 5.20 (1H, dt, 3JHP 7.4, 3JHH 1.1 Hz, H-C2), 4.19 (1H, d, 3JHH 11.5, CH₂-Ph), 4.11 (1H, d, 3JHH 11.5, CH₂-Ph), 4.06-4.09 (1H, m, H-C4), 3.72 (3H, s, O-CH₃), 2.35-2.42 (2H, m, CH₂), 1.95-2.01 (2H, m, CH₂); 13C NMR (125 MHz, CDCl₃) δ (ppm) 159.5 (iPh), 140.6 (d, 3JCP 10.4 Hz, 2x iPh), 140.3 (d, 3JCP 9.9 Hz, iPh), 129.7 (2xPh), 129.1 (iPh), 128.77 (2xPh), 128.76 (2xPh), 128.72 (2xPh), 128.68 (2xPh), 128.53 (Ph), 128.50 (Ph), 128.45 (Ph), 128.34 (Ph), 128.29 (2xPh), 128.25 (2xPh), 113.9 (m, C7), 70.8 (CH₂-Ph), 70.5 (m, CH₂), 67.1 (d, 3JCP 3.3 Hz, C2), 55.4 (O-CH₃), 37.6 (CH₂), 37.3 (CH₂), 36.9 (CH₂); 31P NMR (160 MHz, 1H-decoupled, CDCl₃) δ (ppm) -0.82 (1P), -1.84 (1P); [m/z] (ESI) (M+H)+ C₄₇H₄₉O₁₃P₂ requires 883.2643, found 883.2620.

81b[a]: [α]D25° +43.0 (c 0.35, CHCl₃); 1H NMR (400 MHz, CDCl₃) δ (ppm) 7.43 (2H, d, J 6.9 Hz, Ph), 7.24-7.39 (16H, m, Ph), 6.97 (2H, d, J 8.6 Hz, Ph), 6.67 (2H, d, J 8.6 Hz, Ph), 5.66 (1H, dd, J 9.4, 7.4 Hz, CH-Ph), 5.45-5.54 (2H, m, CH-Ph), 5.43 (1H, d, 3JHH 1.1 Hz, H-C7), 5.28 (1H, dd, J 9.4, 7.4 Hz, CH-Ph), 5.20 (1H, dt, 3JHP 7.4, 3JHH 1.1 Hz, H-C2), 7.05 (d, 3JCP 3.0 Hz, C3), 68.0 (d, 3JCP 5.0 Hz, C5), 67.1 (d, 3JCP 3.3 Hz, C2), 55.4 (O-CH₃), 37.6 (CH₂), 37.3 (CH₂), 36.9 (CH₂); 13C NMR (125 MHz, CDCl₃) δ (ppm) -0.82 (1P), -1.84 (1P); [m/z] (ESI) (M+H)+ C₄₇H₄₉O₁₃P₂ requires 883.2643, found 883.2620.
5. Experimental Part

128.5 (Ph), 128.40 (Ph), 128.39 (Ph), 126.1 (2xPh), 125.84 (2xPh), 125.76 (2xPh), 125.7 (2xPh), 113.9 (2xPh), 102.9 (C7), 81.0 (d, $2J_{CP}$ 6.3 Hz, CH-Ph), 79.8 (d, $2J_{CP}$ 2.9 Hz, CH-Ph), 79.6 (d, $2J_{CP}$ 3.1 Hz, CH-Ph), 72.8 (C6), 71.3 (CH$_2$-Ph), 70.94 (dd, $3J_{CP}$ 8.3 Hz, C3), 70.89 (d, $3J_{CP}$ 3.8 Hz, C1), 70.7 (d, $2J_{CP}$ 4.1 Hz, C4), 67.7 (d, $2J_{CP}$ 1.2 Hz, C5), 67.3 (d, $2J_{CP}$ 4.1 Hz, C2), 55.3 (O-CH$_3$), 37.5 (CH$_2$), 37.3 (CH$_2$), 36.82 (CH$_3$), 36.76 (CH$_3$); $^{31}$P NMR (160 MHz, $^1$H-decoupled, CDCl$_3$) δ (ppm) -0.90 (1P), -1.32 (1P); [m/z (ESI) (M+H)$^+$] $^{13}$C$_{47}$H$_{49}$O$_13$P$_2$ requires 883.2643, found 883.2631.

Synthesis of 2,4-di-O-phosphate myo-inositol 72a and 2,4-di-O-phosphate myo-inositol 72b

See procedure used for (±)-72. 72a and 72b were obtained both as white solid in a yield of 98 % and 87 %, respectively. Analytical data was in agreement with (±)-72.

72a and 72b lithium salts were obtained following the procedure G using LiOH instead of NaOH.

72a: [α]$_D^{25}$ +10.8 (c 0.12, H$_2$O nanopure, pH = 7).

72b: [α]$_D^{25}$ -7.4 (c 0.18, H$_2$O nanopure, pH = 7); [lit. [α]$_D^{22}$ -4.3 (c 0.7, H$_2$O, pH = 10)] (134b).

Chiral synthesis of PSSSPS and SSPSPS

Synthesis of the 2,3,4,6-tetra-O-benzyl-1,5-(di-O-(1R,4R)-1,4-diphenylbutanephospho) myo-inositol 82a and the 1,2,4,6-tetra-O-benzyl-3,5-(di-O-(1R,4R)-1,4-diphenylbutanephospho) myo-inositol 82b

Synthesized from (±)-68 according to procedure B; Eluent: EtOAC/Hexane 30 -> 40%; the diastereomeric mixture was obtained (229 mg, 98%). Crystallization of the mixture by vapor diffusion method (EtOAC/Et$_2$O) afforded diastereomer 82a (d.r. >99:1, 52%$^{[b]}$). Mother liquor was crystallized in iPrOH, and diastereomer 82b was recovered (d.r. 85:15, yield not determined).

[b] Yield based on the diastereomer itself, which represent 50% of the racemic starting material.

82a: [α]$_D^{3}$ +43.1 (c 0.50, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) 7.43 (2H, dd, $J$ 7.8, 1.6 Hz, Ph), 7.29-7.38 (8H, m, Ph), 7.05-7.26 (28H, m, Ph), 7.01 (2H, t, $J$ 7.3 Hz, Ph), 5.59 (1H, dd, $J$ 10.1, 6.9 Hz, CH-Ph), 5.53 (1H, td, $J$ 7.2, 4.9 Hz, CH-Ph), 5.37 (1H, dd, $J$ 10.2, 7.5 Hz, CH-Ph), 5.14 (1H, dd, $J$ 10.4, 6.9
5. Experimental Part

Hz, CH-Ph), 4.97 (1H, d, J 10.3, CH2-Ph), 4.89 (1H, d, J 11.5, CH2-Ph), 4.81 (1H, d, J 11.5, CH2-Ph), 4.63 (1H, d, J 10.3, CH2-Ph), 4.51 (1H, d, J 10.8, CH2-Ph), 4.44-4.51 (1H, m, H-C5), 4.44-4.46 (1H, m, H-C2), 4.43 (1H, d, J 11.3, CH2-Ph), 4.36 (1H, d, J 11.3, CH2-Ph), 4.25 (1H, d, J 10.8, CH2-Ph), 4.08-4.17 (2H, m, H-C1, H-C6), 3.87 (1H, t, J 9.5 Hz, H-C4), 3.30 (1H, dd, J 9.8, 2.3 Hz, H-C3); 13C NMR (125 MHz, CDCl3) δ (ppm) 140.8 (d, 3JCP 10.2 Hz, iPh), 140.4 (d, 3JCP 10.0 Hz, iPh), 140.3 (d, 3JCP 9.0 Hz, iPh), 140.2 (d, 3JCP 10.2 Hz, iPh), 138.2 (iPh), 138.7 (iPh), 138.1 (iPh), 128.69 (2xPh), 128.68 (2xPh), 128.49 (2xPh), 128.45 (3xPh), 128.34 (3xPh), 128.30 (2xPh), 128.14 (Ph), 128.11 (Ph), 128.4 (2xPh), 128.0 (2xPh), 127.89 (2xPh), 127.7 (2xPh), 127.64 (Ph), 127.55 (Ph), 127.54 (2xPh), 127.31 (Ph), 127.0 (Ph), 125.9 (4xPh), 125.74 (2xPh), 125.67 74 (2xPh), 81.32 (dd, 2JCP 6.5, 4JCP 2.6 Hz, C5), 80.71 (d, 2JCP 6.2 Hz, CH-Ph), 80.69 (d, 2JCP 6.2 Hz, CH-Ph), 80.1 (C3), 79.6 (d, 2JCP 3.0 Hz, CH-Ph), 79.3 (d, 2JCP 2.4 Hz, C4), 79.1 (d, 2JCP 2.8 Hz, CH-Ph), 78.6 (dd, 2JCP 9.0, 4.2 Hz, C6), 78.1 (dd, 4JCP 4.8, 2JCP 1.6, Hz, C1), 76.3 (C2), 75.5 (CH2-Ph), 75.3 (CH2-Ph), 74.8 (CH2-Ph), 72.8 (CH2-Ph), 38.1 (CH2), 37.6 (CH2), 37.1 (CH2), 37.0 (CH2); 31P NMR (160 MHz, 1H-decoupled, CDCl3) δ (ppm) -1.49(1P), -2.24 (1P); [m/z] (ESI) (M+H)+ C50H51O12P2 requires 1113.4102, found 1113.4087.

Chiral synthesis of PSSPSS and SSPSSP

Synthesis of the 2,3:5,6-di-isoproylidene-1,4-(di-O-(1R,4R)-1,4-diphenylbutanephospho) myo-inositol 83a and the 1,2:4,5-di-isoproylidene-3,6-(di-O-(1R,4R)-1,4-diphenylbutanephospho) myo-inositol 83b

Synthesized from (±)-70 according to procedure B; Eluent: EtOAC/hexane 30 -> 40%; the diastereomeric mixture was obtained (64 mg, 87%). Crystallization of the mixture by slow evaporation method (iPrOH) afforded diastereomer 83a (d.r. >99:1, 16%[b]). Mother liquor was crystallized in iPrOH, and diastereomer 83b was recovered (d.r. >99:1, yield not determined).

[b] Yield based on the diastereomer itself, which represent 50% of the racemic starting material.

83a: [α]D25 +29.2 (c 0.26, CHCl3); 1H NMR (400 MHz, CDCl3) δ (ppm) 7.28-7.42 (20H, m, Ph), 5.53-5.61 (2H, m, CH-Ph), 5.42 (1H, dd, J 10.0, 7.2 Hz, CH-Ph), 5.34 (1H, d, J 9.1, 7.6 Hz, CH-Ph), 4.66 (1H, ddd, J 9.9, 3JHo 8.8, J 4.1 Hz, H-C3), 4.53-4.61 (2H, m, H-C6, H-C2), 4.17 (1H, dd, J 6.2, 4.6 Hz, H-C1), 3.92 (1H, t, J 9.9 Hz, H-C4), 3.25 (1H, t, J 10.1 Hz, H-C5), 2.20-2.39 (4H, m, CH3), 2.04-2.17 (4H, m, CH3), 1.51 (3H, s, iPr), 1.30 (3H, s, iPr), 0.88 (3H, s, iPr), 0.70 (3H, s, iPr); 13C NMR (125 MHz, CDCl3) δ (ppm) 141.5
5. Experimental Part

(d, $^{3}\text{J}_{CP}$ 10.9 Hz, iPh), 141.1 (d, $^{3}\text{J}_{CP}$ 10.6 Hz, iPh), 140.4 (d, $^{3}\text{J}_{CP}$ 9.9 Hz, iPh), 140.2 (d, $^{3}\text{J}_{CP}$ 10.0 Hz, iPh), 128.6 (2xPh), 128.54 (Ph), 128.47 (2xPh), 128.40 (2xPh), 128.37 (2xPh), 128.3 (Ph), 128.0 (Ph), 127.9 (Ph), 126.0 (2xPh), 125.9 (2xPh), 125.8 (2xPh), 125.6 (2xPh), 113.0 (C(CHR$_2$)$_2$), 111.1 (C(CHR$_2$)$_2$), 80.5 (d, $^{2}\text{J}_{CP}$ 6.0 Hz, CH-Ph), 80.2 (d, $^{2}\text{J}_{CP}$ 6.0 Hz, CH-Ph), 80.1 (d, $^{3}\text{J}_{CP}$ 4.2 Hz, C1), 79.8 (d, $^{2}\text{J}_{CP}$ 2.6 Hz, CH-Ph), 79.6 (d, $^{2}\text{J}_{CP}$ 4.4 Hz, C6), 79.2 (d, $^{2}\text{J}_{CP}$ 2.5 Hz, CH-Ph), 76.8 (C2), 76.5 (d, $^{3}\text{J}_{CP}$ 3.3 Hz, C5), 74.9 (d, $^{3}\text{J}_{CP}$ 6.3 Hz, C4), 73.9 (d, $^{2}\text{J}_{CP}$ 3.6 Hz, C3), 38.5 (CH$_2$), 38.1 (CH$_2$), 37.5 (CH$_3$), 37.1 (CH$_3$), 28.0 (CH$_3$), 26.2 (CH$_3$), 26.1 (CH$_3$), 25.9 (CH$_3$); $^{31}\text{P NMR}$ (160 MHz, ¹H-decoupled, CDCl$_3$) δ (ppm) -0.72 (1P), -1.51 (1P); [m/z] (ESI) (M+H)$^+$ C$_{44}$H$_{51}$O$_{12}$P$_2$ requires 833.2850, found 833.2842.

83b: [α]$_D^{25}$ +9.8 (c 0.17, CHCl$_3$); $^{1}$H NMR (400 MHz, CDCl$_3$) δ (ppm) 7.27-7.45 (20H, m, Ph), 5.53-5.61 (2H, m, CH-Ph), 5.36 (1H, t, J 8.6 Hz, CH-Ph), 5.29 (1H, t, J 8.4 Hz, CH-Ph), 4.80 (1H, ddd, J 11.0, $^{3}\text{J}_{HP}$ 6.8, J 4.3 Hz, H-C3), 4.54 (1H, ddd, J 10.5, 9.0, $^{3}\text{J}_{HP}$ 6.8 Hz, H-C6), 4.35 (1H, t, J 4.5 Hz, H-C2), 4.03 (1H, t, J 9.9 Hz, H-C4), 3.99-4.03 (1H, m, H-C1), 3.46 (1H, dd, J 10.3, 9.9 Hz, H-C5), 2.20-2.36 (4H, m, CH$_2$), 2.02-2.19 (4H, m, CH$_2$), 1.43 (3H, s, iPr), 1.39 (3H, s, iPr), 1.14 (3H, s, iPr), 0.52 (3H, s, iPr); $^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm) 141.2 (d, $^{3}\text{J}_{CP}$ 7.4 Hz, iPh), 141.1 (d, $^{3}\text{J}_{CP}$ 7.6 Hz, iPh), 140.4 (d, $^{3}\text{J}_{CP}$ 9.9 Hz, iPh), 140.2 (d, $^{3}\text{J}_{CP}$ 10.0 Hz, iPh), 128.6 (4xPh), 128.5 (2xPh), 128.42 (Ph), 128.40 (2xPh), 128.3 (Ph), 128.2 (Ph), 126.0 (2xPh), 125.9 (2xPh), 125.8 (2xPh), 113.2 (C(CHR$_2$)$_2$), 110.8 (C(CHR$_2$)$_2$), 80.53 (d, $^{2}\text{J}_{CP}$ 3.8 Hz, CH-Ph), 80.48 (d, $^{2}\text{J}_{CP}$ 3.5 Hz, CH-Ph), 80.2 (d, $^{4}\text{J}_{CP}$ 4.4 Hz, C6), 79.8 (d, $^{3}\text{J}_{CP}$ 3.9 Hz, C1), 79.6 (d, $^{2}\text{J}_{CP}$ 2.8 Hz, CH-Ph), 79.2 (d, $^{2}\text{J}_{CP}$ 2.6 Hz, CH-Ph), 76.7 (d, $^{4}\text{J}_{CP}$ 3.6 Hz, C5), 76.4 (C2), 75.3 (d, $^{3}\text{J}_{CP}$ 7.9 Hz, C4), 74.6 (d, $^{3}\text{J}_{CP}$ 4.0 Hz, C3), 38.3 (CH$_3$), 38.2 (CH$_2$), 37.3 (CH$_3$), 37.1 (CH$_3$), 28.4 (CH$_3$), 27.1 (CH$_3$), 27.0 (CH$_3$), 24.7 (CH$_3$); $^{31}$P NMR (160 MHz, ¹H-decoupled, CDCl$_3$) δ (ppm) -1.53 (1P), -1.76 (1P); [m/z] (ESI) (M+H)$^+$ C$_{44}$H$_{51}$O$_{12}$P$_2$ requires 833.2850, found 833.2849. 
### X-Ray Crystallographic Data

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5.3. Synthesis of glycosyl structures

Synthesis and glycosylation of galactopyranose sugars (Table 2)

Synthesis and glycosylation of the substrate 3,4,6-tri-O-benzyl-2-deoxy-2-fluoro-D-galactopyranose 87

A solution of 3,4,6-tri-O-acetyl-D-galactal (4.08 g, 15 mmol, 1.0 eq.) in MeOH (18 mL, 1 M) at 0 °C, was treated with NaOMe (324 mg, 6 mmol, 0.4 eq.) and stirred at rt for 1 h. The reaction mixture was neutralised with Amberlite® IR120, filtered and concentrated to dryness. The crude material was dissolved in DMF (75 mL, 0.2 M) and cooled to 0 °C under an atmosphere of Ar. NaH [60% in oil (w/w), 2.7 g, 68 mmol, 4.5 eq.] was added portionwise and stirred over a period of 40 min before benzylbromide (8 mL, 4.5 eq.) was added. The reaction mixture was warmed to r.t. and stirred for 16 h. The reaction was quenched by the addition of MeOH (70 mL), stirred for 15 min and evaporated to dryness. The residue was diluted with CH₂Cl₂ (150 mL), washed with H₂O (2×150 mL), and the aqueous layers were back-extracted with CH₂Cl₂. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 12:1) afforded 85 as a white solid (4.93 g, 79%) identical to known material (187).

\[ R_f \, 0.52 \text{ (cyclohexane/EtOAc 3:1); } [\alpha]_D^{23} \, -39.3 \text{ (c 0.2, CH₂Cl₂); } [\alpha]_D^{23} \, -38.0 \text{ (c 1.0, CH₂Cl₂)}; \text{ m.p. } 49.6-52.0^\circ C \text{ (lit m.p. 52-53 }^\circ C); \] ¹H NMR (400 MHz, CDCl₃) \( \delta = 7.38-7.28 \text{ (15H, m, Ph), } 6.41 \text{ (1H, d, } J 6.2, 1.4 \text{ Hz, H-C1), } 4.91 \text{ (1H, d, } J 12.1 \text{ Hz, Bn), } 4.89 \text{ (1H, ddd, } J 6.2, 2.8, 1.4 \text{ Hz, H-C2), } 4.69 \text{ (1H, d, } J \]
5. Experimental Part

12.1 Hz, Bn), 4.68 (1H, d, J 12.1 Hz, Bn), 4.65 (1H, d, J 12.1 Hz, Bn), 4.54 (1H, d, J 12.1 Hz, Bn), 4.46 (1H, d, J 12.1 Hz, Bn), 4.25-4.21 (2H, m, H-C3, H-C5), 3.99 (1H, m, H-C4), 3.82 (1H, dd, J 10.2, 7.3 Hz, H-C6), 3.69 (1H, dd, J 10.2, 5.2 Hz, H-C6).

3,4,6-Tri-O-benzyl-2-deoxy-2-fluoro-d-galactopyranose 87

To a solution of 85 (832 mg, 2.0 mmol, 1.0 eq.) in acetone (9 mL, 0.22 M), H2O (1.85 mL) and Selectfluor® (850 mg, 2.4 mmol, 1.2 eq.) were added. The mixture was stirred at r.t. for 30 h. The reaction mixture was evaporated to dryness, diluted with H2O (60 mL) and sat. aq. solution of NaHCO3 (20 mL) and extracted with CH2Cl2 (2x50 mL). The combined organic phases were washed with H2O, then with brine, dried over Na2SO4, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO2, cyclohexane/EtOAc 4:1) afforded 87 as a colorless oil (638 mg, 71%, α:β 2.7:1) identical to known material (145).

Rf 0.3 (cyclohexane/EtOAc 3:1); 1H NMR (400 MHz, CDCl3) δ = 7.41-7.26 (21H, m, Ph), 5.45 (1H, t, J 3.5 Hz, H-C1α), 4.96 (1H, ddd, 2JHF 50.2, J 9.8, 3.7 Hz, H-C2α), 4.92 (1.4H, d, J 11.5 Hz, Bnα, Bnβ), 4.82 (1H, d, J 11.9 Hz, Bnα), 4.79 (0.4H, d, J 11.9 Hz, Bnβ), 4.76-4.57 (0.4H, m, H-C2β), 4.72-4.70 (0.4H, m, H-C1β), 4.69 (1H, d, J 11.9 Hz, Bnα), 4.68 (0.4H, d, J 11.9 Hz, Bnβ), 4.59 (0.4H, d, J 11.5 Hz, Bnβ), 4.56 (1H, d, J 11.5 Hz, Bnα), 4.49 (1H, d, J 11.8 Hz, Bnα), 4.48 (0.4H, d, J 11.8 Hz, Bnβ), 4.41 (1H, d, J 11.8 Hz, Bnα), 4.41 (0.4H, d, J 11.8 Hz, Bnβ), 4.18 (1H, t, J 6.2 Hz, H-C5α), 4.03 (1H, ddd, 3JHF 9.8, J 9.8, 3.2 Hz, H-C3α), 3.95 (1H, t, J 3.2 Hz, H-C4α), 3.92 (0.4H, t, J 3.2 Hz, H-C4β), 3.64-3.50 (2.6H, m, H-C3β, H-C5β, 2xH-C6β, H-C6α), 3.44 (1H, dd, J 9.5, 6.2 Hz, H-C6α), 3.18 (1H, d, J 3.7Hz, OHα); 19F NMR (376 MHz, CDCl3) δ = -204.7 to -204.8 (m, β-anomer), -206.9 (ddd, 2JHF 52.1, 3JHF 9.8, 4JHF 3.4 Hz, α-anomer).

3,4,6-Tri-O-benzyl-2-deoxy-2-fluoro-α-d-galactopyranosyl trichloroacetimidate 102

To a solution of 87 (226 mg, 0.50 mmol, 1.0 eq.) in dry CH2Cl2 (10 mL, 0.05 M) at 0 °C under an atmosphere of Ar, were added Cl3CCN (503 μL, 5.00 mmol, 10.0 eq.) and DBU (7.5 μL, 0.05 mmol, 0.1 eq.). After 5 min at 0 °C, the reaction mixture was allowed to stir at r.t. for an additional 1 h before
being concentrated \textit{in vacuo}. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 7:1) afforded 102α as a colorless oil (276 mg, 92%, α-anomer) identical to known material (145) and 102β as a colorless oil (23 mg, 8%, β-anomer).

102α: R$_f$ 0.50 (cyclohexane/EtOAc 3:1); [α]$_D^{20}$ +79.1 (c 0.2, CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$) δ = 8.63 (1H, s, NH), 7.40-7.25 (15H, m, Ph), 6.57 (1H, d, J 3.6 Hz, H-C1), 5.17 (1H, d, $^3$J$_{HF}$ 49.2 Hz, H-C2), 4.97 (1H, d, J 11.6 Hz, Bn), 4.82 (1H, d, J 11.9 Hz, Bn), 4.73 (1H, d, J 11.9 Hz, Bn), 4.60 (1H, d, J 11.1 Hz, Bn), 4.48 (1H, d, J 11.6 Hz, Bn), 4.42 (1H, d, J 7.8 Hz, H-C5), 4.16 (1H, dd, J 9.2, 7.8 Hz, H-C6), 3.64 (1H, dd, J 9.2, 5.6 Hz, H-C6); $^1$F NMR (376 MHz, CDCl$_3$) δ = -208.8 (ddd, $^3$J$_{HF}$ 49.2, $^3$J$_{HF}$ 9.4, 4.5 Hz).

102β: R$_f$ 0.34 (Cyclohexane/EtOAc 3:1); [α]$_D^{20}$ +7.8 (c 0.22, CH$_2$Cl$_2$); $v_{\text{max}}$ (neat)/cm$^{-1}$ 2870w, 1674m, 1497s, 1454s, 1367s, 1291m, 1208s, 1093m, 1055m, 910s, 835m, 732m, 695m, 646m, 574s, 540s, 533m; $^1$H NMR (400 MHz, CDCl$_3$) δ = 8.65 (1H, s, NH), 7.38-7.27 (15H, m, Ph), 5.85 (1H, dd, J 7.8, $^3$J$_{HF}$ 4.6 Hz, H-C1), 4.98 (1H, ddd, $^3$J$_{HF}$ 52.1, J 9.4, 7.8 Hz, H-C2), 4.95 (1H, d, J 11.3 Hz, Bn), 4.80 (1H, d, J 12.0 Hz, Bn), 4.72 (1H, d, J 12.0 Hz, Bn), 4.63 (1H, d, J 12.0 Hz, Bn), 4.49 (1H, d, J 11.7 Hz, Bn), 4.44 (1H, d, J 11.7 Hz, Bn), 4.03 (1H, t, J 3.3 Hz, H-C4), 3.79 (1H, dd, J 7.4, 5.7 Hz, H-C5), 3.74 (1H, ddd, $^3$J$_{HF}$ 12.5, J 9.4, 3.3 Hz, H-C3), 3.67 (1H, dd, J 9.2, 7.4 Hz, H-C6), 3.63 (1H, dd, J 9.2, 5.7 Hz, H-C6); $^{13}$C NMR (100 MHz, CDCl$_3$) δ = 161.5 (C7), 138.2 (iPh), 137.8 (iPh), 137.7 (iPh), 128.5-127.6 (Ph), 96.3 (d, $^2$J$_{CF}$ 25.5 Hz, C1), 90.9 (d, $^1$J$_{CF}$ 185.1 Hz, C2), 90.7 (C8), 79.9 (d, $^2$J$_{CF}$ 15.8 Hz, C3), 75.0 (Bn), 74.7 (C5), 74.1 (d, $^3$J$_{CF}$ 8.8 Hz, C4), 73.5 (Bn), 72.9 (d, $^4$J$_{CF}$ 2.2 Hz, Bn), 67.9 (C6); $^{19}$F NMR (376 MHz, CDCl$_3$) δ = -206.1 (dd, $^3$J$_{HF}$ 52.1, $^3$J$_{HF}$ 12.5 Hz); [m/z] (ESI) [M+K]$^+$ C$_{29}$H$_{28}$F$_3$KNO$_5$ requires 634.0727, found 634.0725.

