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PhD Thesis

STEREOSELECTIVE SYNTHESIS OF GLYCOSYL AMIDES BY TRACELESS STAUDINGER LIGATION OF GLYCOSYL AZIDES

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Chapter 1

Introduction

Traceless Staudinger ligation: synthesis of glycosyl amides

Aim of the work

1 Glycopeptides and glycoproteins

Glycoproteins are fundamental to many important biological processes including fertilisation, immune defence, viral replication, parasitic infection, cell growth, cell-cell adhesion, degradation of blood clots, inflammation and protein folding, stability and solubility.[1] The carbohydrate moiety of a glycoprotein may participate directly in recognition events, but it may also modify the properties of the protein. The large size of the carbohydrates is probably the most significant factor in modifying the properties of the proteins to which they are attached. Glycoproteins and glycolipids are major components of the outer surface of mammalian cells. Oligosaccharide structures change dramatically during development, and it has been shown that specific sets of oligosaccharides are expressed at distinct stages of differentiation. Furthermore, alterations in cell surface oligosaccharides are associated with various pathological conditions including malignant transformation.

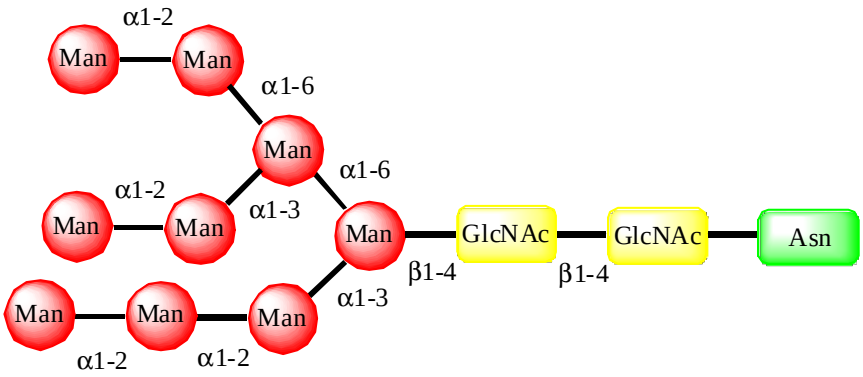
At each glycosylation site on a protein, there can be a set of glycosylated structures. This has led to the concept of a glycoprotein being defined as a set of glycoforms which all have the same amino acids but differ in the sequence or position of the attached sugars. It is the populations in this set of glycoforms that change under a variety of conditions such as disease.

There is no single function for oligosaccharides. Perhaps their major function is to serve as recognition markers. Additionally, oligosaccharides can modify the intrinsic properties of proteins to which they are attached by altering the stability, protease resistance, or quaternary structure. The large size of oligosaccharides may allow them to cover functionally important areas of proteins, to modulate the interactions of glycoconjugates with other molecules, and to affect the rate of processes which involve conformational changes. Glycosylation is highly sensitive to alterations in cellular function, and abnormal glycosylation is diagnostic of a number of disease states including rheumatoid arthritis and cancer. The control of glycosylation by the cell affords, in principle, a means of putting the same recognition markers on quite different proteins without having to code the information into the DNA of that protein. Site-specific glycosylation of a protein also suggests that the 3D structure of the protein plays a role in determining the extent and type of its own glycosylation.

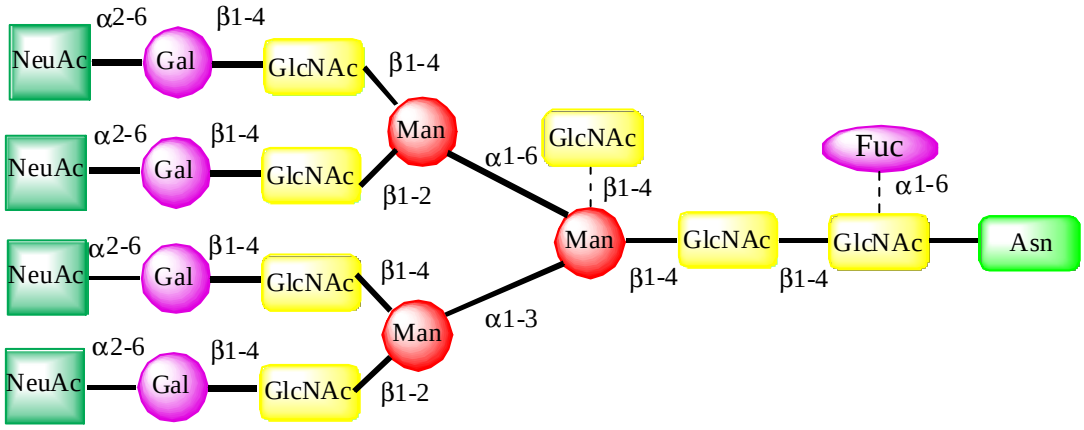
More than 50 % of proteins in humans are glycosylated. Also less complex species such bacteria as *E. coli* have this modification machinery. In mammals, the saccharide residues found in proteins are covalently linked to the protein backbone either *N*- (*via* asparagine) or *O*-glycosidically (*via* serine, threonine, tyrosine or hydroxylysine).

1.1 *N*-Linked glycoproteins

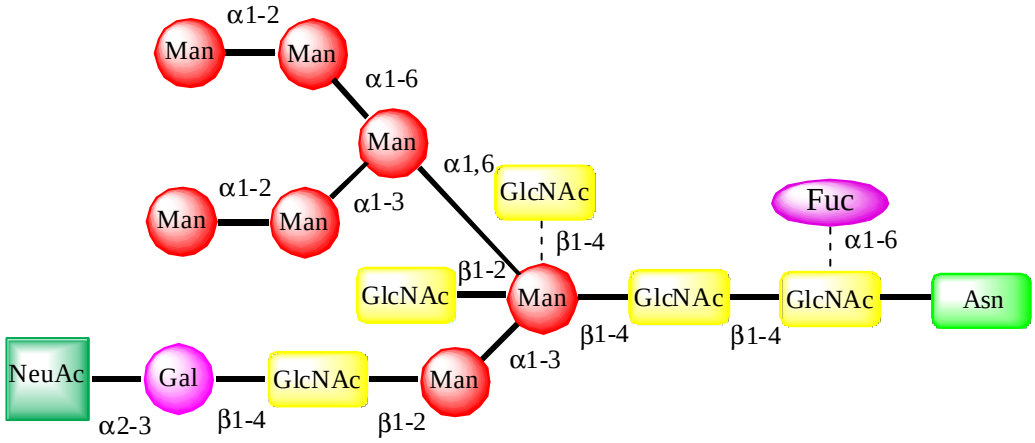
To be glycosylated, an asparagine residue must form part of the tripeptide Asn-Xaa-Ser/Thr (where Xaa is any amino acid other than proline), although the presence of this sequon is not in itself sufficient to ensure glycosylation.