Glycosylation to form 1-O-Isopropyl-3,4,6-tri-O-benzyl-2-deoxy-2-fluoro-D-galactopyranose 111 (Table 2, Entry 1; Table 3, Entry 2)

A solution of 102α (120 mg, 0.20 mmol, 1.0 eq.) and iPrOH (18 µL, 0.24 mmol, 1.2 eq.) in dry CH$_2$Cl$_2$ (4 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (3.6 µL, 0.02 mmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et$_3$N (0.1 mL) and concentrated \textit{in vacuo}. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 7:1) afforded S14β as a colorless oil (84 mg, 85%, β-anomer).
5. Experimental Part

1H NMR (400 MHz, CDCl$_3$) δ = 7.39-7.23 (15H, m, Ph), 4.92 (1H, d, J 11.6 Hz, Bn), 4.78 (1H, d, J 12.0 Hz, Bn), 4.67 (1H, ddd, $^3$J$_{HF}$ 51.5 Hz, J 9.3, 7.7 Hz, H-C2), 4.69 (1H, d, J 12.0 Hz, Bn), 4.62 (1H, d, J 11.6 Hz, Bn), 4.48 (1H, dd, $^3$J$_{HF}$ 4.2 Hz, H-C1), 4.49 (1H, d, J 11.8 Hz, Bn), 4.44 (1H, d, J 11.8 Hz, Bn), 4.0 (1H, sept., J 6.3 Hz, H-C7), 3.95 (1H, t, J 3.1 Hz, H-C4), 3.66-3.57 (4H, m, H-C3, 2xH-C6, H-C5), 1.29 (1H, d, J 6.3 Hz, C8), 1.23 (1H, d, J 6.3 Hz, C8); $^{13}$C NMR (100 MHz, CDCl$_3$) δ = 138.4 (iPh), 138.1 (iPh), 137.9 (iPh), 128.5-127.6 (Ph), 99.4 (d, $^3$J$_{CF}$ 23.7 Hz, C1), 91.9 (d, $^3$J$_{CF}$ 182.5 Hz, C2), 80.5 (d, $^3$J$_{CF}$ 15.8 Hz, C3), 74.7 (Bn), 74.2 (d, $^3$J$_{CF}$ 8.9 Hz, C4), 73.6 (C5), 73.6 (Bn), 72.7 (d, $^3$J$_{CF}$ 2.3 Hz, Bn), 71.9 (C7), 68.6 (C6), 23.4 (C8), 21.8 (C8);$^{19}$F NMR (376 MHz, CDCl$_3$) δ = -204.9 (dd, $^3$J$_{HF}$ 51.6, $^3$J$_{HF}$, 12.7 Hz); [m/z] (ESI) (M+Na)$^+$ C$_{30}$H$_{35}$FNaO$_5$ requires 517.2361, found 517.2354.

Synthesis and glycosylation of the substrate 3,4,6-tri-O-benzyl-2-deoxy-\(\alpha\)-galactopyranose 92

Ph$_3$P·HBr (103 mg, 0.3 mmol, 0.05 eq.) was added to a solution of 85 (2.5 g, 6.0 mmol, 1.00 eq.) in THF (18 mL, 0.33 M) at r.t., and stirred for 10 min. H$_2$O (151 µL, 8.4 mmol, 1.40 eq.) was added and the reaction mixture was stirred for 4 h. The reaction was quenched by the addition of a sat. aq. solution of NaHCO$_3$ (60 mL) and H$_2$O (60 mL) and was extracted with EtOAc (2x40mL). The combined organic phases were washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 3:1) afforded 92 as a white solid (1.94 g, 74%, $\alpha$:$\beta$ 3.3:1) identical to known material (188).
5. Experimental Part

\( \text{Rf} 0.33 \) (cyclohexane/EtOAc 3:1); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta = 7.36-7.25 \) (20H, m, Ph), 5.45 (1H, br, H-C1\(\alpha\)), 4.93 (1H, d, \( J = 11.78 \) Hz, Bn), 4.71 (0.3H, m, H-C1\(\beta\)), 4.70-4.59 (4H, m, 3xBn), 4.51 (1.3H, d, \( J = 11.85 \) Hz, Bn), 4.43 (1.3H, d, \( J = 11.85 \) Hz, Bn), 4.13 (1H, t, \( J = 6.2 \) Hz, H-C5\(\alpha\)), 3.99 (1H, ddd, \( J = 12.04 \), 4.42, 2.36 Hz, H-C3\(\alpha\)), 3.87 (1H, br, H-C4\(\alpha\)), 3.81 (1H, br, H-C4\(\beta\)), 3.61-3.52 (2.3H, m, 2x H-C6\(\beta\), H-C6\(\alpha\), H-C3\(\beta\), H-C5\(\beta\)), 3.47 (1H, dd, \( J = 9.6 \), 5.8 Hz, H-C6\(\alpha\)), 3.2 (0.3H, d, \( J = 7.5 \) Hz, OH\(\beta\)), 2.71 (1H, br, OH\(\alpha\)), 2.20 (1H, m, H-ax-C2\(\alpha\)), 2.15-2.12 (0.3H, m, H-C2\(\beta\)), 2.00 (1.3H, m, H-eq-C2\(\alpha\), H-C2\(\beta\)).

**Synthesis of 3,4,6-tri-O-benzyl-2-deoxy-\(\alpha\)-D-galactopyranosyl trichloroacetimidate 104**

To a solution of 92 (126 mg, 0.30 mmol, 1.0 eq.) in dry CH\(_2\)Cl\(_2\) (6 mL, 0.05 M) at 0 °C under an atmosphere of Ar, were added Cl\(_3\)CCN (298 \( \mu \)L, 3.00 mmol, 10.0 eq.) and DBU (4.5 \( \mu \)L, 0.03 mmol, 0.1 eq.). After 5 min at 0 °C, the reaction mixture was allowed to stir at r.t. for an additional 3 h before being concentrated in vacuo. The crude material was used in the next step without further purification (\( \alpha:\beta \) 1:1.8).

\( \text{Rf} 0.33 \) (cyclohexane/EtOAc 3:1).

**Glycosylation to form 1-O-isopropyl-3,4,6-tri-O-benzyl-2-deoxy-\(\alpha\)-galactopyranose 113 (Table 2, Entry 1; Table 3, Entry 2)**

A solution of 104 (0.30 mmol, 1.0 eq.) and iPrOH (28 \( \mu \)L, 0.36 mmol, 1.2 eq.) in dry CH\(_2\)Cl\(_2\) (6 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (5.5 \( \mu \)L, 0.03 mmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et\(_3\)N (0.5 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO\(_2\), cyclohexane/EtOAc 9:1) afforded 113 as a pale yellow oil (98 mg, 69% over 2 steps, \( \alpha:\beta \) 1:1.7) identical to known material (189).

\( \text{Rf} 0.16 \) (cyclohexane/EtOAc 19:1); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta = 7.38-7.25 \) (42H, m, Ph), 5.11 (1H, d, \( J = 3.2 \) Hz, H-C1\(\alpha\)), 4.95 (1H, d, \( J = 11.7 \) Hz, Bn\(\alpha\)), 4.94 (1.7H, d, \( J = 11.7 \) Hz, Bn\(\beta\)), 4.64-4.60 (6.7H, m, 3xBn\(\alpha\), 2xBn\(\beta\)), 4.57 (1.7H, d, \( J = 12.2 \) Hz, Bn\(\beta\)), 4.52 (1H, J 11.6 Hz, Bn\(\alpha\)), 4.50 (1H, dd, \( J = 9.6 \), 2.3 Hz, H-C1\(\beta\)), 4.49 (1.7H, d, \( J = 11.6 \) Hz, Bn\(\beta\)), 4.45 (1H, J 11.6 Hz, Bn\(\alpha\)), 4.43 (1.7H, d, \( J = 11.6 \) Hz, Bn\(\beta\)), 4.04 3.95 (4.7H, m, H-C7\(\beta\), H-C3\(\alpha\), H-C5\(\alpha\), H-C4\(\alpha\)), 3.89 (1H, sept., \( J = 6.2 \) Hz, H-C7\(\alpha\)), 3.83 (1.7H, br s,
H C4β), 3.68-3.53 (7.1H, m, 2xH-C6α, 2xH-C6β, H-C3β), 3.47 (1.7H, bd t, H-C5β), 2.19 (1H, td, J 12.5, 3.8 Hz, Hax-C2α), 2.11 (1.7H, td, J 12.0, 9.6 Hz, Hax-C2β), 2.02 (1.7H, d, J 12.0 Hz, Heq-C2β), 1.95 (1H, bd d, J 12.5 Hz, Heq-C2α), 1.24 (5.1H, d, J 6.2 Hz, H-C8β), 1.19 (3H, d, J 6.2 Hz, H-C8α), 1.15 (5.1H, d, J 6.2 Hz, H-C8β), 1.14 (3H, d, J 6.2 Hz, H-C8α).

Synthesis and glycosylation of the substrate 2,3,4,6-tetra-O-benzyl-D-galactopyranose 97

Synthesis of allyl 2,3,4,6-tetra-O-benzyl-D-galactopyranoside 96

A solution of D-(+)-galactose (1.80 g, 10.0 mmol, 1.0 eq.) in allyl alcohol (55 mL, 0.18 M) was treated at r.t. with BF$_3$OEt$_2$ (377 μL, 3.0 mmol, 0.3 eq.) and stirred for 10 min. H$_2$O (151 μL, 8.4 mmol, 1.4 eq.) was added and the mixture was stirred for 4 h. The reaction was neutralised by addition of Et$_3$N and evaporated to dryness. The orange oil was dried under high vacuum and used directly in the next step without further purification. The crude material was dissolved in DMF (50 mL, 0.2 M) and NaNH [60% in oil (w/w), 2.8 g, 70.0 mmol, 7.0 eq.] was added portionwise at 0 °C under an atmosphere of Ar over a period of 40 min. Benzyl bromide (7.2 mL, 60.0 mmol, 6.0 eq.) was added and the reaction mixture was warmed to r.t. and stirred over 16 h. The reaction was diluted with EtOAc (100 mL), washed with H$_2$O (2x100 mL) and the aqueous layers were back extracted with EtOAc. The combined organic phases were washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 20:1 to 7:1) afforded 96 as a colorless oil (2.15 g, 37% over 2 steps) identical to known material (190). 

R$_f$ 0.62 (cyclohexane/EtOAc 3:1); $^1$H NMR in accordance with the reported values (190).
5. Experimental Part

Synthesis of 2,3,4,6-tetra-O-benzyl-D-galactopyranoside 97

A solution of 96 (1.90 g, 3.30 mmol, 1.00 eq.) in DMF was treated with tBuOK and heated to 70 °C for 20 min. The reaction mixture was poured onto ice and neutralised with an aq. solution of HCl (6 M). The aqueous phase was extracted with CH₂Cl₂ (3x). The combined organic phases were washed with H₂O, then with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The crude material was dissolved in acetone (12 mL, 0.3 M), treated with a solution of HCl (0.1 M, 1.44 mL, 0.14 mmol, 0.04 eq.) and stirred at reflux for 1.5 h. The reaction mixture was diluted with EtOAc (100 mL) and washed with brine. The organic phase was dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 4/1) afforded 97 as a colorless oil (2.15 g, 37% over 2 steps, α:β 1:1.7) identical to known material (161).

Rf 0.19 (cyclohexane/EtOAc 4/1); ¹H NMR (400 MHz, CDCl₃) δ = 7.40-7.25 (32H, m, Ph), 5.29 (1H, dd, J 3.5, 2.0 Hz, H-C1α), 4.95 (0.65H, d, J 11.4 Hz, Bnβ), 4.94 (1H, d, J 11.7 Hz, Bnα), 4.92 (0.65H, d, J 10.5 Hz, Bnβ), 4.71-4.56 (5.95H, m, 4xBnα, 3xBnβ), 4.66 (0.65H, dd, J 7.4, 6.4 Hz, H-C1β), 4.61 (0.65H, d, J 11.4 Hz, Bnβ), 4.58 (1H, d, J 11.7 Hz, Bnα), 4.48 (1.67H, d, J 11.7Hz, Bnα, Bnβ), 4.41 (1.0H, d, J 11.7 Hz, Bnα), 4.41 (0.65H, d, J 11.7 Hz, Bnβ), 4.16 (1H, dd, J 6.8, 6.2 Hz, H-C5α), 4.04 (1H, dd, J 9.9, 3.5 Hz, H-C2a), 3.97 (1H, d, J 2.8 Hz, H-C4α), 3.91(1H, dd, J 9.9, 2.8 Hz, H-C3α), 3.89 (1H, d, J 1.7 Hz, H-C4β), 3.77 (0.65H, dd, J 9.6, 7.5 Hz, H-C2β), 3.61-3.52 (4.4H, m, 2xH-C6β, H-C5β, H-C3β, H-C6α), 3.52-3.48 (1H, dd, J 9.4, 6.8 Hz, H-C6α), 3.24 (0.65H, d, J 6.5 Hz, OHβ), 2.99 (1H, d, J 2.0 Hz, OHα).

Synthesis of 2,3,4,6-tetra-O-benzyl-D-galactopyranosyl trichloroacetimidate 103

To a solution of 97 (162 mg, 0.30 mmol, 1.0 eq.) in dry CH₂Cl₂ (3 mL, 0.1 M) at 0 °C under an atmosphere of Ar, were added Cl₃CCN (300 μL, 3.00 mmol, 10.0 eq.) and DBU (5 μL, 0.03 mmol, 0.1 eq.). After 5 min at 0 °C, the reaction mixture was allowed to stir at r.t. for an additional 3 h before being concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 7:1) afforded 103 as a colorless oil (179 mg, 87%, α:β 1.5:1) identical to known material (191).

Rf 0.62 α-anomer, 0.46 β-anomer (cyclohexane/EtOAc 3:1); ¹H NMR (400 MHz, CDCl₃) δ = 8.63 (0.75H, s, NHβ), 8.52 (1H, s, NHα), 7.37-7.17 (34H, m, Ph), 6.52 (1H, d, J 3.5 Hz, H-C1α), 5.76 (1H, d,
5. Experimental Part

J 8.1 Hz, H-C1β), 4.98 (1H, d, J 11.7 Hz, Bnα), 4.95 (0.75H, d, J 11.2 Hz, Bnβ), 4.91 (0.75H, d, J 10.7 Hz, Bnβ), 4.82 (1H, d, J 11.7 Hz, Bnα), 4.82 (0.75H, d, J 11.2 Hz, Bnβ), 4.77-4.74 (3.75, m, 3xBnα, Bnβ), 4.64 (0.75H, d, J 11.7 Hz, Bnβ), 4.62 (1H, d, J 11.7 Hz, Bnα), 4.60 (0.75H, d, J 10.7 Hz, Bnβ), 4.48 (0.75H, d, J 12.1 Hz, Bnβ), 4.47 (1H, d, J 11.7 Hz, Bnα), 4.43 (0.75H, d, J 12.1 Hz, Bnβ), 4.40 (1H, d, J 11.7 Hz, Bnα), 4.23 (1H, dd, J 10.0, 3.5 Hz, H-C2α), 4.16 (1H, dd, J 7.5, 5.6 Hz, H-C5α), 4.10 (0.75H, dd, J 9.5, 8.1 Hz, H-C2β), 4.06 (1H, br, H-C4α), 4.02 (1H, dd, J 7.5, 2.8 Hz, H-C3α), 3.99 (1H, br d, J 3.1 Hz, H-C4β), 3.76 (0.75H, dd, J 7.5, 5.6 Hz, H-C5β), 3.70-3.61 (3.25H, m, H-C3β, 2xH-C6β, H-C6α), 3.56 (1H, dd, J 9.5, 5.6 Hz, H-C6α).

Glycosylatlation to form 1-O-Isopropyl-2,3,4,6-tetra-O-benzyl-D-galactopyranose 112 (Table 2, Entry 1)

![Glycosylatlation Reaction](attachment:image.png)

A solution of 103 (156 mg, 0.23 mmol, 1.0 eq.) and iPrOH (21 μL, 0.27 mmol, 1.2 eq.) in dry CH₂Cl₂ (4 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (4.1 μL, 0.02 mmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 7:1) afforded 112 as a colorless oil (110 mg, 83%, α:β 1:7.7) identical to known material (192).

Rf 0.45 (cyclohexane/EtOAc 3:1); ¹H NMR (400 MHz, CDCl₃) δ = 7.39-7.26 (20H, m, Ph), 4.96 (1H, d, J 10.7 Hz, Bn), 4.95 (1H, d, J 11.7 Hz, Bn), 4.78 (1H, d, J 11.7 Hz, Bn), 4.76 (1H, d, J 10.7 Hz, Bn), 4.72 (1H, d, J 11.7 Hz, Bn), 4.64 (1H, d, J 11.7 Hz, Bn), 4.48-4.45 (1H, d, J 11.9 Hz, Bn), 4.44 (1H, d, J 7.7 Hz, H-C1), 4.43 (1H, d, J 11.9 Hz, Bn), 4.01 (1H, sept., J 6.2 Hz, H-C7), 3.89 (1H, d, J 2.9 Hz, H-C4), 3.81 (1H, dd, J 9.7, 7.7 Hz, H-C2), 3.60 (2H, d, J 5.8 Hz, 2xH-C6), 3.53 (1H, m, H-C5), 3.53 (1H, dd, J 9.7, 2.9 Hz, H-C3), 1.29 (1H, d, J 6.2 Hz, H-C8), 1.24 (1H, d, J 6.7 Hz, H-C8).
5. Experimental Part

**Synthesis and glycosylation of the substrate 3,4,6-tri-O-methyl-2-deoxy-2-fluoro-d-glucopyranose 88**

[Chemical structure image]

1. NaOMe, MeOH
2. NaH, Me, DMF

Selectfluor® H2O, Acetone

A solution of 3,4,6-tri-O-acetyl-D-galactal (1.90 g, 7.0 mmol, 1.0 eq.) in MeOH (14 mL, 0.5 M) at 0 °C was treated with NaOMe (151 mg, 2.8 mmol, 0.4 eq.). The mixture was allowed to warm to r.t. and stirred for 1 h. The reaction mixture was neutralised with Amberlite® IR120, filtered and concentrated to dryness. The crude material was dissolved in DMF (35 mL, 0.2 M) and cooled at 0 °C under an atmosphere of Ar. NaH [60% in oil (w/w), 1.26 g, 31.5 mmol, 4.5 eq.] was added portionwise and stirred over a period of 30 min before methyl iodide (2.0 mL, 31.5 mmol, 4.5 eq.) was added. The reaction mixture was warmed to r.t. and stirred over 16 h. The reaction was quenched by the addition of MeOH (30 mL), stirred for 15 min and evaporated to dryness. The residue was diluted with CH2Cl2 (60 mL), washed with H2O (2x60 mL) and the aqueous layers were back-extracted with CH2Cl2 (2x30 mL). The combined organic phases were washed with brine, dried over Na2SO4, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO2, cyclohexane/EtOAc 4:1) afforded 86 as a colorless oil (861 mg, 65%) identical to known material (193).

Rf 0.44 (cyclohexane/EtOAc 1:1); [α]D20 -19.8 (c 0.2, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ = 6.34 (1H, dd, J 6.1, 1.4 Hz, H-C1), 4.82 (1H, ddd, J 6.2, 3.0, 1.2 Hz, H-C2), 4.21-4.19 (1H, m, H-C5), 3.97 (1H, ddd, J 5.6, 3.0, 1.4 Hz, H-C3), 3.73-3.68 (2H, m, H-C6, H-C4), 3.59 (1H, dd, J 10.2, 4.9 Hz, H-C6), 3.52 (3H, s, CH3), 3.42 (3H, s, CH3), 3.40 (3H, s, CH3).

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**Synthesis of 3,4,6-tri-O-methyl-d-galactal 86**

A solution of 3,4,6-tri-O-acetyl-d-galactal (1.90 g, 7.0 mmol, 1.0 eq.) in MeOH (14 mL, 0.5 M) at 0 °C was treated with NaOMe (151 mg, 2.8 mmol, 0.4 eq.). The mixture was allowed to warm to r.t. and stirred for 1 h. The reaction mixture was neutralised with Amberlite® IR120, filtered and concentrated to dryness. The crude material was dissolved in DMF (35 mL, 0.2 M) and cooled at 0 °C under an atmosphere of Ar. NaH [60% in oil (w/w), 1.26 g, 31.5 mmol, 4.5 eq.] was added portionwise and stirred over a period of 30 min before methyl iodide (2.0 mL, 31.5 mmol, 4.5 eq.) was added. The reaction mixture was warmed to r.t. and stirred over 16 h. The reaction was quenched by the addition of MeOH (30 mL), stirred for 15 min and evaporated to dryness. The residue was diluted with CH2Cl2 (60 mL), washed with H2O (2x60 mL) and the aqueous layers were back-extracted with CH2Cl2 (2x30 mL). The combined organic phases were washed with brine, dried over Na2SO4, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO2, cyclohexane/EtOAc 4:1) afforded 86 as a colorless oil (861 mg, 65%) identical to known material (193).

Rf 0.44 (cyclohexane/EtOAc 1:1); [α]D20 -19.8 (c 0.2, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ = 6.34 (1H, dd, J 6.1, 1.4 Hz, H-C1), 4.82 (1H, ddd, J 6.2, 3.0, 1.2 Hz, H-C2), 4.21-4.19 (1H, m, H-C5), 3.97 (1H, dd, J 5.6, 3.0, 1.4 Hz, H-C3), 3.73-3.68 (2H, m, H-C6, H-C4), 3.59 (1H, dd, J 10.2, 4.9 Hz, H-C6), 3.52 (3H, s, CH3), 3.42 (3H, s, CH3), 3.40 (3H, s, CH3).
Synthesis of 3,4,6-tri-O-methyl-2-deoxy-2-fluoro-D-galactopyranose 88

To a solution of 86 (283 mg, 1.5 mmol, 1 eq.) in acetone (6 mL, 0.25 M) and Selectfluor® (638 mg, 1.8 mmol, 1.2 eq.). The mixture was stirred at rt. for 20 h. The reaction mixture was evaporated to dryness, dissolved in CH₂Cl₂ (45 mL) and washed with sat. aq. solution of NaHCO₃ (45 mL). The aqueous phase was extracted with CH₂Cl₂ (3x10 mL). The combined organic phases were dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 1:1) afforded 88 as a white solid (122.6 mg, 37%, α:β 1.8:1).

Rₐ 0.25 (cyclohexane/EtOAc 5:3); m.p. 84.7-86.1 °C; νmax (neat)/cm⁻¹ 3382m, 2899s, 2810s, 1461m, 1399s, 1365s, 1329s, 1247m, 1202m, 1147s, 1228s, 1103s, 1065s, 1043s, 1026s, 984m, 970s, 956s, 932m, 822s, 776s, 724s, 690s, 652m, 621m; ¹H NMR (400 MHz, CDCl₃) δ = 5.44 (1H, br, H-C1α), 4.77 (1H, ddd, 2JHF 50.2, J9.7, 3.8 Hz, H-C2α), 4.70 (0.5H, m, H-C1β), 4.49 (0.5H, ddd, 2JHF 51.7, J9.3, 7.6 Hz, H-C2β), 4.18 (1H, t, J 6.2 Hz, H-C5α), 4.13 (0.5H, d, J 7.0 Hz, OHβ), 3.76 (1H, d, JHF 11.2, J9.7, 2.9 Hz, H-C3α), 3.73-3.68 (2.5H, m, H-C4α, H-C4β, OHα), 3.62 (0.5H, dd, J7.2, 5.1 Hz, H-C5β), 3.58-3.50 (12H, m, 2xH-C6α, 2xH-C6β, 2xCH₃α, 2xCH₃β), 3.57 (4.5H, s, CH₃α, CH₃β), 3.37 (0.5H, m, H-C3β); ¹³C NMR (100 MHz, CDCl₃) δ = 95.2 (d, 2JCF 24.2 Hz, C1β), 92.8 (d, 1JCF 183.2 Hz, C1β), 90.8 (d, 2JCF 22.1 Hz, C1α), 89.1 (d, 1JCF 186.3 Hz, C2α), 82.3 (d, 2JCF 16.1 Hz, C3β), 78.3 (d, 3JCF 16.3 Hz, C3α), 77.2 (d, J 8.8 Hz, C4α), 73.6-75.9 (d, J 8.4 Hz, C4β), 73.6 (C5β), 71.5 (C5α), 70.7 (C6β), 69.3 (C5α), 61.4 (CH3α), 61.4 (CH3β), 59.2 (CH3β), 59.2 (CH3α), 58.6 (CH3β), 58.5 (CH3α); ¹⁹F NMR (376 MHz, CDCl₃) δ = -205.4 (ddt, 3JFH 51.7, 3JFH 12.8, 2JFH 3.4 Hz, β-anomer), -207.2 (ddd, 3JFH 50.2, 2JFH 11.3, 2JFH 4.3 Hz, α-anomer); [m/z] (ESI) (M+Na)⁺ C₉H₁₇FNaO₅ requires 247.0952, measured 247.0954.