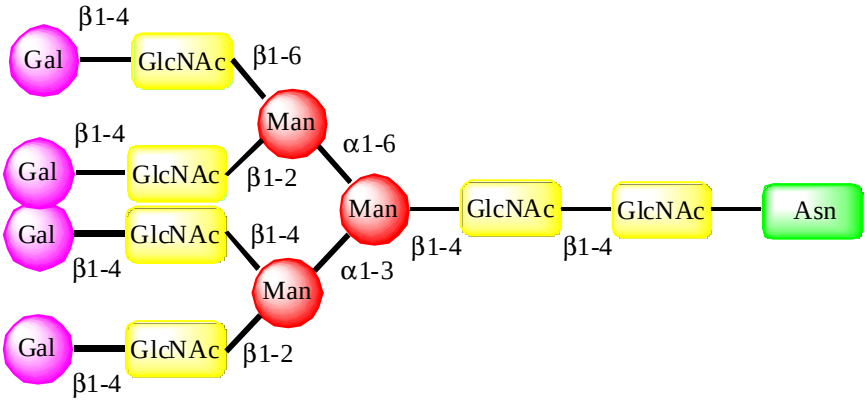
high mannose (a)



complex (b)



hybrid (c)



poly-*N*-acetyllactosamine-type (d)

Figure 1. Classification of *N*-linked oligosaccharides

Some rules have emerged with respect to the factors which control the attachment of oligosaccharides to potential glycosylation sites and the subsequent enzymatic modifications of the glycan chains. While the potential oligosaccharide processing pathways available to a nascent protein are dictated by the cell in which it is expressed, its final glycosylation pattern is also the result of constraints imposed by the 3D structure of the individual protein.

All *N*-linked glycans contain the pentasaccharide Manα1-6-(Manα1-3)-Manβ1-4-GlcNAcβ1-4-GlcNAc as a common core. On the basis of the structure and the location of glycan residues added to the trimannosyl core, *N*-linked oligosaccharides can be classified into four main subgroups:[2] high mannose-type (a), complex-type (b), hybrid-type (c), and poly-*N*-acetyllactosamine-type (d) (Figure 1). High mannose-type glycans (a) contain only α-mannosyl residues attached to the trimannosyl core. Complex-type glycans (b) contain no mannose residues other than those in the trimannosyl core, but have branches with *N*-acetylglucosamine residues at their reducing termini attached to the core. The number of branches normally ranges from two (biantennary) to four (tetraantennary), but a pentaantennary structure has been reported in hen ovomuvoid.[2b] While various monosaccharides can be found in the antennae, the presence or absence of fucose and a bisecting GlcNAc residue on the core contributes to the enormous structural variation of complex-type glycans. The hybrid-type *N*-glycans (c) have the characteristic features of both complex-type and high mannose-type glycans. The fourth group is the poly-*N*-acetyllactosamine *N*-glycans containing repeating units of (Galβ1-4-GlcNAcβ1-3-) attached to the core. These repeats are not necessarily uniformly distributed on the different

antennae and the lactosamine repeat may also be branched. Poly-*N*-acetyllactosamine extensions are most frequently found in tetraantennary glycans.[3]

Although the same glycosylation machinery is available to all the proteins which are translated in a particular cell and use the secretory pathway, it has been estimated that between 10 % and 30 % of potential glycosylation sites are not occupied.[4] Moreover site analysis has shown that the distribution of different classes of *N*-linked oligosaccharide structures is frequently specific for each site on a protein. The 3D structure of the individual protein clearly has a role in determining the type and extent of its glycosylation. A number of mechanisms may be involved. These include the following:

1) The position of the glycosylation site in the protein. *N*-Linked sites at the exposed turns of β-pleated sheets, which are sometimes close to proline residues, are normally occupied while those near the C-terminus are more often vacant.

2) Access to the glycosylation site on the developing oligosaccharide. This may be sterically hindered by the local protein structure or by protein folding which may compete with the initiation of *N*-glycosylation.

3) Interaction of the developing oligosaccharide with the protein surface. This may result in a glycan conformation which may alter the accessibility to specific glycosyltransferases or glycosidases.

4) Interaction of the glycosyl enzymes with the protein structure. This can lead to site-specific processing.

5) Glycosylation at one site in a multiglycosylated protein. This may sterically hinder events at a second site on the same molecule.

6) The interaction of protein subunits to form oligomers. This may prevent glycosylation or restrict the glycoforms at individual sites.

1.2 Biosynthesis of *N*-linked glycoproteins

Many enzymes and proteins are involved in the posttranslational modification of proteins. Their effects often fundamentally alter protein function. Posttranslational modifications create a dynamic combinatorial library of properties that can rapidly respond to systemic stimuli, starting from one basic protein scaffold. *N*-linked protein glycosylation is the most widespread and complex posttranslational modification of secretory proteins in eukaryotes. The dynamic complexity of these modifications is often difficult to elucidate in the laboratory. Working out

the role of each protein component (sometimes very minor but important) requires abundant sources and extensive purification. Furthermore, to continue to precisely exploit the power of proteins in therapeutics requires the creation of pure protein drugs (most today are sold as mixtures). A better understanding of the mechanism by which modifications are formed coupled with methods for creating artificial posttranslational modification mimics may provide a solution to both of these problems. Chemical probes and inhibitors of glycoprocessing enzymes are especially useful in this context.[5]

Aberrant glycosylation occurs in essentially all types of experimental and human cancers, as has been observed for over 40 years, and many glycosyl epitopes constitute tumour-associated antigens.[6] A long-standing debate is whether aberrant glycosylation is a result or a cause of cancer. Many recent studies indicate that some, if not all, aberrant glycosylation is a result of initial oncogenic transformation, as well as a key event in induction of invasion and metastasis. Glycosylation promoting or inhibiting tumour cell invasion and metastasis is of crucial importance in current cancer research. Glycosylation appears to be considered “in the shade” of more popular topics such as oncogenes and antioncogenes, apoptosis, angiogenesis, growth factor receptors, integrin and caderin function, etc., despite the fact that aberrant glycosylation profoundly affects all of these processes.

1.3 *N*-Linked glycopeptides with different linkage

It is now well appreciated that the modification of proteins with carbohydrates plays a very important role in many biological events. For example, the carbohydrate moieties of glycoproteins can be involved in cell-cell communication, immune response, cell adhesion, intracellular targeting, protease resistance, and many other processes.[7] The carbohydrates can also impact several physicochemical properties of proteins including hydration, hydrophilicity, and conformational stability.[8] Additionally, because *N*-linked glycosylation occurs cotranslationally during biosynthesis on the ribosome, the attachment of the carbohydrates may affect the protein folding pathway. Detailed studies of the impact of carbohydrates on glycoprotein structure have been limited due to the flexibility of the carbohydrates, which complicates crystallographic analyses.

Imperiali and co-workers have recently demonstrated by NMR and Molecular Dynamics studies that the stereochemistry of the carbohydrate-peptide linkage has critical and unique conformational effects on the glycopeptide structure.[9] The two *N*-linked glycopeptides **A** and **B** shown in Figure 2 were employed for these conformational studies.

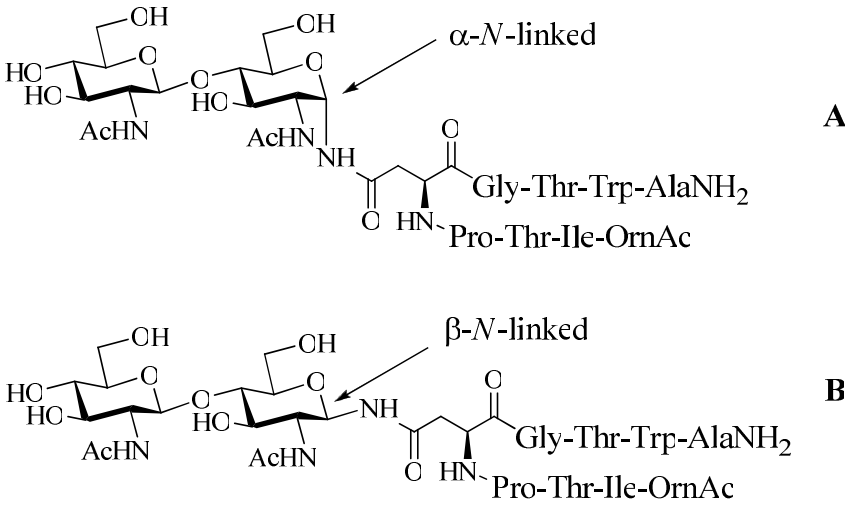


Figure 2. Structure of glycopeptides **A** and **B** [9]

The NMR studies revealed that the stereochemistry at the anomeric center of the *N*-linked carbohydrate dramatically affects the backbone conformation of the glycopeptide, and, indeed, only the β-linked glycopeptide adopts a compact β-turn conformation, while the conformation of the α-glycopeptide is more similar to that of the unglycosylated peptide, featuring an Asx-turn structure (Figure 3).

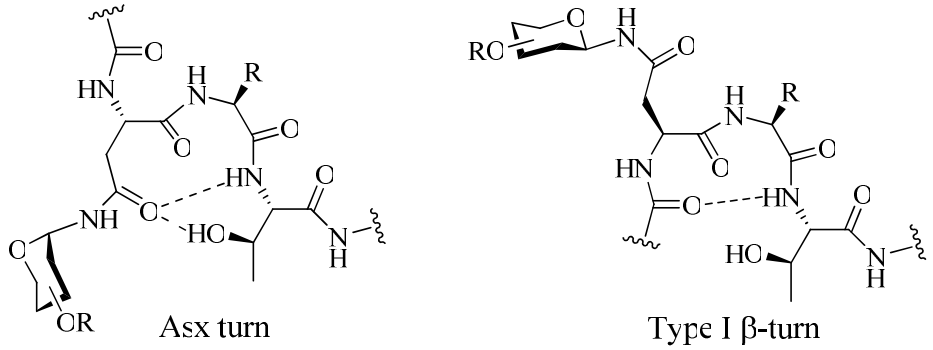
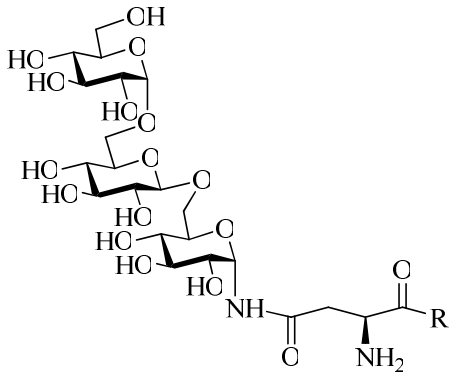


Figure 3. Structure of Asx and Type I β-turn

The conformational consequences of the stereochemistry of the anomeric center in the carbohydrate-peptide linkage reported, provide valuable information for the design of neoglycopeptides and glycopeptide mimetics that may be useful as therapeutic agents.

Nephritogenoside (structure represented in Figure 4) is a glycopeptide isolated as a minor component in the basement membrane of normal animals. This substance is able to induce

glomerulonephritis in homologous animals. The peptide portion is directly and α-*N*-glycosidically bound to the trisaccharide portion (Glcα1-6-Glcβ1-6-Glcα1-Asn) *via* the *N*-terminal amino acid Asn. Nephritogenoside represents the first and so far only example, among natural compounds, of a carbohydrate-peptide linkage having a direct α-*N*-glycosidic linkage.[10]



R = -NH-P-L-F-G-I-A-G-E-D-G-P-T-G-P-S-G-I-V-G-Q-OH

Figure 4. The structure of nephritogenoside

1.4 Neo-glycoconjugates and glycopeptide mimics

The data described so far show how glycoproteins and glycolipids play a central role in human health and disease. *N*-Glycosyl amides are currently under intense scrutiny as potential effectors of carbohydrate-binding proteins.[11] Their mimetics therefore, are interesting candidates to develop drugs and biological probes, useful to clarify the biological roles of these molecules. A major synthetic effort is under way to synthesize so-called “neo-glycoconjugates” where a sugar ring is connected through a N atom to a carbon chain, often to a natural amino acid, using an unnatural linkage.

For instance, Dondoni and co-workers have introduced 1,2,3-triazolo-linked conjugates, exemplified by the structures shown in Figure 5 (1,6)-α-D-oligomannoses (triazolomannoses, Figure 5).[12,13] The choice of mannose as the sugar fragment in these oligomers was suggested by their potential use as mimetics of the manno-oligosaccharide family members which constitute the essential substructure of mycobacteria lipoglycans.