Synthesis of 3,4,6-Tri-O-methyl-2-deoxy-2-fluoro-D-galactopyranosyl trichloroacetimidate 105

To a solution of 88 (123 mg, 0.55 mmol, 1 eq.) in dry CH₂Cl₂ (11 mL, 0.05 M) at 0 °C under an atmosphere of Ar, were added Cl₃CCN (546 μL, 5.5 mmol, 10 eq.) and DBU (8 μL, 0.06 mmol, 0.1 eq.). After 10 min at 0 °C, the reaction mixture was allowed to stir at rt. for an additional 1 h before being concentrated in vacuo. Purification by flash column chromatography (SiO₂,
cyclohexane/EtOAc 4:1 to 5:3) afforded \textbf{105a} as a white solid (154 mg, 76%, α-anomer) and \textbf{105β} as a colorless oil (16 mg, 8%, β-anomer).

\textbf{105a}: \( R_f \) 0.43 (cyclohexane/EtOAc 5:3); \textbf{m.p.} 83.8-84.8 °C; \([\alpha]^{20}_D +43.2\) (c 0.13, CH\(_2\)Cl\(_2\)); \( \nu_{\text{max}} \) (neat)/cm\(^{-1}\) 3317w, 2925w, 1677m, 1448w, 1347m, 1292m, 1208s, 1188s, 1150m, 1133m, 1086s, 1076s, 1061s, 1033m, 1019s, 977s, 953s, 920s, 876s, 848s, 796s, 742m, 654s, 642s, 605s; \( ^1\text{H NMR} \) (400 MHz, CDCl\(_3\)) \( \delta = 8.68 \) (1H, s, NH), 6.55 (1H, d, \( J = 3.9 \) Hz, H-C1), 5.00 (1H, ddd, \( J = 2J_{HF} 49.2, J = 9.8, J = 3.9 \) Hz, H-C2), 4.13 (1H, dd, \( J = 7.9, J = 5.7 \) Hz, H-C5), 3.87 (1H, m, H-C4), 3.85 (1H, ddd, \( J = 9.8, J = 9.8, J = 3.0 \) Hz, H-C3), 3.61 (3H, s, CH\(_3\)), 3.60 (3H, s, CH\(_3\)), 3.61-3.57 (1H, m, H-C6), 3.55 (1H, dd, \( J = 3J_{HF} 9.4, J = 5.7 \) Hz, H-C6), 3.39 (3H, s, CH\(_3\)), 3.36 (3H, s, CH\(_3\)); \( ^{13}\text{C NMR} \) (100 MHz, CDCl\(_3\)) \( \delta = 160.9 \) (C7), 93.9 (d, \( J_{CF} 23.0 \) Hz, C1), 91.1 (C8), 87.9 (d, \( J_{CF} 189.6 \) Hz, C2), 78.7 (d, \( J_{CF} 16.6 \) Hz, C3), 76.1 (d, \( J_{CF} 8.2 \) Hz, C4), 71.8 (C5), 70.1 (C6), 61.5 (CH\(_3\)), 59.2 (CH\(_3\)), 58.5 (CH\(_3\)); \( ^{19}\text{F NMR} \) (376 MHz, CDCl\(_3\)) \( \delta = -209.3 \) (ddd, \( J_{FH} 49.2, J_{FH} 9.8, J_{FH} 4.3 \) Hz; [\( m/z \) (ESI) (M+Na\(^{+}\)) C\(_{11}\)H\(_{17}\)Cl\(_3\)FNNaO\(_5\) requires 390.0049, found 390.0049].

\[ 1 \text{O-Isopropyl-3,4,6-tri-O-methyl-2-deoxy-2-fluoro-D-galactopyranose 114 (Table 2, Entry 2)} \]

A solution of \textbf{105a} (60 mg, 0.16 mmol, 1.0 eq.) and \textbf{iPrOH} (14.5 μL, 0.19 mmol, 1.2 eq.) in dry CH\(_2\)Cl\(_2\) (3 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (3.0 μL, 0.02 mmol, 0.1 eq.) at -50 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et\(_3\)N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO\(_2\), cyclohexane/EtOAc 5:1 and CHCl\(_3\)/MeOH 39:1) afforded \textbf{S27β} as a colorless oil (29 mg, 68%, β-anomer) and a mixture of \textbf{114α} and \textbf{114β} as a colorless oil (4 mg, 9%, α:β 7.4:1).

\textbf{114β}: \( R_f \) 0.27 (cyclohexane/EtOAc 3:1); \([\alpha]^{20}_D -35.0\) (c 0.14, CH\(_2\)Cl\(_2\)); \( \nu_{\text{max}} \) (neat)/cm\(^{-1}\) 2974w, 2932w, 2837w, 1466w, 1383w, 1371w, 1200w, 1154m, 1103s, 1068s, 1024s, 980m, 962m, 926w, 903w, 880w, 828w, 757w, 698w, 660w; \( ^1\text{H NMR} \) (400 MHz, CDCl\(_3\)) \( \delta = 4.46 \) (1H, ddd, \( J = 51.6, J = 9.3, J = 7.4 \) Hz, H-C2), 4.46 (1H, dd, \( J = 4.2, J = 7.4 \) Hz, H-C1), 4.49 (1H, sept., \( J = 6.2 \) Hz, C7), 3.69 (1H, t, \( J = 3.1 \) Hz, H-C4), 3.61 (1H, ddd, \( J = 11.2, J = 9.8 \) Hz, H-C6), 3.55 (3H, s, CH\(_3\)), 3.53 (3H, s, CH\(_3\)), 3.53-3.50 (2H, m, H-C5, H-C6), 3.39 (3H, s, CH\(_3\)), 3.33 (1H, ddd, \( J = 13.2, J = 9.1, J = 3.1 \) Hz, H-C3), 1.24 (1H, d, \( J = 6.2 \) Hz, H-C8), 1.18 (1H, d, \( J = 6.2 \) Hz, H-C8); \( ^{13}\text{C NMR} \) (100 MHz, CDCl\(_3\)) \( \delta = 99.2 \) (d, \( J_{CF} 23.7 \) Hz, C1), 91.4 (d, \( J_{CF} 183.3 \) Hz, C2), 82.6 (d, \( J_{CF} 15.9 \) Hz, C3), 75.6 (d, \( J_{CF} 8.9 \) Hz, C4), 73.2 (C5), 71.7 (C7), 70.5 (C6), 61.3 (CH\(_3\)), 59.2 (CH\(_3\)), 58.5 (CH\(_3\)), 23.4 (C8), 21.7 (C8); \( ^{19}\text{F NMR} \) (376 MHz, CDCl\(_3\)) \( \delta = -209.3 \) (dd, \( J_{FH} 49.2, J_{FH} 9.8, J_{FH} 4.3 \) Hz; [\( m/z \) (ESI) (M+Na\(^{+}\)) C\(_{12}\)H\(_{27}\)FNO\(_5\) requires 284.1868, found 284.1869].
5. Experimental Part

Synthesis and glycosylation of the substrate 3,4,6-tri-O-methyl-2-deoxy-D-galactopyranose 93

\[
\begin{align*}
\text{Ph}_3\text{P} \cdot \text{HBr} & (48 \text{ mg}, 0.14 \text{ mmol}, 0.05 \text{ eq.}) \text{ was added to a solution of } \text{86} (530 \text{ mg}, 2.80 \text{ mmol}, 1.00 \text{ eq.}) \text{ in THF (8 mL, 0.35 M) at rt, and stirred for 10 min. H}_2\text{O} (80 \mu\text{L}, 4.4 \text{ mmol}, 1.60 \text{ eq.}) \text{ was added and the reaction mixture was stirred for 5 h. The reaction was quenched by the addition of a sat. aq. solution of NaHCO}_3 (6 \text{ mL}) \text{ and H}_2\text{O (6 mL) and was extracted with EtOAc (3x5 mL). The combined organic phases were washed with brine, dried over Na}_2\text{SO}_4, \text{ filtered and concentrated in vacuo. Purification by flash column chromatography (SiO}_2, \text{ cyclohexane/EtOAc 3:1) afforded 93 as a colorless oil (82 mg, 14%, } \alpha: \beta \text{ 3.3:1).}
\end{align*}
\]

\[\text{R}_f \text{ 0.09 (cyclohexane/EtOAc 5:3); } \nu_{\text{max}} \text{ (neat)/cm}^{-1} \text{ 3406w, 2911w, 2832s, 1449s, 1378s, 1342s, 1255s, 1197s, 1153m, 1071m, 1027m, 1006m, 964m, 942m, 883s, 842s, 814s, 758s, 747s, 733s, 684s, 646s;}
\]

\[\text{^1H NMR (400 MHz, CDCl}_3 \text{) } \delta = 5.45 \text{ (1H, br s, H-C1}\alpha\text{), 4.74 (0.3H, ddd, J 9.8, 7.5, 2.3 Hz, H-C1}\beta\text{), 4.11 (1H, t, J 6.3 Hz, H-C5}\alpha\text{), 3.72 (1H, ddd, J 11.6, 5.0, 2.5 Hz, H-C3}\alpha\text{), 3.59 (1.5H, m, H-C4}\alpha\text{, 2x H-C6}\beta\text{), 3.87-3.50 (6.25H, m, 2xH-C6}\alpha\text{, CH}_3\text{H, CH}_3\text{C, H-C4}\beta\text{, H-C5}\beta\text{), 3.41 (3.75H, s, CH}_3\text{H, CH}_3\text{C), 3.40-3.37 (4H, m, CH}_3\text{H, CH}_3\text{C, OH}, 3.33 (0.25H, ddd, J 12.2, 4.5, 2.8 Hz, H-C3}\beta\text{), 3.01 (1H, t, J 2.4 Hz, OH}, 2.11 (0.25H, dd, J 12.1, 4.5 Hz, H-C2}\beta\text{), 2.00 (1H, td, J 12.6, 3.7 Hz, H-C2}\beta\text{, 1.94 (1H, dd, J 12.6, 5.0 Hz, H-C2}\alpha\text{, 1.81 (1H, td, J 12.1, 9.8 Hz, H-C2}\beta\text{); ^13C NMR (100 MHz, CDCl}_3 \text{) } \delta = 94.8 \text{ (C1}\beta\text{), 92.6 \text{ (C1}\alpha\text{, 79.2 \text{ (C3}\beta\text{), 75.6 \text{ (C3}\alpha\text{, 75.1 \text{ (C4}\alpha\text{, 74.0-73.6 \text{ (C5}\beta\text{, C4}\beta\text{), 72.4 \text{ (C6}\alpha\text{, 71.6 \text{ (C6}\beta\text{, 69.8 \text{ (C5}\alpha\text{, 60.9 \text{ (CH}_3\text{H}, 60.9 \text{ (CH}_3\text{H, 59.2 \text{ (CH}_3\text{H, 59.2 \text{ (CH}_3\text{H, 56.1 \text{ (CH}_3\text{H, 56.1 \text{ (CH}_3\text{H, 56.3 \text{ (CH}_3\text{H, 34.1 \text{ (C2}\beta\text{, 30.6 \text{ (C2}\alpha\text{; [m/z (ESI) (M+Na)}^+ \text{ C}_9\text{H}_{18}\text{NaO}_5 \text{ requires: 229.1046, found 229.1047].}}
\]
5. Experimental Part

Synthesis of 3,4,6-tri-O-methyl-2-deoxy-α-D-galactopyranosyl trichloroacetimidate 107

To a solution of 93 (82 mg, 0.40 mmol, 1.0 eq.) in dry CH₂Cl₂ (8 mL, 0.05 M) at 0 °C under an atmosphere of Ar, were added Cl₃CCN (412 μL, 4.00 mmol, 10.0 eq.) and DBU (6.0 μL, 0.04 mmol, 0.1 eq.). After 10 min at 0 °C, the reaction mixture was allowed to stir at r.t. for an additional 1 h before being concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 4/1 to 5/3) afforded 107α as a white solid (154 mg, 0.42 mmol 76%) and 107β as a colorless oil (16 mg, 0.04 mmol 8%). The crude material was used in the next step without further purification (α:β 1:1.2).

Rf 0.33 (cyclohexane/EtOAc 3:1)

Glycosylation to form 1-O-isopropyl-3,4,6-tri-O-methyl-2-deoxy-α-D-galactopyranose 116 (Table 2, Entry 2)

A solution of 107 (0.40 mmol, 1.0 eq.) and iPrOH (75 μL, 0.48 mmol, 1.2 eq.) in dry CH₂Cl₂ (8 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (7.3 μL, 0.04 mmol, 0.1 eq.) at -50 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.2 mL) and concentrated in vacuo. NMR analysis of the crude reaction mixture revealed an α:β ratio of 1.4:1.

Synthesis and glycosylation of the substrate 2,3,4,6-tetra-O-methyl-D-galactopyranose 98
5. Experimental Part

Synthesis of methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranose 98

A vigorously stirred solution of methyl α-D-galactopyranoside (1.0 g, 5.2 mmol, 1.0 eq.) in DMSO (18 mL, 0.3 M) was treated with an aq. solution of NaOH [50% (w/v), 3 mL, 35.4 mmol, 7.0 eq.] and methyl iodide (1.94 mL, 31.2 mmol, 6.0 eq.) was added dropwise and the reaction was stirred for 16 h. The reaction mixture was diluted with H₂O (100 mL) and extracted with EtOAc (3x). The combined organic phases were dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 1:1) afforded 98 as a colorless oil (615 mg, 45%) identical to known material (194).

Rf 0.33 (cyclohexane/EtOAc 1:3); [α]D²⁰ +153.7 (c 0.13, CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ = 4.87 (1H, d, J 3.6 Hz, H-C1), 3.85 (1H, t, J 6.4 Hz, H-C5), 3.69 (1H, dd, J 2.9, 1.0 Hz, H-C4), 3.63 (1H, dd, J 9.9, 3.6 Hz, H-C2), 3.59-3.49 (3H, m, H-C6, H-C3), 3.57 (3H, s, CH₃), 3.51 (6H, s, 2xCH₃), 3.41 (3H, s, CH₃).

Synthesis of 2,3,4,6-tetra-O-methyl-D-galactopyranoside 100

A solution of 98 (437 mg, 1.64 mmol, 1.0 eq.) in glacial acetic acid (5.5 mL, 0.3 M) was heated to 80 °C and treated with an aq. solution of triflic acid (1 M, 1.15 mL, 0.7 eq.). The reaction mixture was stirred at 80 °C for 2 h. After cooling, sat. aq. solution of NaHCO₃ was added until gas evolution stopped. The reaction mixture was diluted with H₂O (100 mL) and extracted with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 1:3) afforded 100 as a yellow oil (132 mg, 34%, α:β 1.2:1) identical to known material (194).

Rf 0.3 (cyclohexane/EtOAc 1:3); ¹H NMR (400 MHz, CDCl₃) δ = 5.42 (1H, t, J 3.0 Hz, H-C1α), 4.54 (0.65H, dd, J 7.5, 6.3 Hz, H-C1β), 4.15 (1H, t, J 6.5 Hz, H-C5α), 3.72 (1H, dd, J 2.9, 1.0 Hz, H-C4α), 3.67-3.62 (2.3H, m, H-C2α, H-C4β, OHβ), 3.61-3.54 (5H, m, 2xH-C6α, 2xH-C6β, H-C3α, H-C5β), 3.30 (0.65H, dd, J 9.6, 7.5 Hz, H-C2β), 3.24 (1H, d, J 3.0 Hz, OHα), 3.19 (0.65H, dd, J 9.8, 3.2 Hz, H-C3β), 3.65 (1.95H, s, CH₃β), 3.59 (4.95H, s, CH₃α, CH₃β), 3.55 (3H, s, CH₃α), 3.54 (3H, s, CH₃α), 3.54 (1.95H, s, CH₃β), 3.41 (4.95H, s, CH₃α, CH₃β).
5. Experimental Part

**Synthesis of 2,3,4,6-tri-O-methyl-D-galactopyranosyl trichloroacetimidate 106**

To a solution of 100 (71 mg, 0.30 mmol, 1.0 eq.) in dry CH₂Cl₂ (6 mL, 0.05 M) at 0 °C under an atmosphere of Ar, were added Cl₃CCN (298 μL, 3.00 mmol, 10.0 eq.) and DBU (4.5 μL, 0.03 mmol, 0.1 eq.). After 10 min at 0 °C, the reaction mixture was allowed to stir at r.t. for an additional 1 h before being concentrated *in vacuo*. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 3/1 to 5:3) afforded 106 as a colorless oil (85 mg, 74%, α:β 1.2:1).

Rᵡ 0.59 α-anomer, 0.55 β-anomer (cyclohexane/EtOAc 1:1); νₘₚₐₓ (neat)/cm⁻¹ 3343w, 2931w, 2832w, 1731m, 1672m, 1449w, 1358w, 1287m, 1204m, 1099s, 10 63s, 964s, 915m, 834s, 794s, 729m, 644s;

**¹H NMR** (400 MHz, CDCl₃) δ = 8.62 (1H, s, NHα), 8.58 (0.9H, s, NHβ), 6.56 (1H, d, J 3.6 Hz, H-C1α), 5.62 (0.9H, d, J 8.1 Hz, H-C1β), 4.13-4.09 (1H, m, H-C5α), 3.87-3.8 3 (2H, m, H-C2α, H-C4α), 3.72 (0.9H, d, J 3.2, H-C4β), 3.72-3.69 (0.9H, m, H-C5β), 3.67-3.55 (19H, m, H-C3α, 2xH-C6β, H-C2β, H-C6α), 3.62 (2.7H, s, CH₃β), 3.61 (3H, s, CH₃α), 3.54-3.50 (4H, m, H-C6α, CH₃α), 3.40 (2.7H, CH₃β), 3.39 (3H, s, CH₃α), 3.29 (0.9H, dd, J 9.7, 3.2 Hz, H-C3β);

**¹³C NMR** (100 MHz, CDCl₃) δ = 161.6 (C7β), 161.4 (C7α), 98.6 (C1β), 94.4 (C1α), 91.4 (C8α), 91.0 (C8β), 83.9 (C3β), 79.8 (C3α), 79.7 (C2β), 76.9 (C2α), 75.2 (C4α), 74.7 (C4β), 74.0 (C5β), 71.6 (C5α), 70.4 (C6α), 70.1 (C6β), 61.40 (CH₃β), 61.38 (CH₃α), 61.1 (CH₃β), 59.2 (CH₃β), 59.1 (CH₃α), 58.7 (CH₃α), 58.6 (CH₃β), 58.0 (CH₃α); [m/z] (ESI) (M+Na)⁺ C₁₂H₂₆Cl₃NNaO₆ requires 402.0248, found 402.0238.

**Glycosylation to form 1-O-isopropyl-3,4,6-tri-O-methyl-2-deoxy-2-fluoro-D-galactopyranose 115** *(Table 2, Entry 2)*

A solution of 106 (59 mg, 0.16 mmol, 1.0 eq.) and iPrOH (14.2 μL, 0.19 mmol, 1.2 eq.) in dry CH₂Cl₂ (3 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (2.8 μL, 0.016 mmol, 0.1 eq.) at -50 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N and concentrated *in vacuo*. Purification by flash column chromatography (SiO₂,
5. Experimental Part

cyclohexane/EtOAc 7:3) afforded 115 as a colorless oil (29 mg, 67%, mainly β) and a mixture of 115α and 115β as a colorless oil (3 mg, 7%, α:β 1:2.4) identical to known material (195).

115β: Rf 0.26 (cyclohexane/EtOAc 5:3); \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ = 4.27 (1H, d, \(J = 7.6\) Hz, H-C1), 3.95 (1H, sept., \(J = 6.2\) Hz, C7), 3.63-3.60 (2H, m, H-C4, H-C6), 3.59-3.57 (1H, m, H-C6), 3.58 (3H, s, CH\(_3\)), 3.55 (3H, s, CH\(_3\)), 3.52 (3H, s, CH\(_3\)), 3.52-3.44 (2H, m, H-C6, H-C5), 3.39 (3H, s, CH\(_3\)), 3.28 (1H, dd, \(J = 9.7, 7.6\) Hz, H-C2), 3.10 (1H, dd, \(J = 9.7, 3.2\) Hz, H-C3), 1.23 (1H, d, \(J = 6.2\) Hz, C8), 1.18 (1H, d, \(J = 6.2\) Hz, C8).

Synthesis and glycosylation of the substrate 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-galactopyranose 91

![Chemical structure](image1.png)

To a solution of 3,4,6-tri-O-acetyl-\(\alpha\)-galactal (1.85 g, 6.8 mmol, 1.0 eq.) in acetone (31 mL, 0.22 M), H\(_2\)O (6.2 mL, excess) and Selectfluor® (2.89 g, 8.2 mmol, 1.2 eq.) were added. The mixture was stirred at r.t. for 16 h. The reaction mixture was evaporated to dryness, diluted with H\(_2\)O (150 mL) and sat. aq. solution of NaHCO\(_3\) (60 mL) and extracted with CH\(_2\)Cl\(_2\) (3x200 mL). The combined organic phases were washed with H\(_2\)O, then with brine, dried over Na\(_2\)SO\(_4\), filtered and concentrated in vacuo. Purification by flash column chromatography (SiO\(_2\), cyclohexane/EtOAc 5:3) afforded an inseparable mixture of galacto- and taloconfigured products 89a and 89b as a colorless oil (1.08 g, 51%, Galacto-\(\alpha\):Galacto-\(\beta\):Talo-\(\alpha\) = 16:5:1). The crude material was used directly in the next step without further purification.

Rf 0.16 (cyclohexane/EtOAc 3:1)
5. Experimental Part

Synthesis of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-fluoro-D-galacto/talopyranose 90

To a solution of 89a and 89b (1.07 g, 3.46 mmol, 1.0 eq.) in pyridine (12 mL, 0.3 M) under an atmosphere of Ar were added DMAP (43 mg, 0.35 mmol, 0.1 eq.) and Ac₂O (1.30 mL, 13.8 mmol, 4.0 eq.). The mixture was stirred at r.t. for 16 h. The reaction mixture was diluted with CH₂Cl₂ (150 mL), washed with a sat. aq. solution of NaHCO₃ (2x) and the aqueous phases were back-extracted with CH₂Cl₂. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 3:1) afforded 90 as a white solid (0.95 g, 78%, galacto-α:galacto-β 2.4:1) identical to known material (196) and the talo-configured 90bα as a slightly yellow solid (68 mg, 6%, talo-α) identical to known material (197).

90: Rᵥ 0.31 (cyclohexane/EtOAc 3:1); νₘₐₓ (neat)/cm⁻¹ 1738s, 1431w, 1372m, 1260m, 1222s, 1168m, 1139m, 1111m, 1071s, 1040s, 1012s, 940s, 899m, 877m, 850m, 734w, 699m, 672w, 647w, 627w; ¹H NMR (400 MHz, CDCl₃) δ = 6.49 (1H, d, J=4.0 Hz, H-C₁α), 5.80 (0.4H, dd, J=8.1 Hz, 3J₈F=4.0, H-C₁β), 5.54-5.53 (1H, m, H-C₄α), 5.46 (0.4H, t, J=2.8 Hz, H-C₄β), 5.44 (1H, ddd, 3J₈F=10.8, J₁₀.8, 3.6 Hz, H-C₃α), 5.19 (0.4H, ddd, 3J₈F=12.7, J=9.6, 3.6 Hz, H-C₃β), 4.91 (1H, ddd, 3J₈F=49.4, J=10.2, 4.0 Hz, H-C₂α), 4.72 (0.4H, ddd, 3J₈F=51.5, J=9.6, 8.1 Hz, H-C₂β), 4.33 (1H, t, J=6.7 Hz, H-C₅α), 4.17-4.10 (3.2H, m, 2xH-C₆α, 2xH-C₆β, H-C₅β), 2.21 (1.2H, s, CH₃β), 2.20 (3H, s, CH₃α), 2.17 (4.2H, s, CH₃α, CH₃β), 2.08 (4.2H, s, CH₃α, CH₃β), 2.06 (4.2H, s, CH₃α, CH₃β); ¹³C NMR (100 MHz, CDCl₃) δ = 170.3 (OAcα, OAcβ), 170.0 (OAcα), 169.92 (OAcα), 169.8 (OAcα, OAcβ), 168.8 (OAcα, OAcβ), 91.6 (d, 3Jₑ=23.8 Hz, C₁β), 89.0 (d, 3Jₑ=23.8 Hz, C₁α), 86.7 (d, 3Jₑ=188.5 Hz, C₂β), 84.2 (d, 3Jₑ=191.9 Hz, C₂α), 71.7 (C₅β), 70.9 (d, 3Jₑ=20.6 Hz, C₃β), 68.6 (C₅α), 68.2 (d, 3Jₑ=18.5 Hz, C₃α), 67.8 (d, 3Jₑ=8.4 Hz, C₄α), 67.4 (d, 3Jₑ=8.4 Hz, C₄β), 61.0 (C₆α), 60.8 (C₆β), 20.9 (CH₃α), 20.8 (CH₃β), 20.6 (2xCH₃α, CH₃β), 20.5 (CH₃α, 2xCH₃β); ¹⁹F NMR (376 MHz, CDCl₃) δ = -208.1 (ddd, 3J₉H=51.5, 3J₉F=12.7, 4.1 Hz, β-anomer), -209.1 (ddd, 3J₉H=49.1, 3J₉F=11.4, J=4.0 Hz, α-anomer); [m/z] (ESI) (M+Na)+ C₁₄H₁₉FNaO₉ requires 373.0905, found 373.0906.