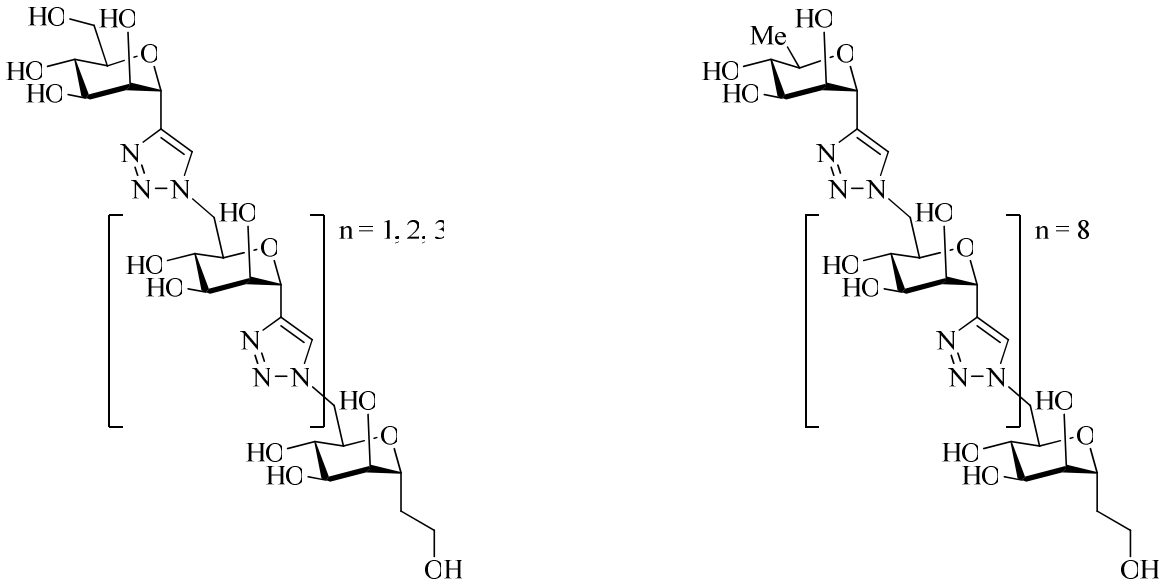


Figure 5. Structures of triazolomannoses synthesized by the Dondoni group[12, 13]

High stability can be foreseen for these oligomers owing to the resistance of anomeric carbon-carbon bond and triazole ring toward chemical and enzymatic degradation.[14] On the other hand, triazole rings can participate in hydrogen bonding and dipole interactions, thereby favoring molecular recognition process and improving solubility. In particular these features were designed as inhibitors of *Mycobacterium tuberculosis* cell-wall biosynthesis.

Glycosyl ureas are found in nature as structural unit of glycocinnamoylspermidine antibiotics.[15, 16] They have been used as stable *N*-linked-glycopeptide mimics,[17] for the synthesis of polyvalent glycoconjugates[18] and for the development of antidiabetic agents and aminoglycoside antibiotics.[19, 20] However, only a few methods for the synthesis of glycosyl ureas have been reported,[21, 22, 23, 24, 25] and in particular, the stereoselective synthesis of α-glycosyl ureas (Figure 6) is still limited.[17b, 21d, 26, 27]

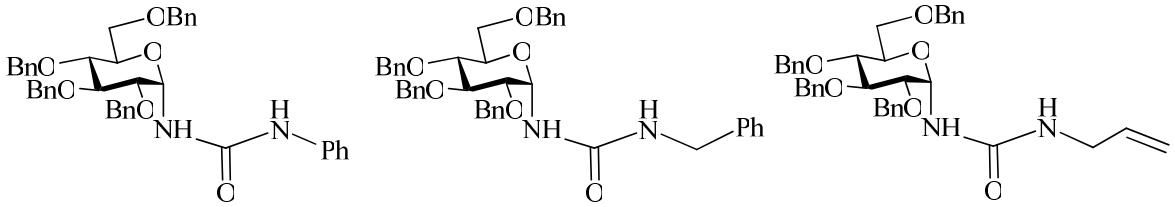


Figure 6. α-glycosyl ureas described from our laboratory (*ref. 26*)

As seen above, natural *N*-linked glycopeptides are almost invariably β-linked,[10] hence, it is plausible that the unnatural, α-linked isomers may be stable to hydrolytic enzymes and may be used for in vivo applications. For these reasons stereoselective synthesis of neo-glycoconjugates in the "unnatural" α *N*-linked configuration is of great interest as a means of designing glycopeptide mimics with altered metabolic stability and virtually unexplored physico-chemical properties. Our laboratory has been actively exploring the synthesis and biological applications of such unnatural glycoconjugate.

A small group of α-fucosyl amides (structure represented in Figure 7) have been tested for their affinity for the carbohydrate recognition domain (CRD) of DC-SIGN [28] and for the PA-II lectin.[29] DC-SIGN is a dendritic cell receptor with mannose and fucose specificities, which has been implicated in the onset of HIV infection. It was brought to the attention of the scientific community by the group of van Kooyk, who reported that HIV-1 targets DC-SIGN but escapes degradation in lytic compartments, and thus uses DCs as a Trojan horse to invade the host organism.[30] After this discovery, it was shown by several groups that many pathogens are recognized by DC-SIGN; this indicates that this lectin could participate in some way during the corresponding infection process.[31] Furthermore, as the detailed molecular mechanisms by which this receptor operates are not known, effective modulators of DC-SIGN are needed to help clarify the different biological processes in which it can be involved. Compound **A** in Figure 7 was the first reported fucose-based glycomimetics to interact with DC-SIGN with an affinity similar to that of the natural DC-SIGN fucose-based ligand, the Lewis-x trisaccharide.[28]

PA-II lectin is a fucose selective lectin from *Pseudomonas aeruginosa*, involved in the formation and stabilization of microbial biofilms.[32] This soluble bacterial lectin binds with an unusually strong micromolar affinity to L-fucose in a tight binding site which requires two Ca^{2+} ions.[33] Compounds **B**-**F** in Figure 7 were found to bind to the lectin in the micromolar range.[29]

Collectively the compounds shown in Figure 7 constitute proof of principle that a α-glycosyl amides can perform as effective mimics of α-fucosides also in high affinity, tight binding proteins such as PA-II lectin.

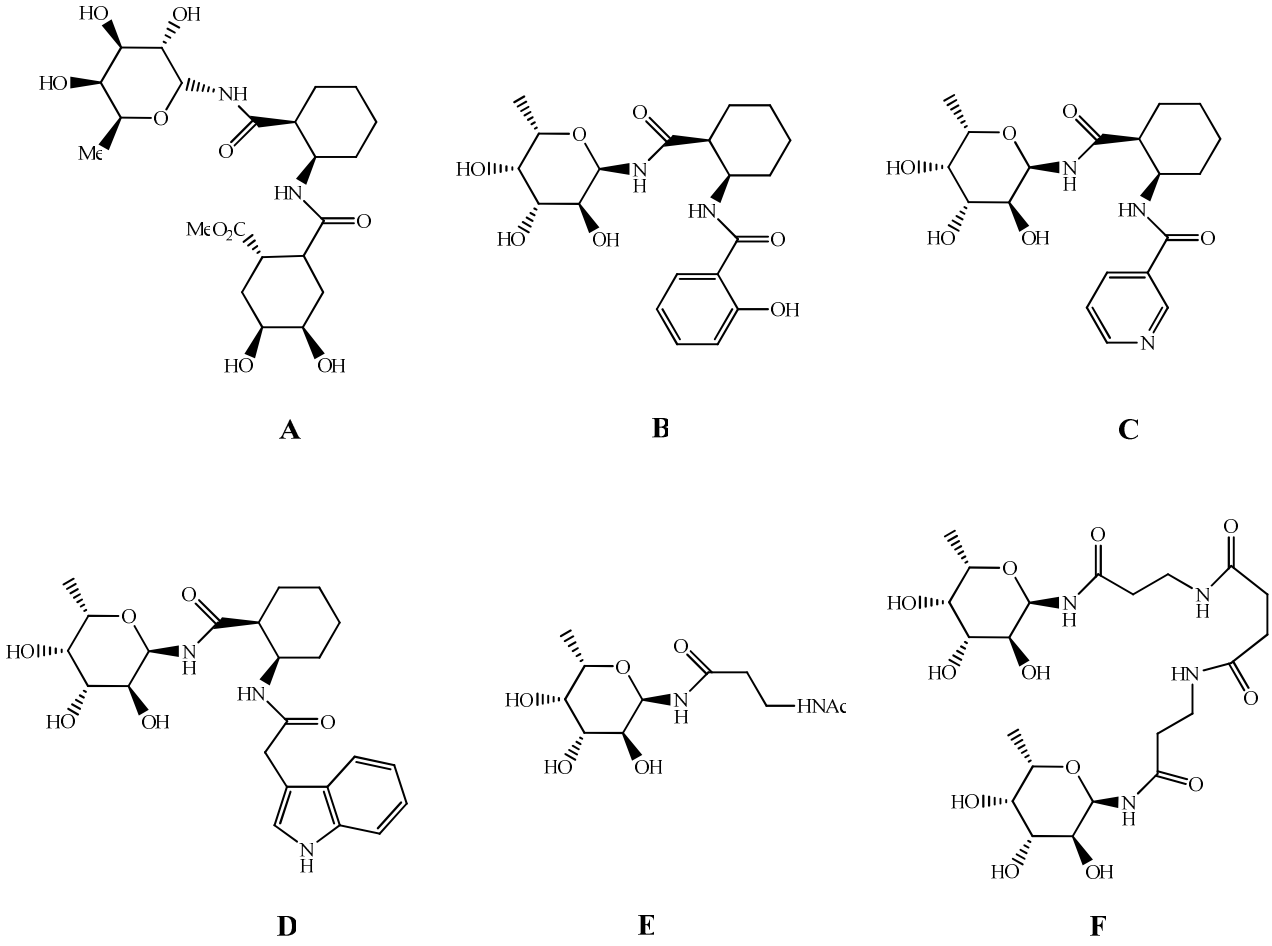


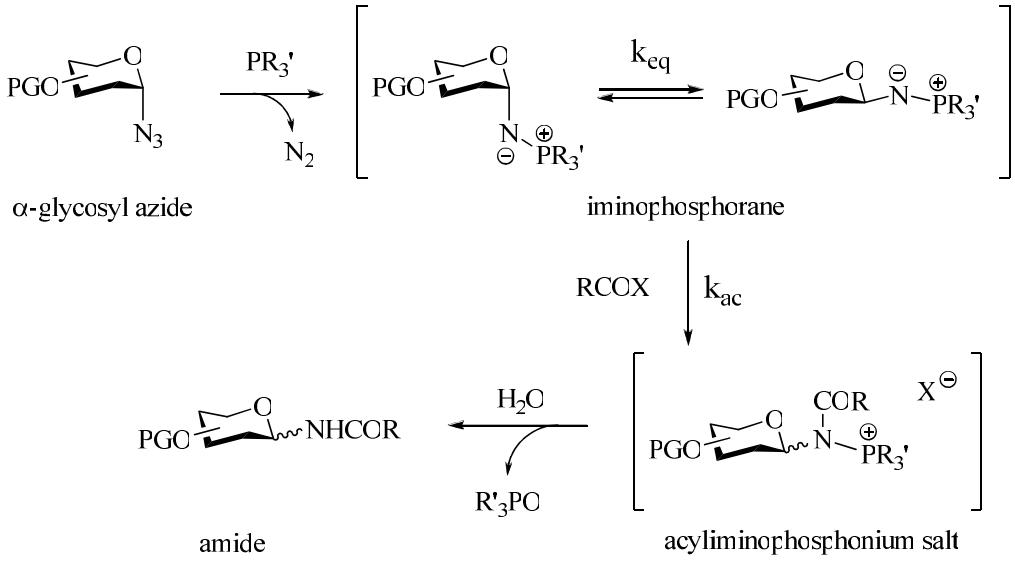
Figure 7. Small library of α-fucosyl amides with affinity for DC-SIGN CRD (**A**) [28] or for PA-II lectin (**B**-**F**) [29]

1.5 Traceless Staudinger ligation of glycosyl azides: stereoselective synthesis of glycosyl amides

The most widely employed method for the synthesis of glycosyl amides is the condensation of protected or unprotected glycosylamines [34, 35] with carboxylic acid derivatives. Several examples of the reduction of glycosyl azides by catalytic hydrogenation followed by acylation of the resulting glycosylamines have been reported.[36, 37, 38] Because glycosylamines rapidly equilibrate to the most stable β-anomer, all the approaches that make use of isolated amine intermediates afford β-glycosyl amides. An alternative methodology attempts to avoid anomeric equilibration by reducing glycosyl azides in the presence of acylating agents.[39, 40, 41, 42, 43]

The group of Györgydeàk investigated in detail the stereochemical course of the reduction-acylation of glycosyl azides by the Staudinger reaction [44] to establish whether α- and β-glycosyl amides can be derived in a stereoselective fashion from the corresponding azides.[45]