90bα: Rᵥ = 0.30 (cyclohexane/EtOAc 3:1); ¹⁹F NMR (282 MHz, CDCl₃) δ = -201.3 (ddd, 3J₉H=48.2, 3J₉F=31.0, 3J=7.5 Hz).
Synthesis of 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-galactopyranose 91

![Chemical Structure](image)

A solution of 90 (525 mg, 1.5 mmol, 1.0 eq.) in THF (20 mL, 0.07 M) at 0 °C under an atmosphere of Ar was treated with a solution of NH₃ in MeOH (7 M, 4.3 mL, 20.0 eq.) and stirred at 0 °C for 5 h. The reaction mixture was concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 5:3) afforded 91 as a colorless oil (323 mg, 70%, α:β 2.3:1) identical to known material (145).

R_f = 0.24 (cyclohexane/EtOAc 5:3); \(^1^H\) NMR (400 MHz, CDCl₃) δ = 5.52 (1H, t, \(J = 3.8\) Hz, H-C1α), 5.49-5.41 (2.4H, m, H-C4α, H-C4β, H-C3α), 5.13 (0.4H, ddd, \(^3^J_{HF} = 13.0, J = 9.8, 3.7\) Hz, H-C3β), 4.90 (0.4H, ddd, \(J = 7.5\) Hz, 6.1, \(^3^J_{HF} = 3.8\) H-C1β), 4.77 (1H, ddd, \(^2^J_{HF} = 49.8, J = 9.3, 3.7\) Hz, H-C2α), 4.95 (0.4H, ddd, \(^2^J_{HF} = 51.4, J = 9.8, 7.5\) Hz, H-C2β), 4.49 (1H, t, \(J = 6.7\) Hz, H-C5α), 4.26 (0.4H, d, \(J = 6.8\) Hz, OHβ), 4.14-4.07 (2.8H, m, H-C6β, H-C6α), 3.98 (0.4H, dd, \(J = 7.0, 6.0\) Hz, H-C5β), 3.75 (1H, d, \(J = 3.8\) Hz, OHα), 2.14 (1.2H, s, CH₃β), 2.13 (3H, s, CH₃α), 2.05 (1.2H, s, CH₃β), 2.04 (4.2H, s, CH₃α, CH₃β), 2.04 (3H, s, CH₃α); \(^1^F\) NMR (376 MHz, CDCl₃) δ = -206.7 (d, \(^2^J_{FH} = 51.3\) Hz, β-anomer), -207.2 (d, \(^2^J_{FH} = 49.8\) Hz, α-anomer).

Synthesis of 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-galactopyranosyl trichloroacetimidate 108

![Chemical Structure](image)

To a solution of 91 (37 mg, 0.12 mmol, 1.0 eq.) in dry CH₂Cl₂ (2.4 mL, 0.05 M) at 0 °C under an atmosphere of Ar, were added Cl₃CCN (121 μL, 1.2 mmol, 10.0 eq.) and DBU (1.8 μL, 12 μmol, 0.1 eq.). After 5 min at 0 °C, the reaction mixture was allowed to stir at r.t. for an additional 2 h before being concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 3:1) afforded 108 as a colorless oil (46 mg, 83%, mainly α) identical to known material (145).

R_f = 0.26 (cyclohexane/EtOAc 3:1); \(^1^H\) NMR (400 MHz, CDCl₃) δ = 8.80 (1H, s, NH), 6.67 (1H, d, \(J = 3.9\) Hz, H-C1), 5.45 (1H, t, \(J = 3.5\) Hz H-C4), 5.50 (1H, ddd, \(^3^J_{HF} = 10.7, J = 10.7, 3.5\) Hz, H-C3), 5.00 (1H, ddd, \(^2^J_{HF} = 48.8, J = 10.3, 3.9\) Hz, H-C2), 4.47 (1H, t, \(J = 6.5\) Hz, H-C5), 4.17 (1H, dd, \(J = 11.4, 6.5\) Hz, H-C6), 4.10 (1H, dd, \(J = 11.4, 6.5\) Hz, H-C6), 2.18 (3H, s, CH₃), 2.09 (3H, s, CH₃), 2.04 (3H, s, CH₃); \(^1^C\) NMR (100 MHz, CDCl₃) δ = 170.3 (OAc), 169.9 (2xOAc), 160.8 (C7), 93.1 (d, \(^2^J_{CE} = 22.2\) Hz, C1), 90.7 (C8), 84.4 (d, \(^1^J_{CE} = 193.5\) Hz, C2), 69.1 (C5), 68.2 (d, \(^1^J_{CE} = 19.1\) Hz, C3), 67.9 (d, \(^1^J_{CE} = 7.6\) Hz, C4), 61.0 (C6), 20.63 (CH₃),...
20.61 (CH₃), 20.5 (CH₃); ¹⁹F NMR (376 MHz, CDCl₃) δ = -207.6 (d, ²JFH 51.4 β-anomer), -207.2 (d, ²JFH 48.8 Hz α-anomer).

**Glycosylation to form 1-O-isopropyl-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-galactopyranose 117 (Table 2, Entry 3)**

A solution of 108 (46 mg, 0.10 mmol, 1.0 eq.) and iPrOH (9.3 μL, 0.12 mmol, 1.2 eq.) in dry CH₂Cl₂ (2 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (1.8 μL, 0.01 mmol, 0.1 eq.) at 0 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, CHCl₃/MeOH 9:1 and cyclohexane/EtOAc 7:1) afforded 117 as a colorless oil (17 mg, 48%, α:β 1.3:1).

**Rf** = 0.16 (cyclohexane/EtOAc 19:1); νmax (neat)/cm⁻¹ 2976w, 2934w, 1744s, 1434w, 1371m, 1214s, 1171m, 1126m, 1070s, 1031s, 985m, 927m, 879w, 844w, 829w, 710w, 676w, 627w; ¹H NMR (400 MHz, CDCl₃) δ = 5.47 (1H, s, H-C4α), 5.40 (0.7H, br, H-C4β), 5.48 (1H, ddd, ³JHF 10.4, J 10.4, 3.4 Hz, H-C3α), 5.18 (1H, d, J 3.9 Hz, H-C1α), 5.19 (0.7H, ddd, ³JHF 13.1, J 9.7, 3.6 Hz, H-C3β), 4.73 (1H, ddd, ²JHF 50.3, 3.4 Hz, H-C2α), 4.61 (0.7H, dd, J 7.6, ²JHF 3.9 Hz, H-C1β), 4.46 (0.7H, ddd, ²JHF 51.1, J 9.7, 7.6 Hz, H-C2β), 4.33 (1H, td, J 6.6, 1.25 Hz, H-C5α), 4.17 (0.7H, dd, J 11.3, 6.7 Hz, H-C6β), 4.09 (0.7H, dd, J 11.3, 6.7 Hz, H-C6β), 4.08 (2H, d, J 6.6 Hz, H-C6α), 4.02 (0.7H, sept., J 6.2 Hz, H-C7β), 3.93 (1H, sept., J 6.2 Hz, H-C7α), 3.90 (0.7H, t, J 6.6 Hz, H-C5β), 2.13 (5.1H, s, CH₃α, CH₃β), 2.05 (2.1H, s, CH₃β), 2.04 (2.1H, s, CH₃β), 2.03 (3H, s, CH₃α), 2.04 (3H, s, CH₃α), 1.29 (2.1H, d, J 6.2 Hz, H-C8β), 1.27 (3H, d, J 6.2 Hz, H-C8α), 1.23 (2.1H, d, J 6.2 Hz, H-C8β), 1.20 (3H, d, J 6.2 Hz, H-C8α); ¹³C NMR (100 MHz, CDCl₃) δ = 170.4 (OAcα, OAcβ), 170.1 (OAcα, 2xOAcβ), 170.0 (OAcα), 99.5 (d, ²JCF 19.4 Hz, C3β), 70.6 (C5β), 68.8 (d, ³JCF 7.6 Hz, C4α), 68.4 (d, ²JCF 19.6 Hz, C3α), 67.7 (d, ³JCF 8.4 Hz, C4β), 33.9 (C5α), 61.7 (d, C6α), 61.2 (C6β), 23.3 (C8α), 23.1 (C8β), 21.9 (C8α), 21.7 (C8β), 20.7 (CH₃α, CH₃β), 16.6 (CH₃α, CH₃β), 20.60 (CH₃α, CH₃β); ¹⁹F NMR (376 MHz, CDCl₃) δ = -206.5 (dd, ²JFH 51.1, ³JFH 13.1 Hz, β-anomer), -207.8 (dd, ²JFH 50.3, ³JFH 10.4, JFH 3.1 Hz, α-anomer); [m/z] (ESI) (M+Na)⁺ C₁₅H₂₃FNaO₈ requires 373.1269, found 373.1275.
Synthesis and glycosylation of the substrate 3,4,6-tri-O-acetyl-2-deoxy-D-galactopyranose 94

A solution of 3,4,6-tri-O-acetyl-D-galactal (817 mg, 3.0 mmol, 1.0 eq.) in CH₃CN (25 mL, 0.12 M) was treated with LiBr (808 mg, 9.3 mmol, 3.1 eq.), Amberlite® IR 120 (817 mg) and H₂O (1 mL, 54.0 mmol, 18.0 eq.) and stirred at r.t. for 3 h. The reaction mixture was filtered, neutralised with Et₃N and evaporated to dryness. The crude material was dissolved in H₂O and extracted with CH₂Cl₂ (3x). The combined organic phases were washed with a cold solution of HCl (1 M), then with a sat. aq. solution of NaHCO₃ and the aq. phases were back-extracted with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 1:1) afforded 94 as a colorless oil (502 mg, 58%, β:α 1:3.3) identical to known material (163).

Rᵣ 0.14 (cyclohexane/EtOAc 5:3); ¹H NMR (400 MHz, CDCl₃) δ = 5.49 (1H, br, H-C1α), 5.38-5.34 (2H, m, H-C3α, H-C4α), 5.25 (0.3H, d, J 2.9 Hz, H-C4β), 4.99 (0.3H, ddd, J 12.5, 4.9, 3.3 Hz, H-C3β), 4.91 (0.3H, ddd, J 9.7, 6.0, 2.1 Hz, H-C1β), 4.40 (1H, t, J 6.5 Hz, H-C5α), 4.15-4.03 (2.6H, m, H-C6β, H-C6α), 3.91 (1H, td, J 6.5, 1.1 Hz, H-C5β), 3.64 (0.3H, d, J 6.1 Hz, OHβ), 3.04 (1H, t, J 2.6 Hz, OHα), 2.13-2.04 (1.3H, m, H-C2α, H-C2β), 1.94-1.85 (1.3H, m, H-C2α, H-C2β), 2.13 (3H, s, CH₃α), 2.12 (0.9H, s, CH₃β), 2.05 (3.9H, s, CH₃α, CH₃β), 2.02 (0.9H, s, CH₃β), 1.98 (3H, s, CH₃α).
5. Experimental Part

### Synthesis of 3,4,6-tri-O-acetyl-2-deoxy-D-galactopyranosyl trichloroacetimidate 110

To a solution of 94 (143 mg, 0.53 mmol, 1.0 eq.) in dry CH$_2$Cl$_2$ (10 mL, 0.05 M) at 0 °C under an atmosphere of Ar, were added Cl$_3$CCN (533 μL, 5.30 mmol, 10.0 eq.) and DBU (7.9 μL, 0.05 mmol, 0.1 eq.). After 5 min at 0 °C, the reaction mixture was allowed to stir at r.t. for an additional 1 h before being concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 3:1) afforded 110 as a colorless oil (77 mg, 33%, mainly α).

R$_f$ 0.54 (cyclohexane/EtOAc 5:3); $\nu_{max}$ (neat)/cm$^{-1}$ 3312w, 2959w, 1742s, 1440w, 1672m, 1440s, 1367m, 1219s, 1203s, 1144m, 1037s, 964m, 948m, 902s, 884s, 827m, 795s, 751m, 716w, 643m; $^1$H NMR (400 MHz, CDCl$_3$) δ = 8.63 (1H, s, NH), 6.49 (1H, d, J 3.5 Hz, H-C1), 5.44 (1H, br, H-C4), 5.36 (1H, ddd, J$_{HF}$ 12.3, 5.1, 3.0 Hz, H-C3), 4.39 (1H, t, J 6.6 Hz, H-C5), 4.16 (1H, dd, J 11.3, 6.4 Hz, H-C6), 2.28 (1H, td, J 12.5, 3.5 Hz, H$_{ax}$-C2), 2.15 (3H, s, CH$_3$), 2.15-2.11 (1H, m, H$_{eq}$-C2), 2.02 (3H, s, CH$_3$), 2.01 (3H, s, CH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) δ = 170.4 (OAc), 170.2 (OAc), 170.0 (OAc), 160.6 (C7), 95.8 (C1), 91.2 (C8), 69.5 (C5), 66.1 (C4), 65.7 (C3), 61.9 (C6), 28.7 (C2), 20.8 (CH$_3$), 20.7 (2xCH$_3$); $m/z$ (ESI) (M+Na)$^+$ requires 455.9990, found 455.9991.

### Glycosylation to form 1-O-isopropyl-3,4,6-tri-O-acetyl-2-deoxy-D-galactopyranose 119 (Table 2, Entry 3)

A solution of 110 (60 mg, 130 μmol, 1.0 eq.) and iPrOH (12.7 μL, 170 μmol, 1.2 eq.) in dry CH$_2$Cl$_2$ (2.6 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (2.4 μL, 13 μmol, 0.1 eq.) at 0 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et$_3$N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, CHCl$_3$/MeOH 9/1, and then cyclohexane/EtOAc 4:1) afforded 119α as a colorless oil (16 mg, 38%, α-anomer), a mixture of 119α and 119β as a colorless oil (9 mg, 21%, α:β 1:2.5) and 119 as a colorless oil (9 mg, 21%, mainly β) identical to known material (198).

R$_f$ 0.23 (cyclohexane/EtOAc 3:1); $\nu_{max}$ (neat)/cm$^{-1}$ 2973w, 1741s, 1441w, 1369m, 1219s, 1182m, 1163m, 1118m, 1078m, 1020s, 982s, 947m, 913w, 883w, 842w, 752w, 706w, 646w, 626w.
5. Experimental Part

119α: $^1$H NMR (400 MHz, CDCl$_3$) δ = 5.32 (1H, br, H-C4α), 5.29 (1H, ddd, J 12.4, 4.9, 3.0 Hz, H-C3α), 5.11 (1H, d, J 3.7 Hz, H-C1α), 4.20 (1H, t, J 6.5 Hz, H-C5α), 4.07 (2H, d, J 6.5 Hz, H-C6α), 3.86 (1H, sept., J 6.2 Hz, H-C7α), 2.12 (3H, s, CH$_3$α), 2.08 (1H, td, J 12.4, 3.7 Hz, H$_{eq}$-C2α), 2.03 (3H, s, CH$_3$α), 1.97 (3H, s, CH$_3$α), 1.79 (1H, dd, J 12.4, 4.9 Hz, H$_{eq}$-C2α), 1.19 (3H, d, J 6.2 Hz, H-C8α), 1.13 (3H, d, J 6.2 Hz, H-C8α); 13C NMR (100 MHz, CDCl$_3$) δ = 170.5 (OAcα), 170.4 (OAcα), 170.1 (OAcα), 98.4 (C1β), 71.6 (C7β), 70.9 (C5β), 68.7 (C3β), 65.4 (C4β), 61.9 (C6β), 32.5 (C2β), 23.4 (C8β), 21.8 (C8β), 20.8 (CH$_3$β), 20.8 (CH$_3$β), 20.7 (CH$_3$β); [m/z] (ESI) (M+Na)$^+$ C$_{15}$H$_{24}$NaO$_8$ requires 355.1363, found 355.1363.

Synthesis and glycosylation of the substrate 2,3,4,6-tetra-O-acetyl-α-D-galactopyranose 101

A solution of α-D-galactose (541 mg, 3.0 mmol, 1.0 eq.) in pyridine (10 mL, 0.3 M) was treated with DMAP (37 mg, 0.3 mmol, 0.1 eq.) and Ac$_2$O (2.8 mL, 30.0 mmol, 10.0 eq.). The reaction mixture was stirred for 16 h at r.t. under an atmosphere of Ar. The reaction mixture was diluted with CH$_2$Cl$_2$, washed with H$_2$O (2x) and the aqueous phases were back-extracted with CH$_2$Cl$_2$. The combined organic phases were washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated in vacuo.

Synthesis of acetyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranose 99
5. Experimental Part

Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 5:3) afforded 99α as a white solid (1.15 g, 98%) identical to known material (199).

Rf 0.45 (cyclohexane/EtOAc 1:1); m.p. 91.8-94.6 °C (lit. m.p. 92-94 °C, (200)); [α]D²¹ +115.1 (c 0.23, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ = 6.38 (1H, br, H-C1), 5.50 (1H, br, H-C5), 5.34 (2H, m, H-C2, H-C3), 4.34 (1H, td, J 6.8, 1.1 Hz, H-C6), 4.12 (1H, dd, J 11.2, 6.8 Hz, H-C6), 4.08 (1H, dd, J 11.2, 6.8 Hz, H-C6), 2.15 (3H, s, CH₃), 2.15 (3H, s, CH₃), 2.04 (3H, s, CH₃), 2.02 (3H, s, CH₃), 2.00 (3H, s, CH₃).

Synthesis of 2,3,4,6-tetra-O-acetyl-D-galactopyranoside 101

A solution of 99 (261 mg, 0.66 mmol, 1.0 eq.) in THF (10 mL, 0.07 M) was treated at 0 °C with a solution of NH₃ in MeOH (7 M, 13.2 mL, 20.0 eq.). The reaction was stirred between 0-10 °C for 5 h. The reaction mixture was concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 1:1) afforded 101 as a colorless oil (177 mg, 77%, α:β 1:2.6) identical to known material (201).

Rf 0.26 (cyclohexane/EtOAc 1:1); ¹H NMR (400 MHz, CDCl₃) δ = 5.53 (1H, d, J 3.6 Hz, H-C1α), 5.48 (1H, d, J 3.3 Hz, H-C4α), 5.42 (1H, dd, J 10.9, 3.3 Hz, H-C3α), 5.41 (0.27H, br, H-C4β), 5.17 (1H, dd, J 10.9, 3.6 Hz, H-C2α), 5.08-5.06 (0.53H, m, H-C3β, H-C2β). 4.69 (0.27H, br, H-C1β) 4.47 (1H, t, J 6.5 Hz, H-C5α), 4.15 (0.54H, d, J 6.6 Hz, H-C6β), 4.12 (1H, dd, J 11.3, 6.5 Hz, H-C6α), 4.08 (1H, dd, J 11.3, 6.5 Hz, H-C6α), 3.95 (0.27H, d, J 6.6 Hz, H-C5β), 3.58 (0.27H, br, OHβ), 3.03 (1H, br, OHα), 2.16 (0.84H, s, CH₃β), 2.15 (3H, s, CH₃α), 2.11 (0.84H, s, CH₃β), 2.10 (3H, s, CH₃α), 2.05 (3.84H, s, CH₃α, CH₃β), 2.00 (0.84H, s, CH₃β), 1.99 (3H, s, CH₃α).

Synthesis of 2,3,4,6-tri-O-acetyl-D-galactopyranosyl trichloroacetimidate 109

To a solution of 101 (105 mg, 0.30 mmol, 1.0 eq.) in dry CH₂Cl₂ (6 mL, 0.05 M) at 0 °C under an atmosphere of Ar, were added Cl₃CCN (302 μL, 3.00 mmol, 10.0 eq.) and DBU (4.5 μL, 0.03 mmol, 0.1 eq.). After 5 min at 0 °C, the reaction mixture was allowed to stir at r.t. for an additional 2 h before being concentrated in vacuo. Purification by flash column chromatography (SiO₂, Cyclohexane/EtOAc 3:1) afforded 109 as a colorless oil (112 mg, 76%, mainly α) identical to known material (202).
5. Experimental Part

\( R_f 0.4 \alpha\text{-anomer (cyclohexane/EtOAc 5:3); }^1H NMR (400 MHz, CDCl}_3 \) \( \delta = 8.66 (1H, s, NH), 6.60 (1H, d, J 3.4 Hz, H-C1), 5.55 (1H, br, J 3.1 Hz, H-C4), 5.40 (1H, dd, J 11.0, 3.1 Hz, H-C3), 5.38 (1H, dd, J 11.0, 3.4 Hz, H-C2), 4.45-4.42 (1H, t, J 6.7 Hz, H-C5), 4.19-4.14 (1H, dd, J 11.3, 6.7 Hz, H-C6), 4.08 (1H, dd, J 11.3, 6.7 Hz, H-C6), 2.16 (3H, s, CH\(_3\)), 2.02 (3H, s, CH\(_3\)), 2.02 (3H, s, CH\(_3\)), 2.01 (3H, s, CH\(_3\)).

**Glycosylation to form 1-\text{O}-isopropyl-2,3,4,6-tetra-\text{O}-acetyl-\text{D}-galactopyranose 118** (Table 2, Entry 3)

A solution of 109 (72 mg, 0.15 mmol, 1.0 eq.) and iPrOH (13 \( \mu \)L, 0.18 mmol, 1.2 eq.) in dry CH\(_2\)Cl\(_2\) (3.5 mL, 0.04 M) under an atmosphere of Ar was treated with TMSOTf (2.6 \( \mu \)L, 0.015 mmol, 0.1 eq.) at 0 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et\(_3\)N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO\(_2\), cyclohexane/EtOAc 30/10 to 5:3) afforded 118\(\beta\) as a colorless oil (22 mg, 39%, \( \beta\)-anomer) identical to known material (203) and 118\(\beta\) (1-\text{O}-isopropyl-3,4,6-tri-\text{O}-acetyl-\text{D}-galactopyranose) (15 mg, 29%, \( \alpha\):\( \beta\) 1:2.9).

118: \( R_f 0.31 \) (cyclohexane/EtOAc 3:1); \( [\alpha]_{D}^{20} -12.6 \) (c 0.2, CH\(_2\)Cl\(_2\)); \(^1H NMR (400 MHz, CDCl}_3 \) \( \delta = 5.40(1H, d, J 3.4 Hz, H-C4), 5.19 (1H, dd, J 10.6, 7.9 Hz, H-C2), 5.04 (1H, dd, J 10.6, 3.4 Hz, H-C3), 4.52 (1H, d, J 7.9 Hz, H-C1), 4.21 (1H, dd, J 11.1, 6.6 Hz, H-C6), 4.13 (1H, dd, J 11.1, 6.9 Hz, H-C6), 3.98-3.89 (2H, m, H-C7, H-C5), 2.16 (3H, s, CH\(_3\)), 2.06 (3H, s, CH\(_3\)), 2.06 (3H, s, CH\(_3\)), 2.00 (3H, s, CH\(_3\)), 1.26 (3H, d, J 6.2 Hz, H-C8), 1.16 (3H, d, J 6.2 Hz, H-C8).

**By-product: 1-\text{O}-isopropyl-3,4,6-tri-\text{O}-acetyl-\text{D}-galactopyranose 118b:**

118b: \( R_f 0.16 \) (cyclohexane/EtOAc 3:1); \( \nu_{\text{max}} \text{(neat)/cm}^{-1} 3485w (OH), 2974w, 2924w, 1742s, 1434w, 1370m, 1218s, 1160m, 1124m, 1071s, 1032s, 950m, 910m, 829w, 731m, 677w, 648w, 628w; \(^1H NMR (400 MHz, CDCl}_3 \) \( \delta = 5.38-5.37 (0.35, m, H-C4\(\alpha\)), 5.37 (1H, d, J 3.5 Hz, H-C4\(\beta\)), 5.09 (0.35, dd, J 10.4, 3.4 Hz, H-C3\(\alpha\)), 5.06 (0.35H, d, J 4.1 Hz, H-C1\(\alpha\)), 4.93 (1H, dd, J 10.3, 3.5 Hz, H-C3\(\beta\)), 4.40 (1H, d, J 8.0 Hz, H-C1\(\beta\)), 4.24 (0.35H, t, J 6.2 Hz, H-C5\(\beta\)), 4.17 (1H, dd, J 11.2, 6.6 Hz, H-C6\(\beta\)), 4.10 (1H, dd, J 11.2, 6.9 Hz, H-C6\(\beta\)), 4.10-4.06 (0.70H, m, 2xH-C6\(\alpha\)), 4.02 (1H, sept., J 6.2 Hz, H-C7\(\beta\)), 3.94 (0.35H, sept., J 6.2 Hz, H-C7\(\alpha\)), 3.91-3.87 (0.35H, m, H-C2\(\alpha\)), 3.89 (1H, t, J 6.7 Hz, H-C5\(\beta\)), 3.76 (1H, dd, J 10.3, 8.0 Hz, H-C2\(\beta\)), 2.28 (1H, br, OH\(\beta\)), 2.13 (1.05H, s, CH\(_3\)\(\alpha\)), 2.12 (3H, s, CH\(_3\)\(\beta\)), 2.05 (1.05H, s, CH\(_3\)\(\alpha\)), 2.04 (3H, s, CH\(_3\)\(\beta\)), 2.04 (4.05H, s, CH\(_3\)\(\alpha\), CH\(_3\)\(\beta\)), 1.89-1.85 (0.35H, m, OH\(\alpha\)), 1.28 (3H, d, J 6.2 Hz, H-C8\(\beta\)), 1.26 (1.05H, d, J 6.2 Hz, H-C8\(\alpha\)), 1.23 (3H, d, J 6.2 Hz, H-C8\(\beta\)), 1.20 (1.05H, d, J 6.2 Hz, H-C8\(\alpha\)); \(^{13}C NMR (100 MHz, CDCl}_3 \) \( \delta = 170.8 \) (Ac\(\alpha\), Ac\(\beta\)).
5. Experimental Part

170.44 (Acα, Acβ), 170.36 (Acβ), 170.3 (Acβ), 170.2 (OAcα), 101.8 (C1β), 97.4 (C1α), 72.8 (C7β), 72.5 (C3β), 71.6 (C7α), 70.9 (C3α), 70.7 (C5β), 69.1 (C2β), 68.4 (C4α), 67.2 (C4β), 66.9 (C2α), 66.8 (C5α), 62.0 (C6α), 61.4 (C6β), 23.4 (C8β), 23.2 (C8α), 22.0 (C8β), 21.9 (C8α), 20.9 (CH3α), 20.8-20.7 (CH3β, CH3α); [m/z (ESI) (M+Na)+ C15H24NaO9 requires 371.1313, found 371.1305].