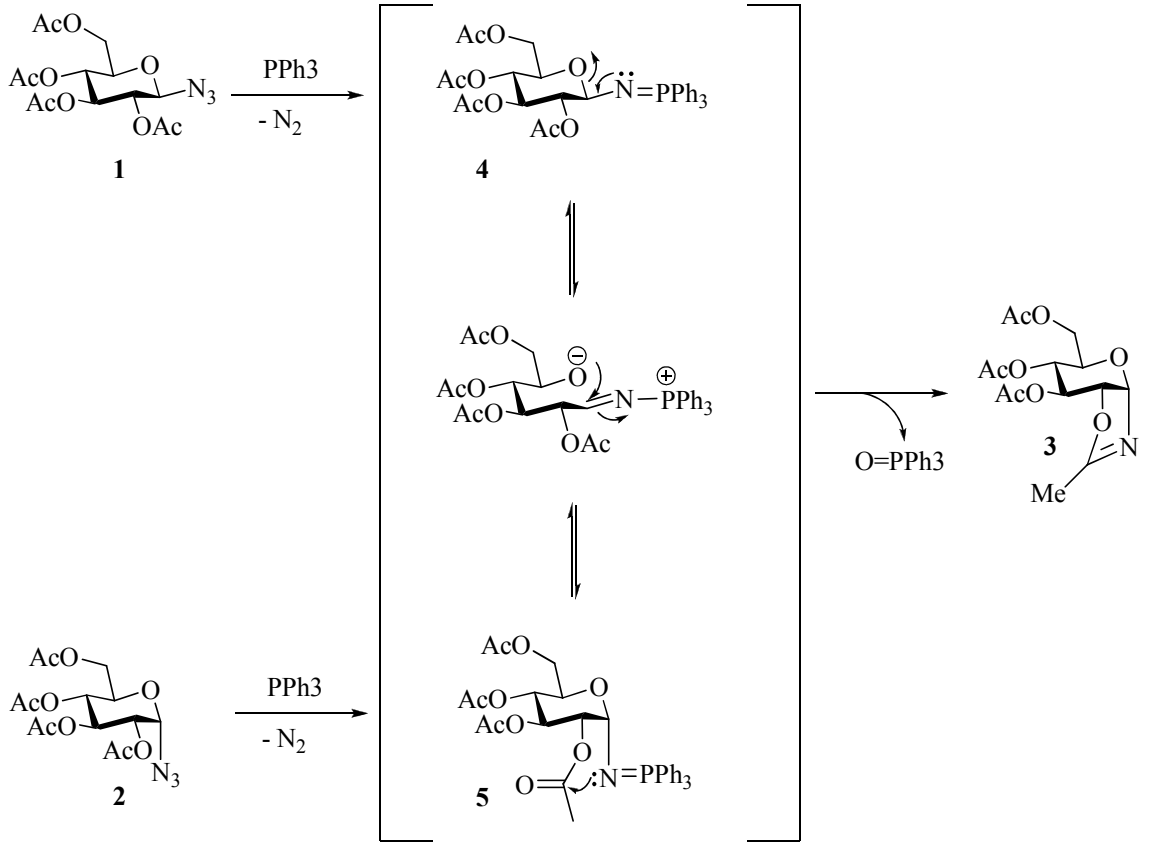
The Staudinger reduction of glycosyl azides affords aza-ylide intermediates (also called iminophosphoranes; Scheme 1), which can be trapped by acylating agents to give configurationally stable acylamino phosphonium salts that, in turn, yield the corresponding amides upon quenching. However, like glycosylamines, the Staudinger's aza-ylides are also subject to anomeric isomerization, which is biased toward the β-anomers. Thus, the synthesis of β-glycosyl amides can be easily achieved in this process, but in most cases, anomerization remains a significant problem during the synthesis of α-glycosyl amides.



Scheme 1. Mechanism of the Staudinger reduction-acylation of the glycosyl azides

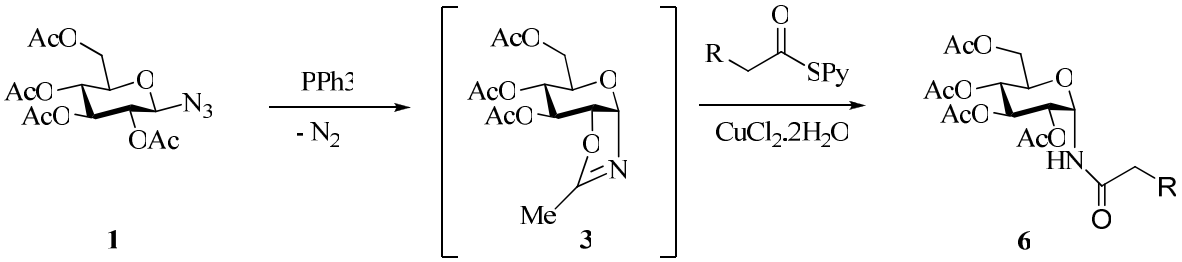
Only a handful of methods have been reported to afford α-glycosyl amides, most of which require two steps and have been described for a limited number of substrates.[46, 47, 48]

Two main synthetic approaches are available for the synthesis of α-glycosyl amides, one, reported by DeShong and coworkers[48] starts from α or β-glucopyranosyl azides **1** and **2** by treatment with PPh_3 in refluxing 1,2-dichloroethane gave oxazoline **3** (Scheme 2). Formation of oxazoline **3** from either azides can be explained by the mechanism shown in Scheme 1 involving α/β anomerization of the intermediate iminophosphorane **4** and **5**.[49, 50] Oxazoline formation from **4** cannot occur due to strain in the resulting product. Accordingly, epimerization followed by cyclization gives exclusively α-oxazoline **3**.



Scheme 2. Mechanism for the formation of isoxazoline **3**

Acylation of oxazoline **3** (formed in situ) with a thiopyridyl ester in the presence of $CuCl_2.2H_2O$ gave exclusively the α-glucopyranosyl amide **6** in a highly stereoselective process (Scheme 3).



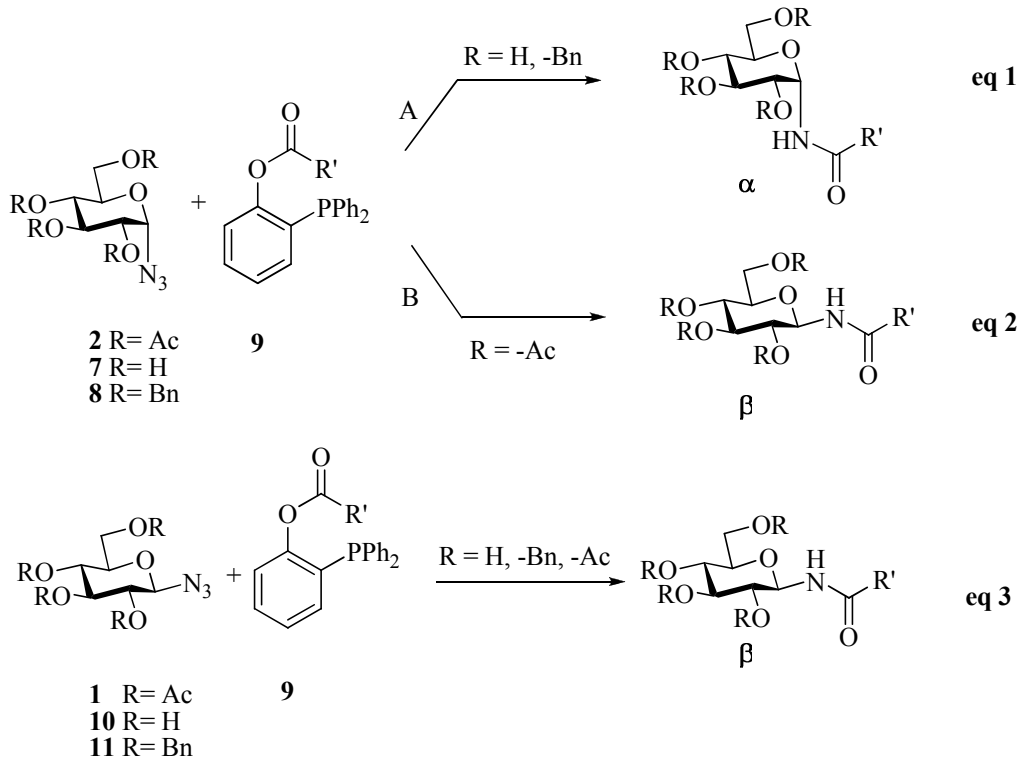
Sceme 3. Coupling reaction of glucopyranosyl isoxazoline **3**

The second method, developed primarily by our group, [47] is based on the traceless Staudinger ligation of glycosyl azides, using functionalized phosphines **9** (Figure 4) described originally by Bertozzi [51] and Kiessling.[52]

The traceless Staudinger ligation of azides employs a Staudinger-like protocol, such as the one described in Scheme 1, but the phosphines used are modified to include an acylating agent, which in **9** is a phenolic ester. Thus, in principle, the reaction allows for reduction of the starting azide and fast intramolecular trapping of the reduction intermediates, resulting in the direct formation of an amide link. We have shown that, in many instances this prevents epimerization and allows retention of configuration at the anomeric carbon. [47]

In 2006 our laboratory reported that the traceless Staudinger ligation of *O*-benzyl-α-glucosyl azide **8** with diphenylphosphanyl-phenyl esters **9** in polar aprotic solvents yields α-glucosyl amides with good yields and selectivity (Scheme 4, path A). The phosphines employed, which are stable to air, allow the fast intramolecular trapping of the reduction intermediates affording direct formation of the amide link and preventing anomerisation. However, the process depends critically on the nature of the sugar protecting groups: the same phosphines react with tetra-*O*-acetyl-α-glucosyl azides **2** in non-stereoconservative fashion and afford β-glucosyl amides (Scheme 4, path B). The dependence of the ligation stereochemistry on the nature of the sugar protecting group appeared to be related to the electron-withdrawing effect of the acetates,[53, 54] which may reduce the rate of the acylation step and favor the anomerization. This effect enforces the use of benzyl ethers as protecting groups in the synthesis of α-glycosyl azides. The method could be applied to *O*-benzyl glycosyl azides in the fuco, gluco, and galacto series to afford the corresponding α-glycosyl amides with good yields and stereoselectivities for a range of acyl chains, how many alkyl and alkenyl groups, both linear and branched, and amino acids with various functional groups. [47d]

More recently, we have developed a protocol for ligation of unprotected α and β glucosyl azides with the same phosphines **9** (Scheme 4, path A). We were able to identify conditions that allowed a clean and stereoconservative reaction to occur in good to moderate yields, affording α or β glucosylamides depending on the configuration of the starting azide.[55] Ligation of the unprotected α- and β-glucosyl azides **7** and **10** was particularly remarkable because in both case it occurred stereoconservatively and allowed simple isolation from the phosphane oxide by-product by water extraction.



Scheme 4. Traceless Staudinger ligation of glycosyl azides with functionalized phosphine **9**.

The overall picture emerging from previous studies on the traceless Staudinger ligation of glycosyl azides are summarized in Scheme 4. β-glycosyl azides can be transformed into the corresponding amides with retention of configuration, irrespective of the nature of the hydroxyl protecting group R (Figure 4, eq 3, R = Bn, Ac, H). On the contrary, the ligation of α-glycosyl azides depends critically on the nature of R: benzyl ethers and free hydroxyl groups allow the reaction to occur with retention of configuration (Figure 4, eq 1), whereas acetates enforce inversion of configuration at the anomeric center and formation of the corresponding β-amide (Figure 4, eq 2).

Phosphines **9** are air stable reagents that can be easily synthesized and purified by flash chromatography, which gives a significant advantage over other ligation reagents, and their application in the stereoselective synthesis of glycosyl amides should be particularly useful.

The process described, however, left various synthetic problems unresolved. The reactivity of α-glycosyl azides was uniformely low, and the corresponding amides were obtained generally in modest yields. Moreover, the yields of the ligation appeared to depend critically also on the nature of the acyl group to be transferred and were specially disappointing for the transfer of

amino acids to α-azides. Thus, in order to further explore the reactivity and stereoselectivity of ligation in the synthesis of α-glycosyl amides, different acyl phosphines were prepared, trying to vary the basicity of the P atom and the nature of the phenyl ester leaving group.

1.6 Aim of the work and plan of the thesis

Our efforts have been initially directed to addressing the issues mentioned above in order to develop more general methods for the stereoselective ligation of unprotected glycosyl azides and to improve the yields of the more difficult ligation reactions.

The first two chapters of the thesis are dedicated to describe the synthesis of the starting material used, i.e. the glycosyl azides (Chapter 2) and the functionalized phosphines (Chapter 3).

Initial results were achieved by screening a set of different phosphines and using as a common substrate α-glucosyl azide **7.** The methods and results are described in Chapter 4. These studies allowed to select appropriate reagents and reaction conditions which led to consistently good yields and stereoselectivity in the ligation of α- and β-glycosyl azides and aminoacids (Chapter 5).

Side-reactions encountered during these initial studies drew our attention to a previously unknown class of compounds, represented by glycofuranosyl amides. In Chapter 6 we describe how these molecules can be selectively synthesized and report preliminary data concerning their biological activity as antibacterial agents.

Finally in Chapter 7 we recapitulate the main mechanistic and stereochemical information collected throughout these studies and use it to formulate a comprehensive mechanistic picture of the traceless Staudinger ligation of unprotected glycosyl azides.