Glycosylation of 3,4,6-tri-O-benzyl-\(\text{D}\)-galactopyranose with various alcohols (Table 3)

**Glycosylation of the substrate 3,4,6-tri-O-benzyl-2-deoxy-2-fluoro-\(\text{D}\)-galactopyranose**

Glycosylation to form 1-O-ethyl-3,4,6-tri-O-benzyl-2-deoxy-2-fluoro-\(\text{D}\)-galactopyranose 120 (Table 3, Entry 1)

A solution of 102a (139 mg, 0.23 mmol, 1.0 eq.) and ethanol (16 µL, 0.28 mmol, 1.2 eq.) in dry CH2Cl2 (4.6 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (4.2 µL, 23 µmol, 0.1 eq.) at -78 °C and stirred for 1.5 h at that temperature. The reaction mixture was quenched by the addition of Et3N (0.2 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO2, cyclohexane/EtOAc, 12:1) afforded 120β as a white solid (90 mg, 80%, β-anomer).

Rf 0.63 (cyclohexane /EtOAc, 3:1); [\(\alpha\)]D\text{20} ^{20} -3.2 (c 0.50, CH2Cl2); m.p. 61.62 °C; \(\nu\)max (neat)/cm\(^{-1}\) 3061w, 3032w, 2983w, 2920w, 2861w, 1604w, 1496w, 1477w, 1454m, 1374w, 1363w, 1306w, 1250w, 1210w, 1169m, 1142m, 1118m, 1069s, 1025s, 1005m, 947m, 917m, 873w, 822m, 754s, 735s, 697s, 678m, 637w, 607m; \(^1\)H NMR (400 MHz, CDCl3) \(\delta\) = 7.24-7.39 (15H, m, Ph), 4.92 (1H, d, \(J\) 11.6, Bn), 4.77 (1H, d, \(J\) 12.0, Bn), 4.70 (1H, ddd, \(J\)FH 51.8, \(J\) 9.4, 7.6 Hz, H-C2), 4.69 (1H, d, \(J\) 12.1 Hz, Bn), 4.61 (1H, d, \(J\) 11.6 Hz, Bn), 4.46 (1H, d, \(J\) 11.7 Hz, Bn), 4.43 (1H, ddd, \(J\) 7.6, \(J\)FH 4.1 Hz, H-C1), 4.41 (1H, d, \(J\) 11.9 Hz, Bn), 3.90-3.99 (2H, m, H-C4, H-C7), 3.54-3.65 (5H, m, H-C3, H-C5, H-C6, H-C7) and 1.26 (3H, t, \(J\) 7.1 Hz, H-C8); \(^{13}\)C NMR (100 MHz, CDCl3) \(\delta\) = 138.3 (iPh), 138.0 (iPh), 137.8 (iPh), 128.4 (Ph), 128.3 (Ph), 128.2 (Ph), 127.9 (Ph), 127.8 (Ph), 127.7 (Ph), 127.60 (Ph), 127.55 (Ph), 100.7 (d, \(J\)CF 23.4 Hz, C1), 91.8 (d, \(J\)CF 183.0 Hz, C2), 80.3 (d, \(J\)CF 15.8 Hz, C3), 74.7 (Bn), 74.1 (d, \(J\)CF 9.0 Hz, C4), 73.5 (C5, Bn), 72.7 (d, \(J\)CF 2.1 Hz, Bn), 68.4 (C6), 65.3 (C7), 15.0 (C8); \(^{19}\)F NMR (376 MHz, CDCl3) \(\delta\)
5. Experimental Part

\[ \text{5. Experimental Part} \]

\[ \text{123} \]

\[ \text{[71x761]} = -205.3 (dddd, } J_{\text{FH}} 51.9, \; J_{\text{FH}} 12.9, \; J_{\text{FH}} 3.7, \; J_{\text{FH}} 3.6); \; [m/z] \; (\text{ESI}) \; (\text{M}+\text{NH}_4)^+ \; \text{C}_{29}\text{H}_{37}\text{FNO}_5 \text{ requires} 498.2650, \text{ found 498.2659}. \]

Glycosylation to form 1-O-tbutyl-3,4,6-tri-O-benzyl-2-deoxy-2-fluoro-\(\beta\)-galactopyranose 122 (Table 3, Entry 3)

A solution of 102\(\alpha\) (139 g, 0.23 mmol, 1.0 eq.) and \(t\)-BuOH (27 µL, 0.28 mmol, 1.2 eq.) in dry CH\(_2\)Cl\(_2\) (4.6 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (4.2 µL, 23 µmol, 0.1 eq.) at -78 °C and stirred for 1.5 h at that temperature. The reaction mixture was quenched by the addition of Et\(_3\)N (0.2 mL) and concentrated \textit{in vacuo}. Purification by flash column chromatography (SiO\(_2\), cyclohexane/EtOAc, 15:1) afforded 122 as a colorless oil (71 mg, 60%, \(\alpha:\beta \; 1:18\)).

\[ \text{R} \; 0.57 \text{ and 0.60 (cyclohexane/EtOAc, 3:1); } \text{v}_{\text{max}} \; \text{(neat)/cm}^{-1} \; 3064\,\text{w}, 3031\,\text{w}, 2976\,\text{w}, 2969\,\text{w}, 1497\,\text{w}, 1454\,\text{m}, 1403\,\text{w}, 1366\,\text{m}, 1306\,\text{w}, 1285\,\text{w}, 1263\,\text{w}, 1240\,\text{w}, 1195\,\text{m}, 1155\,\text{m}, 1068\,\text{s}, 1026\,\text{s}, 911\,\text{m}, 872\,\text{m}, 820\,\text{w}, 732\,\text{s}, 695\,\text{s}, 636\,\text{m}; \; ^1\text{H NMR} \; (400 \text{ MHz, CDCl}_3) \; \delta = 7.23-7.40 \; (15\text{H}, \text{ m, Ph}), 5.29 \; (0.06\text{H, d, } J \; 4.0 \; \text{Hz, H-1}\alpha), 4.92 \; (0.06\text{H, ddd, } J_{\text{FH}} 51.2, \; J \; 9.2, \; J \; 4.0 \; \text{Hz, H-2}\alpha), 4.91 \; (1\text{H, d, } J \; 11.6 \; \text{Hz, Bn}\beta), 4.78 \; (1\text{H, d, } J \; 12.0 \; \text{Hz, Bn}\beta), 4.68 \; (1\text{H, d, } J \; 12.1 \; \text{Hz, Bn}\beta), 4.60 \; (1\text{H, d, } J \; 11.6 \; \text{Hz, Bn}\beta), 4.54-4.73 \; (2\text{H, m, H-1}\beta, \; H-2\beta), 4.46 \; (1\text{H, d, } J \; 11.8 \; \text{Hz, Bn}\beta), 4.40 \; (1\text{H, d, } J \; 11.7 \; \text{Hz, Bn}\beta), 3.92 \; (1\text{H, t, } J \; 3.0 \; \text{Hz, H-4}\beta), 3.52-3.65 \; (4\text{H, m, H-3}\beta, \; H-5\beta, \; H-6\beta), 1.28 \; (9\text{H, s, H-8}\beta), 1.26 \; (0.54\text{H, s, H-8}\alpha), \text{ only some representative signals for the } \alpha\text{-anomer were characterized}; \; ^{13}\text{C NMR} \; (100 \text{ MHz, CDCl}_3) \; \delta = 138.4 \; (i\text{Ph}), 138.2 \; (i\text{Ph}), 137.9 \; (i\text{Ph}), 128.4 \; (\text{Ph}), 128.1 \; (\text{Ph}), 127.8 \; (\text{Ph}), 127.7 \; (\text{Ph}), 127.6 \; (\text{Ph}), 127.55 \; (\text{Ph}), 127.49 \; (\text{Ph}), 95.6 \; (d, \; J_{\text{CF}} \; 23.3 \; \text{Hz, C1}), 91.9 \; (d, \; J_{\text{CF}} \; 183.0 \; \text{Hz, C2}), 80.7 \; (d, \; J_{\text{CF}} \; 15.9 \; \text{Hz, C3}), 76.0 \; (C7), 74.6 \; (Bn), 74.2 \; (d, \; J_{\text{CF}} \; 9.0 \; \text{Hz, C4}), 73.5 \; (Bn), 73.4 \; (C5), 72.7 \; (d, \; J_{\text{CF}} \; 2.2 \; \text{Hz, Bn}), 68.8 \; (C6), 28.6 \; (C8), \text{ only the } \beta\text{-anomer was characterized}; \; ^{19}\text{F NMR} \; (376 \text{ MHz, CDCl}_3) \; \delta = -204.0 \text{ to } -204.2 \; (\text{m, } \beta\text{-anomer}) \text{ and } -205.8 \text{ to } -206.0 \; (\text{m, } \alpha\text{-anomer}); \; [m/z] \; (\text{ESI}) \; (\text{M}+\text{NH}_4)^+ \; \text{C}_{31}\text{H}_{41}\text{FNO}_5 \text{ requires} 526.2963, \text{ found 526.2972}. \]
5. Experimental Part

Glycosylation to form 1-O-allyl-3,4,6-tri-O-benzyl-2-deoxy-2-fluoro-D-galactopyranose 124 (Table 3, Entry 7)

A solution of 102α (128 mg, 214 μmol, 1.0 eq.) and allyl alcohol (17.4 μL, 256 μmol, 1.2 eq.) in dry CH₂Cl₂ (4 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (3.9 μL, 21 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.2 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 19:1) afforded 124 as a white solid (90 mg, 85%, β-anomer).

Rf 0.26 (cyclohexane/EtOAc 7:1); [α]D²⁰ -5.6 (c 0.20, CH₂Cl₂); m.p. 33.3-34.4 °C; νₘₐₓ (neat)/cm⁻¹ 3032w, 2902w, 2874w, 1495w, 1452m, 1408w, 1367m, 1348w, 1207w, 1159m, 1113s, 1071s, 1071s, 1052s, 1023s, 970m, 956m, 929m, 907m, 778w, 729s, 693s, 654m, 611m; ¹H NMR (400 MHz, CDCl₃) δ = 7.29-7.16 (15H, m, Ph), 5.84 (1H, dddd, J 16.9, 10.2, 6.1, 4.8 Hz, H-C₈), 5.23 (1H, d, J 16.9 Hz, H-C₉), 5.11 (1H, d, J 10.2 Hz, H-C₉), 4.84 (1H, dd, J 16.9 Hz, H-C₉), 4.66 (1H, d, J 11.7 Hz, Bn), 4.69 (1H, ddd, JHF 52.0, J 9.1, 7.5 Hz, H-C₂), 4.69 (1H, d, J 11.7 Hz, H-C₃), 4.60 (1H, d, J 11.7 Hz, Bn), 4.52 (1H, dd, J 13.1, 6.0 Hz, H-C₇), 3.85 (1H, br, H-C₄), 3.56-3.46 (4H, m, H-C₅, 2xH-C₆, H-C₃); ¹³C NMR (100 MHz, CDCl₃) δ = 138.3 (iPh), 138.0 (iPh), 137.8 (iPh), 133.7 (C₈), 128.5-127.6 (Ph), 117. 7 (C₉), 99.8 (d, JCS 23.8 Hz, C₁), 91.8 (d, JCS 183.9 Hz, C₂), 80.4 (d, JCS 16.3 Hz, C₃), 74.74 (Bn), 74.2 (d, JCS 9.3 Hz, C₄), 73.6 (CS), 73.6 (Bn), 72.8 (d, JCS 2.5 Hz, Bn), 69.9 (C₇), 68.5 (C₆); ¹⁹F NMR (376 MHz, CDCl₃) δ = -205.2 (dd, JHF 52.0, JHF 12.5 Hz); [m/z] (ESI) (M+H)⁺ C₄₈H₃₄F₅O₅ requires 493.2385, found 493.2373.

Glycosylation to form 1-O-phenyl-3,4,6-tri-O-benzyl-2-deoxy-2-fluoro-D-galactopyranose 126 (Table 3, Entry 5)

A solution of 102α (129 mg, 216 μmol, 1.0 eq.) and phenol (244 μL, 260 μmol, 1.2 eq.) in dry CH₂Cl₂ (4 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (3.9 μL, 22 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.2 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 19:1) afforded 126 as a white solid (90 mg, 85%, β-anomer).
cyclohexane/EtOAc 19:1) afforded 126α as a white syrup (8 mg, 7%, α-anomer) and 126β as a white solid (75 mg, 66%, β-anomer).

126β: R<sub>f</sub> 0.29 (cyclohexane:EtOAc 7:1); [α]<sub>D</sub><sup>20</sup> -21.4 (c 0.21, CH<sub>2</sub>Cl<sub>2</sub>); m.p. 82.3-83.2 °C; ν<sub>max</sub> (neat)/cm<sup>-1</sup> 3064w, 3032w, 2872w, 1601w, 1496m, 1454m, 1393w, 1371m, 1345w, 1313w, 1276w, 1222m, 1150m, 1119s, 1062s, 1025s, 1001m, 970m, 941m, 904m, 837w, 815w, 776m, 734s, 691s, 636m, 620m; 1<sup>H</sup> NMR (400 MHz, CDCl<sub>3</sub>) δ = 7.30-7.93 (20H, m, Ph), 5.05-5.01 (1H, m, H-C1), 4.98 (1H, ddd, 2J<sub>HF</sub> 48.8, 2J<sub>HF</sub> 9.2, 7.6 Hz, H-C2), 4.97 (1H, d, 2J<sub>HF</sub> 11.6 Hz, Bn), 4.82 (1H, d, 2J<sub>HF</sub> 12.0 Hz, Bn), 4.73 (1H, d, 2J<sub>HF</sub> 11.6 Hz, Bn), 4.64 (1H, d, 2J<sub>HF</sub> 11.6 Hz, Bn), 3.99 (1H, br, H-C4), 3.74-3.67 (2H, m, H-C5, H-C3), 3.67-3.60 (2H, m, H-C6); 1<sup>3</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ = 157.2 (iPh), 138.3 (iPh), 137.9 (iPh), 137.8 (iPh), 129.4-117.2 (Ph), 99.4 (d, 2J<sub>CF</sub> 24.0 Hz, C1), 88.3 (d, 2J<sub>CF</sub> 184.7 Hz, C2), 80.2 (d, 2J<sub>CF</sub> 15.9 Hz, C3), 74.8 (Bn), 74.1 (d, 2J<sub>CF</sub> 8.8 Hz, C4), 74.1 (C5), 73.7 (Bn), 72.8 (d, 2J<sub>CF</sub> 1.6 Hz, Bn), 68.5 (C6); 1<sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>) δ = -205.0 (m); m/z (ESI) (M+NH<sub>4</sub>)<sup>+</sup> C<sub>33</sub>H<sub>37</sub>FNO<sub>5</sub> requires 546.2650, found 546.2646.

126α: R<sub>f</sub> 0.39 (Cyclohexane/EtOAc 7/1); [α]<sub>D</sub><sup>20</sup> +92.0 (c 0.23, CH<sub>2</sub>Cl<sub>2</sub>); ν<sub>max</sub> (neat)/cm<sup>-1</sup> 3031w, 2921w, 2855w, 1730w, 1598m, 1496m, 1357m, 1221m, 1120s, 1095s, 1069s, 1047s, 1027s, 966m, 909m, 861m, 804m, 752s, 733s, 693s; 1<sup>H</sup> NMR (400 MHz, CDCl<sub>3</sub>) δ = 7.44-7.21 (17H, m, Ph), 7.10 (2H, d, 2J<sub>HF</sub> 7.5 Hz, Ph), 7.05 (1H, t, 2J<sub>HF</sub> 7.5 Hz, Ph), 5.69 (1H, d, 2J<sub>HF</sub> 3.9 Hz, H-C1), 5.27 (1H, ddd, 2J<sub>HF</sub> 50.0, 2J<sub>HF</sub> 10.0, 2J<sub>HF</sub> 3.9 Hz, H-C2), 4.98 (1H, d, 2J<sub>HF</sub> 11.3 Hz, Bn), 4.89 (1H, d, 2J<sub>HF</sub> 11.8 Hz, Bn), 4.77 (1H, d, 2J<sub>HF</sub> 11.8 Hz, Bn), 4.60 (1H, d, 2J<sub>HF</sub> 11.3 Hz, Bn), 4.44 (1H, d, 2J<sub>HF</sub> 11.5 Hz, Bn), 4.38 (1H, d, 2J<sub>HF</sub> 11.5 Hz, Bn), 4.23 (1H, ddd, 2J<sub>HF</sub> 10.0, 2J<sub>HF</sub> 10.0, 2J<sub>HF</sub> 3.3 Hz, H-C3), 4.16 (1H, dd, ddd, 2J<sub>HF</sub> 7.4, 5.8 Hz, H-C5), 4.10 (1H, t, 2J<sub>HF</sub> 3.3 Hz, H-C4), 3.63 (1H, dd, 2J<sub>HF</sub> 9.2, 7.4 Hz, H-C6), 3.54 (1H, dd, 2J<sub>HF</sub> 9.2, 5.8 Hz, H-C6); 1<sup>3</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ = 156.9 (iPh), 138.4 (iPh), 138.3 (iPh), 129.6-117.2 (Ph), 95.9 (d, 2J<sub>CF</sub> 21.3 Hz, C1), 88.3 (d, 2J<sub>CF</sub> 189.4 Hz, C2), 77.2 (m, C3), 75.5 (d, 2J<sub>CF</sub> 8.3 Hz, C4), 75.1 (Bn), 73.4 (Bn), 73.2 (d, 2J<sub>CF</sub> 2.1 Hz, Bn), 70.2 (C5), 68.4 (C6); 1<sup>19</sup>F NMR (100 MHz, CDCl<sub>3</sub>) δ = -207.6 (ddd, 2J<sub>HF</sub> 50.0, 2J<sub>HF</sub> 10.0, 2J<sub>HF</sub> 4.3 Hz); m/z (ESI) (M+Na)<sup>+</sup> C<sub>33</sub>H<sub>33</sub>FNaO<sub>5</sub> requires 551.2204, found 551.2202.

Glycosylation to form 1-O-benzyl-3,4,6-tri-O-benzyl-2-deoxy-2-fluoro-D-galactopyranose 128 (Table 3, Entry 6)

A solution of 102α (129 mg, 193 μmol, 1.0 eq.) and benzyl alcohol (23.8 μL, 230 μmol, 1.2 eq.) in dry CH<sub>2</sub>Cl<sub>2</sub> (4 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (3.5 μL, 19 μmol, 0.1 eq.)
at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et$_3$N (0.2 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 19:1) afforded **128β** as a white solid (93 mg, 89%, β-anomer).

R$_f$ 0.31 (Cyclohexane/EtOAc 7:1); [α]$_{D}^{20}$ -19.2 (c 0.21, CH$_2$Cl$_2$); m.p. 91.8-92.5 °C; ν$_{max}$ (neat)/cm$^{-1}$ 3063w, 3031w, 2913w, 2860w, 1496m, 1453m, 1367m, 1347w, 1308w, 1267w, 1206w, 1156m, 1118s, 1105s, 1086s, 1070s, 1052s, 1028s, 957m, 906 m, 806w, 777s, 730s, 693s, 644m, 609m;

**1H NMR** (400 MHz, CDCl$_3$) δ = 7.39-7.27 (20 H, m, Ph), 4.94 (1H, d, J 11.5 Hz, Bn), 4.93 (1H, d, J 12.3 Hz, Bn), 4.80 (1H, ddd, $^3$J$_{HF}$ 51.8 Hz, J 9.3, 7.6 Hz, H-C2), 4.78 (1H, d, J 12.1 Hz, Bn), 4.68 (1H, d, J 12.1 Hz, Bn), 4.67 (1H, d, J 12.3 Hz, Bn), 4.63 (1H, d, J 11.5 Hz, Bn), 4.51 (1H, dd, $^3$J$_{HF}$ 4.2 Hz, J 7.6 Hz, H-C1), 4.49 (1H, d, J 11.7 Hz, Bn), 4.44 (1H, d, J 11.7 Hz, Bn), 3.94 (1H, t, J 3.1 Hz, H-C4), 3.64-3.55 (4H, m, H-C6, H-C6, H-C3, H-C5);

**13C NMR** (100 MHz, CDCl$_3$) δ = 138.3 (iPh), 138.0 (iPh), 137.9 (iPh), 137.1 (iPh), 128.5-127.6 (Ph), 99.5 (d, $^2$J$_{CF}$ 23.3 Hz, C1), 91.9 (d, $^2$J$_{CF}$ 184.8 Hz, C2), 80.4 (d, $^2$J$_{CF}$ 15.8 Hz, C3), 74.8 (Bn), 74.2 (d, $^3$J$_{CF}$ 9.1 Hz, C4), 73.7 (C5), 73.6 (Bn), 72.8 (d, $^2$J$_{CF}$ 2.0 Hz, Bn), 70.5 (Bn), 68.8 (C6);

**19F NMR** (376 MHz, CDCl$_3$) δ = -205.2 (dd, $^2$J$_{HF}$ 51.8 Hz, $^2$J$_{HF}$ 12.9 Hz); [m/z] (ESI) (M+NH$_4$)$^+$ C$_{34}$H$_{39}$FNO$_5$ requires 560.2807, found 560.2796.

Glycosylation of the 3,4,6-tri-O-benzyl-2-deoxy-D-galactopyranose

![Glycosylation reaction](image)

Glycosylation to form 1-O-ethyl-3,4,6-tri-O-benzyl-2-deoxy-D-galactopyranose **121** (Table 3, Entry 1)

A solution of **104** (116 g, 0.20 mmol, 1.0 eq.) and ethanol (14 µL, 0.24 mmol, 1.2 eq.) in dry CH$_2$Cl$_2$ (4 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (3.6 µL, 20 µmol, 0.1 eq.) at -78 °C and stirred for 1.5 h at that temperature. The reaction mixture was quenched by the addition of Et$_3$N (0.2 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc, 12:1) afforded **121** as a colorless oil (66 mg, 71% over 2 steps, α:β 1:1.35).

R$_f$ 0.50 (cyclohexane/EtOAc, 3:1); ν$_{max}$ (neat)/cm$^{-1}$ 3064w, 3030w, 2975w, 2866w, 1607w, 1497w, 1454m, 1376m, 1359m, 1307w, 1255w, 1204m, 1166m, 1094s, 1056s, 1027s, 957m, 908m, 876m,
5. Experimental Part

819w, 733s, 695s, 652m; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta = 7.20-7.42 (37.5H, m, Ph), 5.00 (1H, d, J = 3.5\text{ Hz}, H-C1\alpha), 4.94 (1H, d, J = 11.6\text{ Hz}, Bn\alpha), 4.93 (1.5H, d, J = 11.8\text{ Hz}, Bn\beta), 3.67-4.40 (14H, m, H-C1\beta, Bn), 3.90-4.00 (4.5H, m, H-C3\alpha, H-C4\alpha, H-C5\alpha, H-C7\beta), 3.82-3.84 (1.5H, d, J = 11.8\text{ Hz}, H-C7\alpha), 3.67-4.40 (14H, m, H-C1\beta, H-C3\beta, H-C4\beta, H-C5\beta, H-C6\alpha, H-C6\beta, H-C7\alpha, H-C7\beta), 2.19-2.28 (1H, m, H-C2\alpha), 2.04-2.16 (3H, m, H-C2\beta), 1.96-2.03 (1H, m, H-C2\alpha), 1.21 (4.5H, t, J = 7.1\text{ Hz}, H-C8\beta), 1.19 (3H, t, J = 7.1\text{ Hz}, H-C8\alpha); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta = 138.93 (\text{iPh}\alpha), 138.87 (\text{iPh}\beta), 138.6 (\text{iPh}\alpha), 138.3 (\text{iPh}\beta), 138.12 (\text{iPh}\alpha), 138.06 (\text{iPh}\beta), 128.4-127.3 (\text{Ph}), 100.1 (C1\beta), 97.5 (C1\alpha), 77.4 (C3\beta), 75.0 (C3\alpha), 74.3 (Bn\alpha), 74.12 (Bn\beta), 74.08 (C5\beta), 73.53 (Bn\beta), 73.47 (Bn\alpha), 73.0 (C4\alpha), 71.7 (C4\beta), 70.5 (Bn\alpha), 70.2 (Bn\beta), 69.7 (C5\alpha), 69.6 (C6\alpha), 69.4 (C6\beta), 64.5 (C7\beta), 62.7 (C7\alpha), 32.9 (C2\beta), 31.3 (C2\alpha), 15.08 (C8\beta), 15.07 (C8\alpha); [m/z (ESI) (M+NH\textsubscript{4})\textsuperscript{+}] C\textsubscript{29}H\textsubscript{38}NO\textsubscript{5} requires 480.2744, found 480.2738].

Glycosylation to form 1-O-t-butyl-3,4,6-tri-O-benzyl-2-deoxy-D-galactopyranose 123 (Table 3, Entry 3)

A solution of 104 (116 g, 0.20 mmol, 1.0 eq.) and tBuOH (23 µL, 0.24 mmol, 1.2 eq.) in dry CH\textsubscript{2}Cl\textsubscript{2} (4 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (3.6 µL, 20 µmol, 0.1 eq.) at -78 °C and stirred for 1.5 h at that temperature. The reaction mixture was quenched by the addition of Et\textsubscript{3}N (0.2 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO\textsubscript{2}, cyclohexane/EtOAc, 15:1) afforded 123 as a colorless oil (82 mg, 84% over 2 steps, α:β 1:1.3) identical to known material (204).
69.2 (C5α), 34.2 (C2β), 32.8 (C2α), 28.65 (C8β), 28.64 (C8α); [m/z (ESI) (M+NH₄)⁺, C₃₁H₄₂NO₅ requires 508.3057, found 508.3057].

Glycosylation to form 1-O-allyl-3,4,6-tri-O-benzyl-2-deoxy-D-galactopyranose 125 (Table 3, Entry 4)

A solution of 104 (98.4 mg, 170 μmol, 1.0 eq.) and allyl alcohol (11.9 μL, 204 μmol, 1.2 eq.) in dry CH₂Cl₂ (3.4 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (3.1 μL, 17 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 12:1) afforded 125 as a colorless oil (9 mg, 85% over 2 steps, α:β 1:1) identical to known material (205).

Rf 0.47 β-anomer, 0.51 α-anomer (cyclohexane/EtOAc 7:1); ¹H NMR (400 MHz, CDCl₃) δ = 7.39-7.25 (30H, m, Ph), 5.92 (2H, m, H-C8α, H-C8β), 5.28 (2H, d, J 17.6 Hz, H-C9α, H-C9β), 5.17 (2H, d, J 10.2 Hz, H-C9α, H-C9β), 5.04 (1H, d, J 3.5 Hz, H-C1α), 4.95 (1H, d, J 11.4 Hz, Bn), 4.94 (1H, d, J 11.4 Hz, Bn), 4.66 (1H, d, J 11.9 Hz, Bn), 4.63 (1H, d, J 11.9 Hz, Bn), 4.64-4.67 (4H, d, J 12.0 Hz, Bn), 4.54-4.43 (5H, m, Bn, H-C1β) 4.38 (1H, ddd, J 12.8, 4.9, 1.5 Hz, H-C7β), 4.15 (1H, ddd, J 12.8, 5.1, 1.5 Hz, H-C7β), 4.05 (1H, ddd, J 12.8, 6.5, 1.2 Hz, H-C7α), 4.00-3.93 (4H, m, H-C3α, H-C4α, H-C5α, H-C7α), 3.85 (1H, br, H-C4β), 3.69-3.59 (4H, m, H-C6α, H-C6β), 3.57 (1H, ddd, J 12.0, 4.9, 2.6 Hz, H-C3β), 3.49 (1H, dd, J 6.8, 5.6 Hz, H-C5β), 2.26 (1H, td, J 12.2, 3.5 Hz, H eq-C2α), 2.15 (0.9H, td, J 12.0, 9.6 Hz, H ax-C2β), 2.13-2.07 (1H, m, H eq-C2β), 2.04 (1H, dd, J 12.2, 4.5 Hz, H eq-C2α).

Glycosylation to form 1-O-phenyl-3,4,6-tri-O-benzyl-2-deoxy-D-galactopyranose 127 (Table 3, Entry 5)

A solution of 104 (98 mg, 170 μmol, 1.0 eq.) and phenol (19 mg, 204 μmol, 1.2 eq.) in dry CH₂Cl₂ (4 mL, 0.04 M) under an atmosphere of Ar was treated with TMSOTf (3.1 μL, 17 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂,
cyclohexane/EtOAc 12:1) afforded **127α** as a colorless oil (58 mg, 67% over 2 steps, α-anomer) identical to known material (206).

**Rf** 0.31 (Cyclohexane/EtOAc 7:1); [α]$_D^{20}$ +95.3 (c 0.22, CH$_2$Cl$_2$); **1H NMR** (400 MHz, CDCl$_3$) δ = 7.41-7.21 (17H, m, Ph), 7.07 (2H, d, J 8.8 Hz, Ph), 7.00 (1H, t, J 7.6 Hz, Ph), 5.73 (1H, d, J 3.5 Hz, H-C1), 4.99 (1H, d, J 11.7 Hz, Bn), 4.71 (1H, d, J 11.9 Hz, Bn), 4.68 (1H, d, J 11.9 Hz, Bn), 4.67 (1H, d, J 11.7 Hz, Bn), 4.43 (1H, d, J 11.6 Hz, Bn), 4.37 (1H, d, J 11.6 Hz, Bn), 4.16 (1H, ddd, J 12.4, 4.8, 2.6 Hz, H-C3), 4.07 (1H, dd, J 7.5, 5.5 Hz, H-C5), 4.03 (1H, br, H-C4), 3.67 (1H, dd, J 9.3, 7.5 Hz, H-C6), 3.54 (1H, dd, J 9.3, 5.5 Hz, H-C6), 2.42 (1H, ddd, J 12.4, 3.5 Hz, H$_{ax}$-C2), 2.22 (1H, dd, J 12.4, 4.6 Hz, H$_{eq}$-C2).

**Glycosylation to form 1-O-benzyl-3,4,6-tri-O-benzyl-2-deoxy-D-galactopyranose 129 (Table 3, Entry 6)**

A solution of **102** (67 mg, 116 μmol, 1.0 eq.) and benzyl alcohol (14.4 μL, 139 μmol, 1.2 eq.) in dry CH$_2$Cl$_2$ (2.3 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (2.1 μL 12 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et$_3$N (0.1 mL) and concentrated *in vacuo*. Purification by flash column chromatography (SiO$_2$, Cyclohexane/EtOAc 19:1) afforded **129** as a colorless oil (34 mg, 56% over 2 steps, α:β 1.1:1) identical to known material (205).

**Rf** 0.40 (cyclohexane/EtOAc 3:1); **1H NMR** (400 MHz, CDCl$_3$) δ = 7.39-7.25 (38H, m, Ph), 5.11 (1H, d, J 3.4 Hz, H-C1α), 4.97 (1.9H, d, J 11.8 Hz, Bnα, Bnβ), 4.93 (0.9H, d, J 12.0 Hz, Bnβ), 4.71-4.56 (1H, ddd, J 12.3, 4.6, 2.4 Hz, H-C3α), 4.01-3.97 (1H, m, H-C5α), 3.97 (1H, br, H-C4α), 3.85 (0.9H, br s, H-C4β), 3.69 (1H, d, J 6.3 Hz, H-C6β), 3.64 (1H, dd, J 9.2, 6.9 Hz, H-C6α), 3.59 (1H, dd, J 9.2, 6.1 Hz, H-C6α), 3.56 (0.9H, ddd, J 12.0, 4.6, 2.6 Hz, H-C3β), 3.55 (0.9 H, t, J 6.3 Hz, H-C5β), 2.28 (1H, dd, J 12.3, 3.7 Hz, H$_{ax}$-C2α), 2.20 (0.9H, dd, J 12.0, 9.7 Hz, H$_{ax}$-C2β), 2.13-2.08 (0.9H, m, H$_{eq}$-C2β), 2.07 (1H, dd, J 12.3, 4.6 Hz, H$_{eq}$-C2α).
Glycosylation with glycosyl acceptors to form dissacharides (Table 4)

Synthesis of the glycosyl acceptor methyl 2-O-benzyl-4,6-O-benzylidene-α-D-galactopyranoside 130

Camphor sulfonic acid (186 mg, 0.8 mmol, 0.1 eq.) and benzaldehyde dimethyl acetal (1.5 mL, 10 mmol, 1.3 eq.) were added to a solution of 1-methyl-α-D-glucopyranoside (1.55 g, 8 mmol, 1.0 eq.) in acetonitrile (80 mL, 0.1 M). The reaction mixture was heated to reflux and stirred for 45 min under an atmosphere of Ar. The reaction mixture was neutralised with Et₃N, stirred 20 min and evaporated to dryness. The crude material was diluted with CH₂Cl₂ (80 mL), washed with H₂O (80 mL) and the aqueous layer was back-extracted with CH₂Cl₂ (2x30 mL). The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was washed with cyclohexane/EtOAc (1:1, 70 mL) and filtered to afford 137 as a white solid (814 mg, 37%) identical to known material (169).

Rᵣ 0.4 (CH₂Cl₂/MeOH 12:1); m.p. 170.3-171.0 °C (lit. 166-168 °C; (169)); [α]D²⁰+152.1 (c 0.2, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ = 7.51-7.48 (2H, m, Ph), 7.39-7.36 (3H, m, Ph), 5.56 (1H, s, CH-Ph), 4.94 (1H, d, J 3.1 Hz, H-C1), 4.30 (1H, dd, J 12.6, 1.8 Hz, H-C6), 4.27-4.26 (1H, m, H-C4), 4.09 (1H, dd, J 12.6, 1.8 Hz, H-C6), 3.93-3.89 (2H, m, H-C2, H-C3), 3.71 (1H, br s, H-C5), 3.46 (3H, s, CH₃), 2.42 (1H, d, J 8.9 Hz, OH), 2.19 (1H, d, J 7.8 Hz, OH).

Synthesis of methyl 4,6-O-benzylidene-α-D-galactopyranoside 137

[Diagram of synthesis]
Synthesis of methyl 2-O-benzyl-4,6-O-benzylidene-α-D-galactopyranoside 138 and methyl 3-O-benzyl-4,6-O-benzylidene-α-D-galactopyranoside 139

A mixture of 137 (914 mg, 3.2 mmol, 1.0 eq.), nBu4NI (274 mg, 0.74 mmol, 0.2 eq.), benzyl bromide (456 μL, 4.0 mmol, 1.2 eq.) and an aq. solution of NaOH (0.68 M, 7.3 mL, 1.5 eq.) in CH2Cl2 (23 mL, 0.14 M) was stirred at r.t. for 24 h. The organic phase was separated, washed with brine, dried over Na2SO4, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO2, dry loading, cyclohexane/EtOAc 7:1) afforded a mixture of 138 and 139 (491 mg, 41%).

Synthesis of methyl 2-O-benzyl-3-O-acetyl-4,6-O-benzylidene-α-D-galactopyranoside 140

A mixture of 138 and 139 (491 mg, 1.3 mmol, 1.0 eq.), Ac2O (490 μL, 5.2 mmol, 4.0 eq.) and DMAP (16 mg, 0.3 mmol, 0.1 eq.) in pyridine (5 mL, 0.3 M) was stirred at r.t. for 24 h. The reaction mixture was diluted with CH2Cl2, washed with a sat. aq. solution of NaHCO3, then with H2O, and the aq. phases were back-extracted with CH2Cl2. The combined organic phases were washed with brine, dried over Na2SO4, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO2, cyclohexane/EtOAc 7:1) afforded 140 as a colorless oil (236 mg, 44%) and 141 as a colourless oil (171 mg, 32%) identical to known material (207).

Rf 0.56 (cyclohexane/EtOAc 1:1); [α]D20 +162.8 (c 0.22, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ = 7.50-7.27 (10H, m, Ph), 5.50 (1H, s, CH-Ph), 5.29 (1H, dd, J 10.5, 3.5 Hz, H-C3), 4.81 (1H, d, J 3.5 Hz, H-C1), 4.75 (1H, d, J 12.3 Hz, Bn), 4.63 (1H, d, J 12.3 Hz, Bn), 4.46 (1H, d, J 3.6 Hz, H-C4), 4.24 (1H, dd, J 12.5, 1.6 Hz, H-C6), 4.09 (1H, dd, J 10.5, 3.5 Hz, H-C2), 4.05 (1H, dd, J 12.5, 1.9 Hz, H-C6), 3.73 (1H, m, H-C5), 3.41 (3H, s, CH3), 2.09 (3H, s, CH3).

Synthesis of methyl 2-O-benzyl-4,6-O-benzylidene-α-D-galactopyranoside 130

A solution of 140 (236 mg, 0.57 mmol, 1.0 eq.) in MeOH (5.7 mL, 0.1 M) was treated with MeONa (12 mg, 0.23 mmol, 0.4 eq.) and stirred at r.t. for 2 h. The reaction mixture was neutralised with
5. Experimental Part

Amberlite® IR 120, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 5:3) afforded 130 (49 mg, 23%) identical to known material (207). 

R$_f$ 0.2 (cyclohexane/EtOAc 1:1); m.p. 93.0-95.0 °C (lit. m.p 100.6-101.5 °C; $[\alpha]_{D}^{20} +15.4$ (c 0.22, CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta = 7.50-7.27$ (10H, m, Ph), 5.55 (1H, s, CH-Ph), 4.81 (1H, d, J 11.9 Hz, Bn), 4.80 (1H, d, J 3.5 Hz, H-C1), 4.67 (1H, d, J 11.9 Hz, Bn), 4.29 (1H, d, J 3.7 Hz, H-C4), 4.25 (1H, dd, J 12.7, 1.1 Hz, H-C6), 4.14 (1H, m, H-C3), 4.07 (1H, dd, J 12.7, 1.9 Hz, H-C6), 3.83 (1H, dd, J 10.0, 3.5 Hz, H-C2), 3.68 (1H, m, H-C5), 3.37 (3H, s, CH$_3$), 2.42 (1H, d, J 7.5 Hz, OH).

Glycosylation to form methyl (2-deoxy-2-fluoro-3,4,6-tri-O-benzyl-D-galactopyranosyl)-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-D-galactopyranoside 144 (Table 4, Entry 1)

A solution of 102α (30 mg, 50 μmol, 1.0 eq.) and 130 (22 mg, 60 μmol, 1.2 eq.) in dry CH$_2$Cl$_2$ (1 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.9 μL, 5 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et$_3$N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 3:1) afforded 144 as a white solid (19 mg, 48%, α:β 1:30).

R$_f$ 0.37 (cyclohexane/EtOAc 5:3); $[\alpha]_{D}^{20} +72.7$ (c 0.19, CH$_2$Cl$_2$); m.p. 62.0-64.5 °C; $\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3032w, 2918w, 1725w, 1497w, 1454m, 1399w, 1366m, 1345w, 1314w, 1274w, 1249w, 1196w, 1150m, 1093s, 1047s, 1026s, 991s, 912m, 836w, 793w, 735s, 695s, 649m; $^1$H NMR (400 MHz, CDCl$_3$) $\delta = 7.53-7.28$ (25H, m, Ph), 5.54 (1H, s, CH-Ph), 4.94 (1H, d, J 11.6 Hz, Bn), 4.85 (1H, d, J 11.8 Hz, Bn), 4.84 (1H, d, J 7.8 Hz, H-C1), 4.78 (1H, ddd, $^2$J$_{HF}$ 50.3, J 9.3, 7.8 Hz, H-C2), 4.80 (1H, J 12.0 Hz, Bn), 4.70 (1H, d, J 3.5 Hz, H-C1'), 4.69 (1H, d, J 12.0 Hz, Bn), 4.63 (1H, d, J 11.6 Hz, Bn), 4.58 (1H, d, J 11.8 Hz, Bn), 4.46 (1H, d, J 11.8 Hz, Bn), 4.42 (1H, d, J 11.6 Hz, Bn), 4.29 (1H, d, J 3.3 Hz, H-C4'), 4.18 (1H, dd, J 12.5, 1.3 Hz, H-C6'), 4.187 (1H, dd, J 10.2, 3.3 Hz, H-C3'), 4.07 (1H, ddd, J 10.2, 3.5, Hz, H-C2'), 3.96 (1H, dd, J 12.5, 1.3 Hz, H-C6'), 3.92 (1H, t, J 3.1 Hz, H-C4), 3.60-3.53 (5H, m, H-C3, H-C5, C-H-C5), 3.38 (3H, s, CH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta = 138.5$ (iPh), 138.3 (iPh), 138.1 (iPh), 138.05 (iPh), 137.96 (iPh), 128.7-126.4 (Ph), 102.3 (d, $^2$J$_{CF}$ 22.9 Hz, C1), 100.8 (CH-Ph), 99.4 (C1'), 92.5 (d, $^1$J$_{CF}$ 184.6 Hz, C2), 80.5 (d, $^2$J$_{CF}$ 15.6 Hz, C3), 75.7, 76.9 (C4'), 75.7 (C2'), 75.1 (C3'), 74.9 (Bn), 74.3 (d, $^1$J$_{CF}$ 8.7 Hz, C4), 74.0 (d, J 2.3 Hz, Bn), 73.41-73.36 (Bn, C5), 72.9 (d, J 2.1 Hz, Bn), 69.2 (C6'), 68.3 (C6), 62.8 (C5'), 55.5
5. Experimental Part

$^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ = -203.8 (m); [m/z (ESI) (M+NH$_4$)+] $^{C_{4b}H_{55}FNO_{10}}$ requires 824.3805, found 824.3796.

Glycosylation to form methyl (2-deoxy-3,4,6-tri-O-benzyl-ß-galactopyranosyl)-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-ß-glucopyranoside 145 (Table 4, Entry 1)

A solution of 104 (29 mg, 50 μmol, 1.0 eq.) and 130 (22 mg, 60 μmol, 1.2 eq.) in dry CH$_2$Cl$_2$ (1 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.9 μL, 5 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et$_3$N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 3:1) afforded 145α as a white solid (22 mg, 56% over 2 steps, α-anomer).

$\text{R}_f$ 0.36 (cyclohexane/EtOAc 5:3); $[\alpha]^{20}_D$ +68.4 (c 0.15, CH$_2$Cl$_2$); m.p. 51.3-51.9 °C; $\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3063w, 3032w, 2915w, 1722w, 1604w, 1497w, 1454ms, 1398w, 1360m, 1314w, 1276w, 1248w, 1203m, 1153s, 1096m, 1045s, 1025s, 991s, 953m, 919m, 831m, 794m, 734s, 696s, 647m; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 7.50-7.22 (25H, m, Ph), 5.50 (1H, s, CH-Ph), 5.29 (1H, d, $J$ 3.6 Hz, H-C1), 4.94 (1H, d, $J$ 11.5 Hz, Bn), 4.74 (1H, d, J 3.5 Hz, H-C1'), 4.71 (1H, d, $J$ 12.2 Hz, Bn), 4.62 (1H, d, J 11.5 Hz, Bn), 4.59 (1H, d, $J$ 12.4 Hz, Bn), 4.56 (1H, d, $J$ 12.4 Hz, Bn), 4.54 (1H, d, $J$ 12.2 Hz, H-Bn), 4.44 (2H, s, Bn), 4.37 (1H, d, J 3.4 Hz, H-C4'), 4.24-4.20 (2H, m, H-C6', H-C3'), 4.18 (1H, t, $J$ 6.6 Hz, H-C5), 4.03 (1H, dd, $J$ 12.2, 4.4 Hz, H-C6), 3.98 (1H, ddd, $J$ 12.2, 4.4, 2.2 Hz, H-C3), 3.94 (1H, dd, $J$ 10.2, 3.5 Hz, H-C2'), 3.89 (1H, br, J 3.4 Hz, H-C6'), 3.64 (1H, dd, $J$ 9.6, 6.4 Hz, H-C6), 3.60-3.55 (2H, m, H-C6, H-C5'), 3.35 (3H, s, CH$_3$), 2.31-2.24 (1H, td, $J$ 12.2, 3.6 Hz, H$_{ax-C2}$), 2.05-2.01 (1H, dd, $J$ 12.2, 4.4 Hz, H$_{eq-C2}$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 139.0 (iPh), 138.7 (iPh), 138.0 (iPh), 138.42 (iPh), 138.7 (iPh), 129.8-126.2 (Ph), 100.8 (CH-Ph), 99.4 (C1'), 92.6 (C1), 74.4 (C3, C2'), 74.3 (Bn), 73.6 (Bn), 73.1 (C4), 73.0 (Bn), 72.1 (C4'), 70.3 (CBr), 70.2 (C3'), 69.8 (C5), 69.6 (C6'), 69.2 (C6), 62.1 (C5'), 55.5 (CH$_3$), 31.0 (C2); [m/z (ESI) (M+Na)+] $^{C_{48}H_{65}NaO_{10}}$ requires 811.3453, found 811.3451.
5. Experimental Part

**Synthesis of the glycosyl acceptor methyl 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside 131**

![Chemical structure image]

**Synthesis of methyl 4,6-O-benzylidene-α-D-glucopyranoside 142**

Camphor sulfonic acid (186 mg, 0.8 mmol, 0.1 eq.) and benzaldehyde dimethyl acetal (1.5 mL, 10.0 mmol, 1.3 eq.) were added to a solution of 1-methyl-α-D-glucopyranoside (1.55 g, 8.0 mmol, 1.0 eq.) in acetonitrile (80 mL, 0.1 M). The reaction mixture was heated to reflux and stirred for 45 min under an atmosphere of Ar. The reaction mixture was neutralised with Et₃N, stirred for 20 min and evaporated to dryness. The crude material was diluted with CH₂Cl₂ (80 mL), washed with H₂O (80 mL) and the aqueous layer was back-extracted with CH₂Cl₂ (2x30 mL). The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was washed with cyclohexane/EtOAc (1:1, 70 mL) and filtered to afford 142 as a white solid (1.82 g, 81%) identical to known material (169).

Rᵥ 0.24 (cyclohexane/EtOAc 1:7); ¹H NMR (400 MHz, CDCl₃) δ = 7.50-7.45 (2H, m, Ph), 7.40-7.36 (3H, m, Ph), 5.55 (1H, s, CH-Ph), 4.82 (1H, d, J 3.9 Hz, H-C1), 4.32 (1H, dd, J 9.7, 4.9 Hz, H-C6), 3.95 (1H, t, J 9.3 Hz, H-C3), 3.83 (1H, ddd, J 10.3, 9.3, 4.4 Hz, H-C5), 3.77 (1H, dd, J 10.3, 9.7 Hz, H-C6), 3.64 (1H, br, H-C2), 3.52 (1H, t, J 9.3 Hz, H-C4), 3.48 (3H, s, CH₃), 2.83 (1H, br, OH), 2.35 (1H, br, OH).

**Synthesis of methyl 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside 131**

A mixture of 142 (1.41 g, 5.00 mmol, 1.0 eq.), n-Bu₄NI (425 mg, 1.15 mmol, 0.2 eq.), benzyl bromide (706 μL, 6.15 mmol, 1.2 eq.) and NaOH aq. solution (0.68 M, 11.25 mL, 1.5 eq.) in CH₂Cl₂ (35 mL, 0.14 M) was stirred at r.t. for 24 h. The organic phase was separated, washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 3:1) afforded 131 as a white solid (746 mg, 40%) identical to known material (208).
5. Experimental Part

R\textsubscript{f} 0.5 (Cyclohexane/EtOAc 1:1); [\alpha]\textsubscript{D}\textsuperscript{20} +52.1 (c 0.2, CH\textsubscript{2}Cl\textsubscript{2}); m.p. 129.7-130.4 °C (lit. 122-124 °C, (209)); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta = 7.53-7.32 \) (10 H, m, Ph), 5.54 (1H, s, CH-Ph), 4.82 (1 H, d, J 12.2 Hz, Bn), 4.74 (1H, d, J 12.2 Hz, Bn), 4.65 (1 H, d, J 3.6 Hz, H-C1), 4.29 (1H, dd, J 10.1, 4.8 Hz, H-C6), 4.16 (1H, td, J 9.3, 2.2 Hz, H-C3), 3.82 (1H, td, J 9.9, 4.8 Hz, H-C5), 3.71 (1H, t, J 10.2 Hz, H-C6), 3.50 (1H, t, J 10.1 Hz, H-C4), 3.48 (1H, dd, J 9.4, 3.6 Hz, H-C2), 3.38 (3 H, s, CH\textsubscript{3}), 2.57 (1H, br, OH).

Glycosylation to form methyl (2-deoxy-2-fluoro-3,4,6-tri-O-benzyl-\( \alpha \)-galactopyranosyl)\( \rightarrow 3 \)-2-O-benzyl-4,6-O-benzylidene-\( \alpha \)-D-glucopyranoside 146 (Table 4, Entry 2)

![Chemical structure](image)

A solution of 102\( \alpha \) (105 mg, 170 μmol, 1.0 eq.) and 131 (79 mg, 210 μmol, 1.2 eq.) in dry CH\textsubscript{2}Cl\textsubscript{2} (3.4 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (3.1 μL, 17 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et\textsubscript{3}N (0.1 mL) and concentrated \textit{in vacuo}. Purification by flash column chromatography (SiO\textsubscript{2}, cyclohexane/EtOAc 4:1 and toluene/acetone 19:1) afforded 146\( \beta \) as a white thick solid (49 mg, 36%, \( \beta \)-anomer).

R\textsubscript{f} 0.40 (cyclohexane/EtOAc 3/1); [\alpha]\textsubscript{D}\textsuperscript{20} +9.4 (c 0.19, CH\textsubscript{2}Cl\textsubscript{2}); m.p. 124-125 °C; \( \nu \max \) (neat)/cm\textsuperscript{-1} 3064w, 3032w, 2920w, 2874w, 1731w, 1497w, 1452m, 1369m, 1331w, 1282w, 1214m, 1176m, 1156m, 1076s, 1053s, 1028s, 1001s, 967s, 909m, 745s, 731s, 694s, 654m, 638m, 616m; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta = 7.50-7.20 \) (25H, m, Ph), 5.51 (1H, s, CH-Ph), 4.93 (1H, d, J 11.7 Hz, Bn), 4.92 (1H, d, J 12.2 Hz, Bn), 4.77 (1H, ddd, \( ^{2}J_{HF} \) 52.8, \( ^{3}J_{HF} \) 9.2, 7.6 Hz, H-C2), 4.77 (1H, d, J 7.6, \( ^{3}J_{HF} \) 2.5 Hz, H-C1), 4.75 (1H, d, J 12.1 Hz, Bn), 4.68 (1H, d, J 12.2 Hz, Bn), 4.65 (1H, d, J 12.1 Hz, Bn), 4.60 (1H, d, J 11.7 Hz, Bn), 4.49 (1H, d, J 3.7, H-C1’), 4.34 (1H, d, J 11.8 Hz, Bn), 4.26 (1H, d, J 11.8 Hz, Bn), 4.23 (1H, dd, J 10.0, 4.5 Hz, H-C6’), 4.18 (1H, t, J 9.2 Hz, H-C3’), 3.95 (1H, t, J 3.0 Hz, H-C4), 3.78 (1H, td, J 10.0, 4.5 Hz, H-C5’), 3.70 (1H, t, J 10.0 Hz, H-C6’), 3.66 (1H, t, J 8.5 Hz, H-C6), 3.61-3.55 (3H, m, H-C3, H-C2’, H-C4’), 3.48 (1H, dd, J 8.5, 5.2 Hz, H-C5), 3.39-3.35 (1H, m, H-C6), 3.37 (3H, s, CH\textsubscript{3}); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \( \delta = 138.7 \) (iPh), 138.4 (iPh), 138.1 (iPh), 137.9 (iPh), 137.5 (iPh), 128.7-126.1 (Ph), 101.8 (d, \( ^{3}J_{CF} \) 23.2 Hz, C1), 100.9 (CH-Ph), 99.3 (C1’), 92.1 (d, \( ^{3}J_{CF} \) 184.7 Hz, C2), 80.3 (d, \( ^{3}J_{CF} \) 15.9 Hz, C3), 80.1 (C4’), 79.0 (C2’), 75.8 (C3’), 74.8 (Bn), 74.3 (Bn), 74.3 (d, \( ^{3}J_{CF} \) 9.4 Hz, C4), 73.5 (Bn), 73.2 (C5), 72.6 (d, \( ^{3}J_{CF} \) 1.9 Hz, Bn), 69.0 (C6’), 67.9 (C6), 62.4 (C5’), 55.4 (CH\textsubscript{3}); \textsuperscript{19}F NMR (376 MHz, CDCl\textsubscript{3}) \( \delta = -204.6 \) (dd, \( ^{3}J_{HF} \) 52.8, \( ^{3}J_{HF} \) 12.6); \[m/z\] (ESI) (M+NH\textsubscript{4})\textsuperscript{+} C\textsubscript{48}H\textsubscript{55}FNO\textsubscript{10} requires 824.3805, found 824.3809.

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Glycosylation to form methyl (2-deoxy-3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside 147 (Table 4, Entry 2)

A solution of 104 (29 mg, 50 μmol, 1.0 eq.) and S69 (22 mg, 60 μmol, 1.2 eq.) in dry CH₂Cl₂ (1 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.9 μL, 5 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 12/1 to 7:1) afforded 147α as a white solid (21 mg, 54% over 2 steps, α-anomer).

Rᶠ 0.16 (cyclohexane/EtOAc 3:1); [α]²⁰⁺D +60.6 (c 0.20, CH₂Cl₂); m.p. 129.0-130.2 °C; νmax (neat)/cm⁻¹ 3065w, 3032w, 2909w, 1497w, 1452m, 1386w, 1368m, 1333w, 1311w, 1286w, 1236w, 1208w, 1179w, 1163w, 1133m, 1088s, 1075s, 1062s, 1038s, 1025s, 1005s, 963m, 899m, 875w, 851w, 833w, 775w, 733s, 694s, 656m, 641w, 619w; ¹H NMR (400 MHz, CDCl₃) δ = 7.45-7.25 (25 H, m), 5.56 (1H, d, J 3.1 Hz, H-C1), 5.53 (1H, s, CH-Ph), 4.93 (1 H, d, J 11.5 Hz, Bn), 4.73(1H, d, J 11.5 Hz, Bn), 4.65(1H, d, J 11.5 Hz, Bn), 4.60 (1H, d, J 12.1 Hz, Bn), 4.57 (1H, d, J 12.1 Hz, Bn), 4.55 (1H, d, J 3.1 Hz, H-C1’), 4.50 (1H, d, J 12.1 Hz, Bn), 4.43 (1H, d, J 11.7 Hz, Bn), 4.38 (1H, d, J 11.7 Hz, Bn), 4.28 (1H, d, J 12.1 Hz, H-C3’), 4.28-4.23 (1H, m, H-C5), 4.23 (1H, d, d, J 10.2, 4.8 Hz, H-C6’), 3.97 (1H, br, H-C4), 3.95 (1H, d, d, J 11.9, 4.0 Hz, H-C3), 3.82 (1H, td, J 9.8, 4.8 Hz, H-C5’), 3.72 (1H, d, J 10.2 Hz, H-C6’), 3.69-3.63 (2H, m, 2xH-C6), 3.58 (1H, t, J 9.4 Hz, H-C4’), 3.47 (1H, dd, J 9.4, 3.6 Hz, H-C2’), 3.36 (3H, s, CH₃), 2.24 (1H, td, J 11.9, 3.1 Hz, Hax-C2), 2.06 (1H, dd, J 11.3, 4.0 Hz, Heq-C2); ¹³C NMR (100 MHz, CDCl₃) δ = 139.0 (iPh), 138.7 (iPh), 138.5 (iPh), 138.1 (iPh), 137.2 (iPh), 129.0-126.0 (Ph), 101.3 (CH-Ph), 99.0 (C1’), 97.8 (C1), 83.0 (C4’), 78.1 (C2’), 74.4 (C3), 74.3 (Bn), 73.5 (Bn), 73.1 (Bn), 73.1 (C4), 72.5 (C3’), 70.1 (Bn), 69.5 (C5), 69.1 (C6’), 69.0 (C6), 62.0 (C5’), 55.2 (CH₃), 31.2 (C2); [m/z] (ESI) (M+NH₄)⁺ C₄₈H₆₆NₒO₁₀ requires 806.3904, found 806.3894].
Glycosylation to form methyl (2-deoxy-2-fluoro-3,4,6-tri-O-benzyl-D-galactopyranosyl)-(1→3)-2-deoxy-4,6-O-benzylidene-α-D-glucopyranoside 148 (Table 4, Entry 3)

A solution of 102α (30 mg, 50 μmol, 1.0 eq.) and 4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (172) (16 mg, 60 μmol, 1.2 eq.) in dry CH$_2$Cl$_2$ (1 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.9 μL, 5 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et$_3$N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 4:1) afforded 148 as a white solid (33 mg, > 66%, α:β 1:38).

R$_f$ 0.27 (cyclohexane/EtOAc 3:1); [α]$_D^{20}$ +28.3 (c 0.20, CH$_2$Cl$_2$); m.p. 109.5-109.9 °C; $\nu$$_{max}$ (neat)/cm$^{-1}$ 13032w, 2868w, 1497w, 1452m, 1409w, 1373m, 1308w, 1272w, 1210m, 1159m, 1119s, 1096s, 1076s, 1052s, 1016s, 979s, 912s, 852m, 746s, 732s, 694s, 668m, 654s, 619m; $^1$H NMR (400 MHz, CDCl$_3$) δ = 7.49-7.20 (20H, m, Ph), 5.56 (1H, s, CH-Ph), 4.88 (1H, d, $J_{11.6}$ Hz, Bn), 4.79 (1H, d, $J_{3.8}$ Hz, H-C1'), 4.66 (1H, m, H-C2'), 4.72 (1H, d, $J_{11.9}$ Hz, Bn), 4.65 (1H, d, $J_{11.9}$ Hz, Bn), 4.61-4.57 (1H, m, H-C1), 4.55 (1H, d, $J_{11.6}$ Hz, Bn), 4.30 (1H, d, $J_{11.6}$ Hz, Bn), 4.27 (1H, d, $J_{11.6}$ Hz, Bn), 4.26-4.19 (2H, m, H-C3', H-C6'), 3.90 (1H, t, $J_{2.9}$ Hz, H-C4'), 3.79-3.72 (2H, m, H-C5', H-C6'), 3.68 (1H, t, $J_{9.5}$ Hz, H-C4'), 3.63-3.52 (2H, m, H-C3, H-C6), 3.42 (1H, ddd, $J_{7.5}$, 5.4 Hz, H-C5S), 3.37 (1H, ddd, $J_{8.7}$, 5.4 Hz, H-C6S), 3.33 (3H, s, CH$_3$), 2.30 (1H, dd, $J_{13.5}$, 5.3 Hz, H$_{exo}$C2'), 1.85 (1H, dddd, $J_{13.5}$, 11.1, 3.8 Hz, H$_{exo}$C2'); $^{13}$C NMR (100 MHz, CDCl$_3$) δ = 138.5 (iPh), 138.1 (iPh), 137.81 (iPh), 137.7 (iPh), 128.8-126.1 (Ph), 101.4 (CH-Ph), 100.9 (d, $^2$J$_{CF}$ 24.8 Hz, C1), 99.0 (C1'), 92.2 (d, $^2$J$_{CF}$ 182.5 Hz, C2), 82.3 (C4'), 80.3 (d, $^2$J$_{CF}$ 16.2 Hz, C3), 74.7 (Bn), 74.2 (C3'), 74.0 (d, $^2$J$_{CF}$ 9.0 Hz, C4), 73.5 (Bn), 73.4 (C5), 72.5 (d, $J_{1.9}$ Hz, Bn), 69.1 (C6'), 68.0 (C6), 62.8 (C5'), 54.8 (CH$_3$), 36.1 (C2'); $^{19}$F NMR (376 MHz, CDCl$_3$) δ = -205.1 (m); [m/z] (ESI) (M+NH$_4$)$^+$ C$_{41}$H$_{49}$FNO$_9$ requires 718.3386, found 718.3394.
Glycosylation to form methyl (2-deoxy-3,4,6-tri-O-benzyl-D-galactopyranosyl)-(1→3)-2-deoxy-4,6-O-benzylidene-D-glucopyranoside 149

A solution of 104 (29 mg, 50 μmol, 1.0 eq.) and 4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (172) (16 mg, 60 μmol, 1.2 eq.) in dry CH₂Cl₂ (1 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.9 μL, 5 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 7:1) afforded 149α as a white solid (16 mg, 47% over 2 steps, α-anomer).

Rf 0.42 (cyclohexane/EtOAc 5:3); [α]D²⁰ +67.3 (c 0.20, CH₂Cl₂); m.p. 115.5-118.7 °C; νmax (neat)/cm⁻¹ 3034w, 2906w, 2860w, 1497w, 1468w, 1454m, 1386w, 1357m, 1347m, 1271w, 1209m, 1191w, 1149m, 1122m, 1094s, 1056s, 1043s, 1026s, 989s, 971s, 921m, 907m, 875m, 854m, 830w, 804w, 785m, 744s, 734s, 696s, 668m, 654m, 630m; ¹H NMR (400 MHz, CDCl₃) δ = 7.49-7.25 (20H, m, Ph), 5.60 (1H, s, CH-Ph), 5.39 (1H, d, J 3.2 Hz, H-C1), 4.95 (1H, d, J 11.8 Hz, 2xBn), 4.77 (1H, d, J 3.6 Hz, H-C1'), 4.64 (1H, d, J 12.8 Hz, Bn), 4.63 (2H, s, Bn), 4.56 (1H, d, J 11.7 Hz, Bn), 4.46 (1H, d, J 11.7 Hz, Bn), 4.27 (2H, m, H-C6', H-C3'), 3.96 (3H, m, H-C3, H-C4, H-C5), 3.86-3.80 (1H, td, J 9.8, 4.6 Hz, H-C5'), 3.77 (1H, t, J 9.5 Hz, H-C6'), 3.33 (3H, s, CH₃), 2.26-2.18 (2H, m, Hax-C2', Heq-C2), 2.06 (1H, dd, J 12.4, 4.8 Hz, Hax-C2), 1.79 (1H, ddd, J 13.5, 11.4, 3.6 Hz, Hax-C2'); ¹³C NMR (100 MHz, CDCl₃) δ = 138.9 (iPh), 138.6 (iPh), 138.3 (iPh), 137.6 (iPh), 128.9 (Ph), 128.4-126.0 (Ph), 101.34 (C-Ph), 99.1 (C1'), 98.9 (C1), 83.5 (C4'), 74.8 (C3), 74.3 (Bn), 73.6 (Bn), 73.1 (C4), 70.5 (C3), 70.4 (Bn), 70.3 (C5), 69.8 (C6), 69.2 (C6'), 62.8 (C5'), 54.7 (CH₃), 36.9 (C2'), 31.2 (C2); [m/z] (ESI) (M+NH₄)⁺ C₄₁H₅₀NO₉ requires 700.3480, found 700.3485.
Glycosylation to form 2-deoxy-2-fluoro-3,4,6-tri-O-benzyl-\(\beta\)-galactopyranosyl-(1→3)-1,2,5,6-di-O-isopropylidene-\(\alpha\)-\(\delta\)-allofuranose 150 (Table 4, Entry 4)

A solution of 102\(\alpha\) (30 mg, 50 \(\mu\)mol, 1.0 eq.) and 1,2,5,6-di-O-isopropylidene-\(\alpha\)-\(\delta\)-allofuranose (16 mg, 60 \(\mu\)mol, 1.2 eq.) in dry CH\(_2\)Cl\(_2\) (1 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.9 \(\mu\)L, 5 \(\mu\)mol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et\(_3\)N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO\(_2\), Cyclohexane/EtOAc 7:1 and toluene/acetone 19:1) afforded 150 as a colorless oil (33 mg, > 66%, \(\alpha:\beta\) 1:44).

\(R_f\) 0.27 (cyclohexane/EtOAc 3:1); \([\alpha]^D_{20}\) +10.6 (c 0.21, CH\(_2\)Cl\(_2\)); \(v_{\text{max}}\) (neat)/cm\(^{-1}\) 3030w, 2987w, 2936w, 2878w, 1497w, 1455m, 1371m, 1306m, 1256m, 1213m, 1160m, 1066s, 1024s, 912w, 887m, 848m, 804w, 750s, 734s, 696s, 667m, 632m; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 7.35-7.27\) (15H, m, Ph), 5.92 (1H, d, \(J = 3.8\) Hz, H-C1'), 4.92 (1H, d, \(J = 11.6\) Hz, Bn), 4.75 (1H, d, \(J = 12.1\) Hz, Bn), 4.65 (1H, ddd, \(J_{HF} = 51.4, J = 9.5, 7.5\) Hz, H-C2), 4.67 (1H, d, \(J = 12.1\) Hz, Bn), 4.59 (1H, d, \(J = 11.6\) Hz, Bn), 4.55 (1H, d, \(J = 3.8\) Hz, H-C2'), 4.52 (1H, dd, \(J = 7.5, J_{HF} = 5.0\) Hz, H-C1), 4.46 (1H, d, \(J = 12.1\) Hz, Bn), 4.43 (1H, d, \(J = 12.1\) Hz, Bn), 4.39 (1H, m, H-C5'), 4.30-4.28 (2H, m, H-C3', H-C4'), 4.05 (1H, dd, \(J = 8.5, 6.4\) Hz, H-C6'), 3.94 (1H, t, \(J = 3.1\) Hz, H-C4), 3.62-3.54 (4H, m, H-C3, H-C5, 2xH-C6); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta = 138.32\) (iPh), 137.78 (iPh), 137.72 (iPh), 128.5-127.6 (Ph), 111.9 (Cq), 105.5 (Cq), 105.2 (Cq), 100.0 (d, \(J_{CF} = 24.0\) Hz, C1), 91.7 (d, \(J_{CF} = 183.1\) Hz, C2), 83.1 (C2'), 81.1-80.5 (C3', C4'), 80.1 (d, \(J_{CF} = 15.9\) Hz, C3), 74.8 (Bn), 74.1 (d, \(J_{CF} = 9.6\) Hz, C4), 74.0 (C5), 76.60 (Bn), 73.1 (C5'), 72.7 (d, \(J_{CF} = 1.8\) Hz, Bn), 68.1 (C6), 66.2 (C6'), 26.8 (Ch3), 26.5 (Ch3), 26.3 (Ch3), 25.4 (Ch3); \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) \(\delta = -205.01\) to -205.20 (dd, \(J_{HF} = 51.4, J_{HF} = 12.7\) Hz); \([m/z]\) (ESI) (M+NH\(_4\))\(^+\) C\(_{39}\)H\(_{51}\)FNO\(_{10}\) requires 712.3492, found 712.3485.
Glycosylation to form 2-deoxy-3,4,6-tri-O-benzyl-D-galactopyranosyl-(1→3)-1,2,5,6-di-O-isopropylidene-α-D-allofuranose 151 (Table 4, Entry 4)

A solution of 104 (29 mg, 50 μmol, 1.0 eq.) and 1,2,5,6-di-O-isopropylidene-α-D-allofuranose (16 mg, 60 μmol, 1.2 eq.) in dry CH₂Cl₂ (1 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.9 μL, 5 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 7:1 to 3:1) afforded 151α as a colorless oil (17 mg, 51% over 2 steps, α-anomer).

R_f 0.41 (cyclohexane/EtOAc 3:1); [α]_D^20 +38.6 (c 0.19, CH₂Cl₂); ν_{max} (neat)/cm⁻¹ 2987w, 2934w, 1733w, 1497w, 1454m, 1372m, 1253s, 1163m, 1130s, 1090s, 1060s, 1023s, 964m, 912s, 884m, 845m, 789w, 734s, 696s, 642m, 615m; ¹H NMR (400 MHz, CDCl₃) δ = 7.36-7.24 (15H, m, Ph), 5.83 (1H, d, J 3.5 Hz, H-C1’), 5.25 (1H, d, J 3.7 Hz, H-C1), 4.94 (1H, d, J 11.7 Hz, Bn), 4.69 (1H, d, J 3.5 Hz, H-C2’), 4.62 (1H, d, J 12.1 Hz, Bn), 4.61 (1H, d, J 11.7 Hz, Bn), 4.59 (1H, d, J 12.1 Hz, Bn), 4.53 (1H, d, J 11.8 Hz, Bn), 4.44 (1H, d, J 11.8 Hz, Bn), 4.22 (1H, d, J 2.9 Hz, H-C3’), 4.17 (1H, dt, J 8.2, 5.8 Hz, H-C5’), 4.05-3.94 (2H, m, H-C6’, H-C4’), 3.96 (1H, dd, J 8.6, 5.7 Hz, H-C6’), 3.92 (1H, m, H-C5), 3.90 (1H, br, H-C4), 3.86 (1H, ddd, J 12.2, 4.5, 2.4 Hz, H-C3), 3.62 (1H, dd, J 9.5, 6.2 Hz, H-C6), 3.61 (1H, dd, J 9.5, 6.1 Hz, H-C5), 2.24 (1H, td, J 12.5, 3.7 Hz, H₃-C2’), 2.00 (1H, dd, J 12.7, 4.5 Hz, H₃-C2), 1.47 (3H, s, CH₃), 1.40 (3H, s, CH₃), 1.33 (3H, s, CH₃), 1.20 (3H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ = 138.7 (iPh), 138.3 (iPh), 138.0 (iPh), 128.5-127.3 (Ph), 111.8 (C₉), 109.1 (C₆), 105.3 (C₁’), 99.5 (C₁), 83.5 (C₂’), 81.3 (C₄’), 80.9 (C₃’), 74.4 (C₃), 74.3 (Bn), 73.6 (Bn), 73.0 (C₄), 72.6 (C₅’), 71.0 (C₅), 70.5 (Bn), 70.0 (C₆), 67.6 (C₆’), 31.0 (C₂), 26.9 (CH₃), 26.8 (CH₃), 26.1 (CH₃), 25.4 (CH₃); [m/z] (ESI) (M+Na)^+ C₃₉H₄₅NaO₁₀ requires 699.3140, found 699.3145.
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**Synthesis of the glycosyl acceptor methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside 134**

![Chemical Structure]

To a solution of 142 (402 mg, 1.42 mmol, 1 eq.) in DMF (10 mL, 0.2 M) under an atmosphere of Ar at 0 °C, NaH [60% in oil (w/w), 172 mg, 4.3 mmol, 3 eq.] was added portionwise over a period of 15 min before benzyl bromide (515 μL, 4.3 mL, 3 eq.) was added. The reaction was warmed to r.t. and stirred for 16 h. The reaction mixture was quenched by the addition of MeOH (10 mL), stirred for 5 min and evaporated to dryness. The residue was diluted with CH₂Cl₂ (30 mL), washed with H₂O (2x30 mL) and the aqueous layers were back-extracted with CH₂Cl₂. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 7:1) afforded 143 as a white solid (632 mg, 96%) identical to known material (210).

\[ R_f 0.19 \text{ (Cyclohexane/EtOAc 7:1), } [\alpha]^{20}_D +15.4 \text{ (c 2.2, CH₂Cl₂) [lit. } [\alpha]^{20}_D +20.0 \text{ (c 0.885, CH₂Cl₂), (211)]; m.p. 93.0-95.0^\circ C \text{ (lit. m.p. 98-99 °C); } \]

**Synthesis of methyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside 143**

![Chemical Structure]

To a solution of 142 (402 mg, 1.42 mmol, 1 eq.) in DMF (10 mL, 0.2 M) under an atmosphere of Ar at 0 °C, NaH [60% in oil (w/w), 172 mg, 4.3 mmol, 3 eq.] was added portionwise over a period of 15 min before benzyl bromide (515 μL, 4.3 mL, 3 eq.) was added. The reaction was warmed to r.t. and stirred for 16 h. The reaction mixture was quenched by the addition of MeOH (10 mL), stirred for 5 min and evaporated to dryness. The residue was diluted with CH₂Cl₂ (30 mL), washed with H₂O (2x30 mL) and the aqueous layers were back-extracted with CH₂Cl₂. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 7:1) afforded 143 as a white solid (632 mg, 96%) identical to known material (210).

\[ R_f 0.19 \text{ (Cyclohexane/EtOAc 7:1), } [\alpha]^{20}_D +15.4 \text{ (c 2.2, CH₂Cl₂) [lit. } [\alpha]^{20}_D +20.0 \text{ (c 0.885, CH₂Cl₂), (211)]; m.p. 93.0-95.0^\circ C \text{ (lit. m.p. 98-99 °C); } \]

**Synthesis of methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside 134**

![Chemical Structure]

To a solution of 143 (116 mg, 0.25 mmol, 1.0 eq.) and NaBH₃CN (79 mg, 1.25 mmol, 5.0 eq.) in dry acetonitrile (2.5 mL, 0.1 M) at r.t. under an atmosphere of Ar, I₂ (222 mg, 0.88 mmol, 3.5 eq.) was added portionwise over a period of 15 min and the reaction was stirred an additional 30 min. The reaction mixture was diluted with CH₂Cl₂ and filtered through a pad of celite. The organic phase was washed with a sat. aq. solution of Na₂CO₃ and the aq. phase was back-extracted with CH₂Cl₂. The
combined organic phases were dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 4:1) afforded **134** as a colorless oil (45 mg, 38%) identical to known material (194).

$R_f$ 0.22 (cyclohexane/EtOAc 3:1); $[\alpha]_D^{20} +42.7$ (c 0.21, CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 7.38-7.27 (15H, m, Ph), 4.78 (1H, d, $J$ 12.1 Hz, Bn), 4.55 (1H, d, $J$ 12.2 Hz, Bn), 3.79 (1H, t, $J$ 9.1 Hz, H-C3), 3.74-3.67 (3 H, m, H-C5, 2xH-C6), 3.61 (1H, dt, $J$ 9.1, 2.1 Hz, H-C4), 3.54 (1H, dd, $J$ 3.6 Hz, H-C1), 3.47 (1H, d, $J$ 12.1 Hz, Bn), 3.39 (3H, s, s, CH$_3$), 2.33 (1H, d, $J$ 9.1 Hz, H-C4), 3.04-2.95 (4H, m, H-C4, H-C4', H-C3', H-C6').

**Glycosylation to form methyl (2-deoxy-2-fluoro-3,4,6-tri-O-benzyl-o-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside 152 (Table 4, Entry 5)**

A solution of **102α** (21 mg, 34.5 μmol, 1.0 eq.) and **134** (20 mg, 42 μmol, 1.2 eq.) in dry CH$_2$Cl$_2$ (0.5 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.6 μL, 3.5 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et$_3$N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO$_2$, cyclohexane/EtOAc 7:1) afforded **152α** as a white solid (7 mg, 22%, α-anomer) and **152β** as a thick oil (8.6 mg, 27%, β-anomer).

**152β**: $R_f$ 0.38 (cyclohexane/EtOAc 3:1); $[\alpha]_D^{20} +26.3$ (c 0.2, CH$_2$Cl$_2$); $\nu_{max}$ (neat)/cm$^{-1}$ 3031w, 2923w, 2868w, 1731w, 1497w, 1454m, 1366m, 1308w, 1261w, 1208w, 1173m, 1153m, 1045s, 1026s, 911m, 804m, 732s, 695s; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 7.36-7.17 (30H, m, Ph), 4.94 (1H, d, $J$ 11.0 Hz, Bn), 4.91 (1H, d, $J$ 11.3 Hz, Bn), 4.81 (1H, d, $J$ 12.2 Hz, Bn), 4.69 (1H, d, $J$ 12.1 Hz, Bn), 4.61 (1H, ddd, $J_{HF}$ 51.6, 7.6 Hz, H-C2), 4.59 (1H, d, $J$ 11.7 Hz, Bn), 4.53 (1H, d, $J$ 11.3 Hz, Bn), 4.47 (1H, dd, $J$ 7.6, 3.1 Hz, H-C1'), 4.32 (1H, d, $J$ 11.7 Hz, Bn), 4.19 (1H, d, $J$ 11.7 Hz, Bn), 3.92-3.84 (4H, m, H-C4, H-C4', H-C3', H-C6'), 2.77-2.75 (1H, m, H-C5'), 3.67 (1H, d, $J$ 10.6, 1.6 Hz, H-C6'), 3.51-3.46 (2H, m, H-C2', H-C6), 3.38 (1H, ddd, $J_{HF}$ 12.5 J 9.4, 3.1 Hz, H-C3), 3.36 (3H, s, CH$_3$), 3.28 (1H, dd, $J$ 12.1 Hz, H-C5), 3.20 (1H, dd, $J$ 8.7, 4.9 Hz, H-C6); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 139.5 (iPh), 138.7 (iPh), 138.3 (iPh), 138.1 (iPh), 138.0 (iPh), 128.5-127.0 (Ph), 101.1 (d, $J_{CF}$ 22.9 Hz, C1), 98.3 (C1), 92.6 (d, $J_{CF}$ 181.8 Hz, C2), 80.3 (C3'), 80.1 (d, $J_{CF}$ 15.7 Hz, C3), 79.3 (C2'), 77.5
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(C4'), 75.22 (Bn), 74.9 (Bn), 74.2 (d, 3JCF 8.7 Hz, C4), 73.6 (Bn), 73.4 (2xBn), 73.2 (C5), 72.4 (Bn), 69.7 (C5'), 68.1 (d, 2JCF 8.7 Hz, C5'), 67.6 (C6), 55.2 (CH3); 19F NMR (376 MHz, CDCl3) δ = -203.95 to -204.14 (d, 2JFH 51.6 Hz); [m/z (ESI) (M+NH4)+] C55H63FNO10 requires 916.4431, found 916.4441.

152α: Rf 0.38 (cyclohexane/EtOAc 3/1); [α]D20 +64.7 (c 0.1, CH2Cl2); m.p. 98.7-102.1 °C; 1H NMR (400 MHz, CDCl3) δ = 7.42-7.23 (30H, m, Ph), 5.76 (1H, d, J 4.3 Hz, H-C1), 4.94 (1H, ddd, 2JCF 50.4, J 10.0, 4.3 Hz, H-C2), 4.92 (1H, d, J 11.4 Hz, Bn), 4.81 (1H, d, J 10.3 Hz, Bn), 4.81 (1H, d, J 11.6 Hz, Bn), 4.80 (1H, d, J 12.1 Hz, Bn), 4.69 (1H, d, J 1.6 Hz, Bn), 4.66 (1H, d, J 12.1 Hz, Bn), 4.61 (1H, d, J 3.6 Hz, H-C1'), 4.57 (1H, d, J 11.4 Hz, Bn), 4.55 (1H, d, J 12.1 Hz, Bn), 4.43 (1H, d, J 12.1 Hz, Bn), 4.35 (1H, d, J 11.7 Hz, Bn), 4.29 (1H, d, J 11.7 Hz, Bn), 4.02-4.00 (1H, m, H-C3'), 3.96-3.87 (3H, m, H-C4, H-C3, H-C5), 3.85-3.79 (3H, m, H-C5', H-C4'), 3.72-3.66 (2H, m, 2xH-C6'), 3.56 (1H, dd, J 9.7, 3.6 Hz, H-C2'), 3.49-3.38 (2H, m, 2xH-C6'), 3.42 (3H, s, CH3); 13C NMR (100 MHz, CDCl3) δ = 138.6 (iPh), 138.4 (iPh), 138.3 (iPh), 138.1 (iPh), 137.9 (iPh), 128.5-127.4 (Ph), 97.9 (C1'), 97.4 (d, 3JCF 20.9 Hz, C1), 88.7 (d, 1JCF 189.0 Hz, C2), 81.6 (C3'), 80.2 (C2'), 77.2 (m, C3), 77.5 (C4'), 75.3 (d, 3JCF 8.7 Hz, C4), 75.2 (d, 4JCF 3.2 Hz, Bn), 75.0 (Bn), 73.5 (Bn), 73.4 (Bn), 73.0 (Bn), 72.9 (d, J 2.2 Hz, Bn), 70.0 (C5), 69.6 (C5', C6'), 68.6 (C6), 55.3 (CH3); 19F NMR (376 MHz, CDCl3) δ = -206.1 (ddd, 2JFH 51.6, 9.4, 4.0 Hz); [m/z (ESI) (M+NH4)+] C55H63FNO10 requires 916.4431, found 916.4434.

Glycosylation to form methyl (2-deoxy-3,4,6-tri-O-benzyl-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside 153 (Table 4, Entry 5)

A solution of 104 (16 mg, 28 μmol, 1.0 eq.) and S77 (16 mg, 34 μmol, 1.2 eq.) in dry CH2Cl2 (420 μL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.5 μL, 3 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et3N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO2, cyclohexane/EtOAc 9:1 to 7:1) afforded 153α a colorless oil (12 mg, 46% over 2 steps, α-anomer) identical to known material (189).

152α: Rf 0.38 (cyclohexane/EtOAc 3/1); [α]D20 +64.7 (c 0.1, CH2Cl2); m.p. 98.7-102.1 °C; 1H NMR (400 MHz, CDCl3) δ = 7.42-7.23 (30H, m, Ph), 5.76 (1H, d, J 4.3 Hz, H-C1), 4.94 (1H, ddd, 2JCF 50.4, J 10.0, 4.3 Hz, H-C2), 5.00(1H, d, J 10.3 Hz, Bn), 4.92 (1H, d, J 11.4 Hz, Bn), 4.81 (1H, d, J 10.3 Hz, Bn), 4.81 (1H, d, J 11.6 Hz, Bn), 4.80 (1H, d, J 12.1 Hz, Bn), 4.69 (1H, d, J 1.6 Hz, Bn), 4.66 (1H, d, J 12.1 Hz, Bn), 4.61 (1H, d, J 3.6 Hz, H-C1'), 4.57 (1H, d, J 11.4 Hz, Bn), 4.55 (1H, d, J 12.1 Hz, Bn), 4.43 (1H, d, J 12.1 Hz, Bn), 4.35 (1H, d, J 11.7 Hz, Bn), 4.29 (1H, d, J 11.7 Hz, Bn), 4.02-4.00 (1H, m, H-C3'), 3.96-3.87 (3H, m, H-C4, H-C3, H-C5), 3.85-3.79 (3H, m, H-C5', H-C4'), 3.72-3.66 (2H, m, 2xH-C6'), 3.56 (1H, dd, J 9.7, 3.6 Hz, H-C2'), 3.49-3.38 (2H, m, 2xH-C6'), 3.42 (3H, s, CH3); 13C NMR (100 MHz, CDCl3) δ = 138.6 (iPh), 138.4 (iPh), 138.3 (iPh), 138.1 (iPh), 137.9 (iPh), 128.5-127.4 (Ph), 97.9 (C1'), 97.4 (d, 3JCF 20.9 Hz, C1), 88.7 (d, 1JCF 189.0 Hz, C2), 81.6 (C3'), 80.2 (C2'), 77.2 (m, C3), 77.5 (C4'), 75.3 (d, 3JCF 8.7 Hz, C4), 75.2 (d, 4JCF 3.2 Hz, Bn), 75.0 (Bn), 73.5 (Bn), 73.4 (Bn), 73.0 (Bn), 72.9 (d, J 2.2 Hz, Bn), 70.0 (C5), 69.6 (C5', C6'), 68.6 (C6), 55.3 (CH3); 19F NMR (376 MHz, CDCl3) δ = -206.1 (ddd, 2JFH 51.6, 9.4, 4.0 Hz); [m/z (ESI) (M+NH4)+] C55H63FNO10 requires 916.4431, found 916.4434.
5. Experimental Part

\[ J \text{ 11.7 Hz, Bn), 4.37 (1H, d, } J \text{ 12.0 Hz, Bn), 4.32 (1H, d, } J \text{ 12.0 Hz, Bn), 3.88 (1H, t, } J \text{ 9.6 Hz, H-C3'), 3.85 (1H, br, H-C4), 3.85-3.82 (1H, m, H-C5), 3.80 (1H, ddd, } J \text{ 12.4, 4.5, 2.4 Hz, H-C3), 3.75-3.60 (4H, m, H-C5', H-C4', 2xH-C6'), 3.51 (1H, dd, J 9.6, 3.6 Hz, H-C2'), 3.49 (1H, dd, J 9.3, 6.9 Hz, H-C6), 3.46 (1H, dd, J 9.3, 6.0 Hz, H-C6), 3.40 (3H, s, CH}_3, 2.24 (1H, td, J 12.4, 3.7 Hz, H_{ax-C2}), 1.88 (1H, dd, J 12.4, 4.5 Hz, H_{eq-C2}). \]

Synthesis of the substrates methyl 2,3,4-tri-O-benzyl-\( \alpha \)-D-glucopyranoside 135

A solution of 143 (115 mg, 0.25 mmol, 1.0 eq.) in dry CH\(_2\)Cl\(_2\) (2.5 mL, 0.1 M) under an atmosphere of Ar was treated at r.t. with a solution of BH\(_3\) in THF (1 M, 1.25 mL, 1.25 mmol, 5.0 eq.) and TMSOTf (7 μL, 0.04 mmol, 0.2 eq.) and stirred for 2.5 h. The reaction mixture was neutralised with Et\(_3\)N (250 μL), and MeOH was added until gas evolution stopped. The mixture was concentrated \textit{in vacuo} and the crude material was co-evaporated with MeOH. Purification by flash column chromatography (SiO\(_2\), cyclohexane/EtOAc 3:1) afforded 135 as a colorless oil (96 mg, 83%) identical to known material (194).

R\(_f\) 0.19 (cyclohexane/EtOAc 3:1); \([\alpha]^{20}_D +47.8 \ (c \ 0.14, \text{CH}_2\text{Cl}_2); \text{ }^1\text{H NMR} \ (400 \text{ MHz, CDCl}_3) \delta = 7.37-7.27 \ (15H, m, Ph), 4.99 \ (1H, d, J 10.9 Hz, Bn), 4.88 \ (1H, d, J 11.0 Hz, Bn), 4.84 \ (1H, d, J 10.9 Hz, Bn), 4.81 \ (1H, d, J 12.2 Hz, Bn), 4.67 \ (1H, d, J 12.2 Hz, Bn), 4.64 \ (1H, d, J 11.0 Hz, Bn), 4.57 \ (1H, d, J 3.6 Hz, H-C1), 4.01 \ (1H, d, J 9.3 Hz, H-C3), 3.77 \ (1H, ddd, J 11.7, 5.1, 2.6 Hz, H-C6), 3.70 \ (1H, ddd, J 11.7, 7.1, 3.8 Hz, H-C6), 3.66 \ (1H, ddd, J 9.5, 3.8, 2.6 Hz, H-C5), 4.52 \ (1H, dd, J 9.5, 9.3 Hz, H-C4), 3.50 \ (1H, dd, J 9.3, 3.6 Hz, H-C2), 3.37 \ (3H, s, CH\(_3\)).

Glycosylation to form methyl (2-deoxy-2-fluoro-3,4,6-tri-O-benzyl-\( \alpha \)-D-galactopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-\( \alpha \)-D-glucopyranoside 154 (Table 4, Entry 6)

A solution of 102α (21 mg, 34.5 μmol, 1.0 eq.) and 135 (20 mg, 42 μmol, 1.2 eq.) in dry CH\(_2\)Cl\(_2\) (0.5 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.6 μL, 3.5 μmol, 0.1 eq.) at -
5. Experimental Part

78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 7:1) afforded 154β as a white solid (7 mg, 58%, mainly β).

Rf 0.36 (cyclohexane/EtOAc 3:1); [α]D²⁰ +26.9 (c 0.21, CH₂Cl₂); m.p. 101.7-104.0 °C; νmax (neat)/cm⁻¹ 3064w, 3031w, 2914w, 1731w, 1497m, 1454m, 1391m, 1327w, 1286w, 1257w, 1212w, 1195s, 1174m, 1133m, 1061s, 1044s, 1027s, 997s, 910 m, 821w, 733s, 694s, 627m, 612m; ¹H NMR (400 MHz, CDCl₃) δ = 7.36-7.24 (30H, m, Ph), 4.98 (1H, d, J 11.0 Hz, Bn), 4.91 (1H, d, J 11.2 Hz, Bn), 4.89 (1H, d, J 11.2 Hz, Bn), 4.82 (1H, d, J 11.0 Hz, Bn), 4.74 (1H, ddd, JHF 51.7, J 9.2, 7.6 Hz, H-C2), 4.79 (1H, d, J 12.8 Hz, Bn), 4.76 (1H, d, J 12.1 Hz, Bn), 4.67 (1H, d, J 12.1 Hz, Bn), 4.65 (1H, d, J 12.8 Hz, Bn), 4.62 (1H, d, J 11.4 Hz, Bn), 4.60 (1H, d, J 3.4 Hz, H-C1'), 4.55 (1H, d, J 11.4 Hz, Bn), 4.44 (1H, d, J 11.8 Hz, Bn), 4.40 (1H, d, J 11.8 Hz, Bn), 4.36 (1H, dd, J 7.6, JHF 4.1 Hz, H-C1), 4.08 (1H, dd, J 10.7, 1.4 Hz, H-C6'), 3.98 (1H, t, J 9.2 Hz, H-C3'), 3.92 (1H, t, J 3.1 Hz, H-C4), 3.78 (1H, dd, J 10.0, 4.3 Hz, H-C5'), 3.71 (1H, dd, J 10.7, 4.3 Hz, H-C6'), 3.61 (1H, t, J 10.0 Hz, H-C4'), 3.60-3.35 (5H, m, 2xH-C6, H-C3, H-C5, H-C2'), 3.35 (3H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ = 139.9 (iPh), 138.6 (iPh), 138.4 (iPh), 138.2 (iPh), 138.0 (iPh), 137.8 (iPh), 128.5-127.6 (Ph), 101.5 (d, JCF 23.7 Hz, C1), 98.1 (C1'), 91.5 (d, JCF 183.3 Hz, C2), 82.2 (C3'), 80.2 (d, JCF 16.0 Hz, C3), 79.8 (C5), 77.6 (C4'), 75.7 (Bn), 74.9 (Bn), 74.8 (Bn), 74.3 (d, JCF 8.9 Hz, C4), 73.6 (Bn, C2'), 72.4 (Bn), 72.6 (Bn), 69.9 (C5'), 68.3 (C6), 68.2 (C6'), 55.1 (CH₃); ¹⁹F NMR (376 MHz, CDCl₃) δ = -204.8 (dd, JHF 51.7, JHF 12.7 Hz); [m/z] (ESI) (M+NH₄)+ C₅₅H₆₃FNO₁₀ requires 916.4431, found 916.4424.

Glycosylation to form methyl (2-deoxy-3,4,6-tri-O-benzyl-D-galactopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-α-D-glucopyranoside 155 (Table 4, Entry 6)

A solution of 104 (29 mg, 50 μmol, 1.0 eq.) and 135 (28 mg, 60 μmol, 1.2 eq.) in dry CH₂Cl₂ (1.0 mL, 0.05 M) under an atmosphere of Ar was treated with TMSOTf (0.9 μL, 5 μmol, 0.1 eq.) at -78 °C and stirred for 2 h at that temperature. The reaction mixture was quenched by the addition of Et₃N (0.1 mL) and concentrated in vacuo. Purification by flash column chromatography (SiO₂, cyclohexane/EtOAc 7:1 and CH₂Cl/MeOH 40:1) afforded 155 as a white solid (24 mg, 54% over 2 steps, α:β 1:2.4).
Experimental Part

\( R_f 0.29 \) \( \alpha \)-anomer, 0.27 \( \beta \)-anomer (cyclohexane/EtOAc 3:1); \( \nu_{\max} \) (neat)/cm\(^{-1}\) 3029w, 2910w, 1496m, 1453m, 1358m, 1328w, 1209w, 1168m, 1084s, 1059s, 1045s, 1027s, 909m, 821w, 733s, 694s, 644m, 615m; \( ^{1}H \) NMR (400 MHz, CDCl\(_3\)) \( \delta = \) 7.36-7.22 (30H, m, Ph), 5.03 (1H, dd, J 3.4 Hz, H-C1\( \alpha \)), 4.99 (1H, d, J 10.8 Hz, Bn\( \alpha \)), 4.98 (2.4H, d, J 10.7 Hz, Bn\( \beta \)), 4.92 (3.4H, d, J 11.7 Hz, Bn\( \alpha \), Bn\( \beta \)), 4.86 (2.4H, d, J 11.3 Hz, Bn\( \beta \)), 4.85 (1H, d, J 10.8 Hz, Bn\( \alpha \)), 4.82-4.77 (6.8H, m, 2xBn\( \alpha \), 2xBn\( \beta \)), 4.69 (1H, d, J 12.0 Hz, Bn\( \alpha \)), 4.66 (2.4H, d, J 12.0 Hz, Bn\( \beta \)), 4.62-4.51 (17H, m, H-C1’\( \beta \), H-C1’\( \alpha \), 4xBn\( \alpha \), 4xBn\( \beta \)), 4.44 (2.4H, d, J 11.7 Hz, Bn\( \beta \)), 4.42 (1H, d, J 11.7 Hz, Bn\( \alpha \)), 4.39 (2.4H, d, J 11.7 Hz, Bn\( \beta \)), 4.35 (1H, d, J 11.7 Hz, Bn\( \alpha \)), 4.32 (2.4H, dd, J 9.9, 1.9 Hz, H-C1\( \beta \)), 4.07 (2.4H, dd, J 10.8, 2.1 Hz, H-C6’\( \beta \)), 3.99 (1H, t, J 9.2 Hz, H-C3’\( \alpha \)), 3.98 (2.4H, t, J 9.2 Hz, H-C3’\( \beta \)), 3.89-3.85 (3H, m, H-C3\( \alpha \), H-C4\( \beta \), H-C5\( \alpha \)), 3.82 (1H, dd, J 10.2, 4.9 Hz, H-C6’\( \alpha \)), 3.81 (2.4H, br, H-C4\( \beta \)), 3.77 (2.4H, dd, J 10.2, 4.9, 1.6 Hz, H-C5’\( \beta \)), 3.75-3.70 (1H, m, H-C5’\( \alpha \)), 3.65 (2.4H, dd, J 9.3, 7.5 Hz, H-C6\( \beta \)), 3.63 (1H, m, H-C6’\( \beta \)), 3.58-3.45 (16H, m, H-C6’\( \beta \), H-C6\( \beta \), 2xH-C6\( \alpha \), H-C2’\( \beta \), H-C3’\( \beta \), H-C4’\( \beta \), H-C4’\( \alpha \)), 3.41 (2.4H, dd, J 7.5, 5.5 Hz, H-C5\( \beta \)), 3.35 (7.2H, s, CH\( _3 \)\( \beta \)), 3.35 (3H, s, CH\( _3 \)\( \alpha \)), 2.21 (1H, td, J 12.7, 3.6 Hz, H\textsubscript{ax}-C2\( \alpha \)), 2.11 (2.7H, td, J 12.0, 9.9 Hz, H\textsubscript{ax}-C2\( \beta \)), 2.02 (1H, dd, J 12.7, 4.2 Hz, H\textsubscript{eq}-C2\( \alpha \)), 1.94 (2.7H, d, J 12.0 Hz, H\textsubscript{eq}-C2\( \beta \)); \( ^{13}C \) NMR (100 MHz, CDCl\(_3\)) \( \delta = \) 140.0 (iPh\( \beta \)), 138.9 (iPh\( \alpha \)), 138.83 (iPh\( \beta \)), 138.76 (iPh\( \alpha \)), 138.5 (iPh\( \beta \)), 138.4 (iPh\( \alpha \)), 138.32 (iPh\( \beta \)), 138.27 (iPh\( \alpha \)), 138.18 (iPh\( \beta \), iPh\( \alpha \)), 138.16 (iPh\( \alpha \)), 138.0 (iPh\( \beta \)), 128.4-127.3 (Ph), 100.8 (C1\( \beta \)), 98.3 (C1\( \alpha \)), 97.9 (C1’\( \beta \)), 97.9 (C1’\( \alpha \)), 82.2 (C3’\( \beta \)), 82.1 (C3’\( \alpha \)), 80.0 (C2’\( \alpha \)), 79.9 (C2’\( \beta \)), 77.9 (C4’\( \alpha \)), 77.8 (C3\( \beta \)), 77.2 (C4’\( \beta \)), 75.8 (Bn\( \alpha \)), 75.8 (Bn\( \beta \)), 76.0 (Bn\( \alpha \)), 74.8 (Bn\( \beta \)), 74.3 (Bn\( \alpha \)), 74.2 (Bn\( \beta \), C3\( \alpha \)), 74.1 (C5\( \beta \)), 73.5 (Bn\( \beta \)), 73.4 (Bn\( \beta \), Bn\( \alpha \)), 73.3 (Bn\( \alpha \)), 72.9 (C4\( \alpha \)), 71.8 (C4\( \beta \)), 70.2 (Bn\( \alpha \)), 70.2 (Bn\( \beta \)), 70.1 (C5\( \alpha \)), 69.8 (C5’\( \alpha \)), 69.8 (C5’\( \beta \)), 69.4 (C6\( \alpha \)), 69.1 (C6\( \beta \)), 67.4 (C6’\( \beta \)), 66.0 (C6’\( \alpha \)), 55.1 (CH\( _3 \)\( \beta \)), 55.0 (CH\( _3 \)\( \alpha \)), 32.5 (C2\( \beta \)), 31.0 (C2\( \alpha \)); \( m/z \) (ESI) (M+NH\(_4^+\)) \( C_{55}H_{64}NO_{10} \) requires 898.4525, found 898.4517.
6. References


6. References


6. References


6. References


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6. References


6. References


6. References


Curriculum Vitae

PROFESSIONAL EXPERIENCE

2010-2015  ETH Zurich  Zurich, Switzerland
Pharmaceutical Sciences Institute, Drug formulation and delivery, Leroux group
Project: Drug development of Clostridium difficile infection based on inositol phosphate/sulfate hybrids
  • Overcome synthetic challenges owing to peculiar physicochemical properties of inositol phosphate
  • Development of a chiral resolution methodology for the synthesis of inositol phosphates
  • Scale-up synthesis of the inositol phosphate candidate for in vivo studies

Organic Chemistry Laboratory, Gilmour group
Project: Fluorinated Carbohydrates Synthesis
  • Investigation on the role of fluorine in stereoselectivity of glycosylation reaction: achievement of an excellent β-stereoselectivity
  • Stabilization of dioxocarbenium ion with weakly coordinating anion in the goal of studying glycosylation reaction intermediate (oxocarbenium ion)

2009  Novartis, R&D, Chemistry Development  Basel, Switzerland
Project: Process Development of “Groebke-Blackburn-Bienaymé” multicomponent
  • Development of a scalable multicomponent process under cGMP used for drug development project
  • Investigation of scopes and limits of the process

2009  Ludwig-Maximilians University, Knochel group  Munich, Germany
Project: Studies towards the total synthesis of Frondosin B via cupration and a key Heck coupling

2008  sanofi-aventis, R&D, Internal Medicine  NJ, USA
Project: Synthesis of heterocyclic amine derivatives for therapeutic targets

2007  sanofi-aventis, R&D, Oncology  Paris, France
Project: Synthesis of platinium complexes for cancer therapy

2005  sanofi-aventis, R&D, Oncology  Budapest, Hungary
Project: Synthesis of heterocyclic amine derivatives for oncology therapeutic targets

EDUCATION

2010 – 2014  PhD in Chemistry, ETH Zurich, Prof. Leroux and Prof. Gilmour
Fellowships: Novartis and Swiss National Science Foundation
Title: “Chemical Synthesis of Biomolecules Analogs: Inositol Phosphate/Sulfate Hybrids & Fluorinated Carbohydrates”, Diss.-No. ETH 22532

2008 – 2009  Master in Chemistry, National Postgraduate School of Chemistry, École Nationale Supérieure de Chimie (ENSCM), Montpellier (France)
Specialized in Biomolecular Chemistry

2005 – 2009  Engineer in Chemistry National Postgraduate School of Chemistry, Ecole Nationale Supérieure de Chimie (ENSCM), Montpellier (France)
Specialized in Organic Chemistry

2003 – 2005  Technical University diploma in Chemistry (DUT), University of Montpellier II (France)

2003  Advanced Level in Science (Baccalauréat), Lycée J. Renou, La Réole (France)
**Skills**

- **Technical Skills**
  - **Expertise in organic synthesis**
    - Handling air and moisture sensitive chemicals, Schlenk technique, glovebox, solvent degassing, ...
  - **Expertise in purification techniques**
    - Chromatography (silica, reverse phase, size exclusion, ion exchange, high pressure liquid chromatography), distillation, precipitation, crystallization
  - **Expertise in analytical techniques**
    - NMR, MS, IR, X-Ray crystallography

- **Interpersonal Skills**
  - **Management**
    - Project management: planning, risk evaluation, monitor/control progress, analysis
    - Management of Master student projects
    - Responsible for laboratory safety and organization
  - **Communication**
    - Presenting Sciences and writing scientific report
    - Collaboration in multidisciplinary project and group
  - **Language**
    - French mother tongue
    - English fluent
    - Spanish proficient
    - German beginner

**Bibliographical References**


Manuscripts in preparation
- *Therapeutic Potential of Triggering Pre-Emptive* *Clostridium difficile* *Toxin B Auto-Proteolysis*, M. E. Ivansson, E. Durantie, C. Huberli, S. Huwiler, J. Lu, E. F. Verdu, P. Bercik, J.-C. Leroux, B. Castagner