1.7 Reference

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Chapter 2

Synthesis of glycosyl azides

In order to develop a stereoselective Staudinger ligation for the synthesis of α-glycosyl amides, it was decided to employ the procedure based on the reduction-acylation of the corresponding α-glycosyl azides. Our methods to achieve a stereoselective synthesis of α-glycosyl amides are described in Chapters 4-6. Before that, the stereoselective procedures employed for the synthesis of α-glycosyl azides are described in the following sections.

2.1 Stereoselective synthesis of α-glycosyl azides

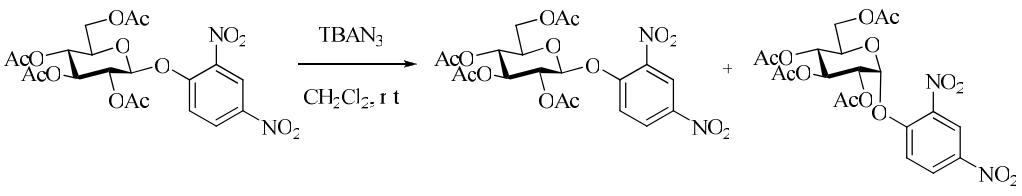
Generally, 1,2-*trans* glycosyl azides can be synthesised using trimethylsilyl azide. Trimethylsilyl azide is an excellent azide donor and enables direct conversion, under Lewis acid catalysis, of acylated (mainly acetylated) monosaccharides. The high stereoselectivity observed in these reactions is due to intermediate formation of acyloxonium ions whose ring opening by the azide reactant yields 1,2-*trans* products (β-azide for glucosyl and galactosyl derivatives and α-azide for mannosyl derivatives).[1] 2-acetamido-3,4,6-tri-*O*-acetyl glucosamine represents an exception because no reaction occurs treating the acetate with trimethylsilyl azide. In this case, the 1,2-*trans* azide is synthesised starting from the corresponding α-chloride. [2]

1,2-*cis* Azides of peracetylated substrates must be prepared by S_N2 nucleophilic substitution from the corresponding β-halides.[3]

Monosaccharides acylated at position C1 and bearing a non-participating group in position C2 mainly give 1,2-*cis* azides upon reaction with trimethylsilyl azide under Lewis acid-catalysed conditions. In this case, α/β mixtures are obtained (as in the case of tetra-*O*-benzyl-glucose and galactose) because the azido group can act as a leaving group.*

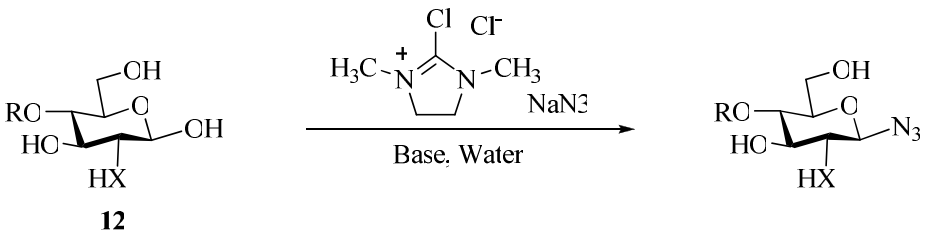
Recently, one procedure has been developed for the one-pot transformation of unprotected monosaccharides into peracetylated β-glycosyl azides under phase-transfer catalysis conditions,

* This effect was observed during our attempt to synthesise the 2,3,4,6-tetra-*O*-acetyl-α-glucosyl azide **2** from the corresponding β-DISAL (methyl *di*nitro*sal*icylate, 2-hydroxy-3,5-dinitrobenzoate) donor. Either using sodium azide with tetrabutylammonium iodide or tetrabutylammonium azide in CH_2Cl_2, β to α equilibration was observed in during the reaction: the 2,4-dinitro phenoxide is a stronger nucleophile than the azide and the epimerisation of the starting material occurred without formation of the corresponding azide.



in the presence of HBr/AcOH (30%), sodium azide and tetrabutylammonium hydrogen sulphate (Scheme 3).[4]

In 2009 Shoda and coworkers showed the direct synthesis of various glycosyl azides *via* an intermolecular nucleophilic attack of the azide ion on the anomeric center of an unprotected sugar **12** mediated by 2-chloro-1,3 dimethylimidazolinium chloride in aqueous solution (Scheme 1).[5]



Scheme 1. Direct synthesis of glycosyl azides by using DCM

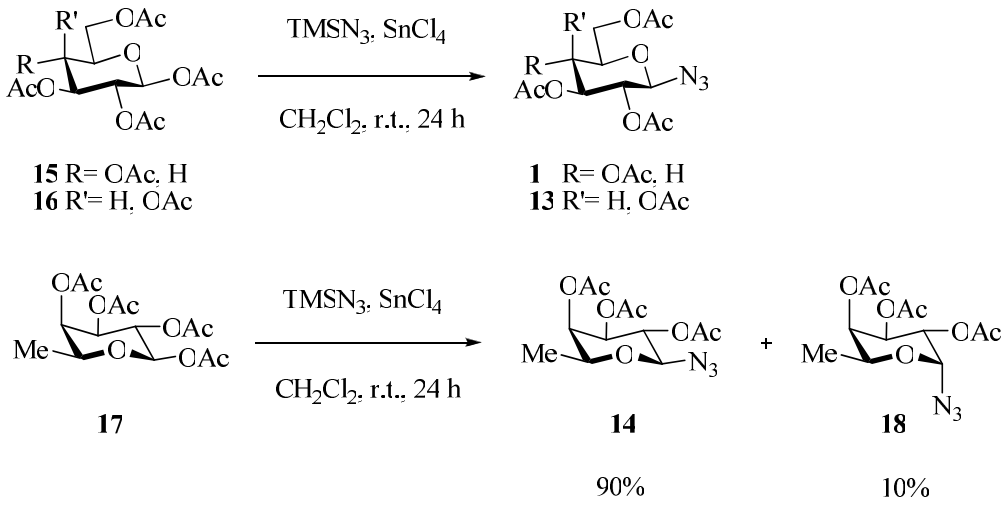
The reactions proceed through a reactive intermediate formed by preferential attack of the anomeric hydroxyl group towards 2-chloro-1,3 dimethylimidazolinium chloride, based on the fact that the pK_a values of hemiacetal anomeric hydroxy groups are much lower than those of other hydroxy groups and water.[6]

Several methods for the direct azidation of unprotected carbohydrates have been reported in the literature, for example using PPh_3/NaN_3[7] or under Mitsunobu conditions,[8] but both these methodologies presented low stereoselectivity on the anomeric carbon.

2.2 1,2-*trans*-per-*O*-Acetyl glycopyranosyl azides

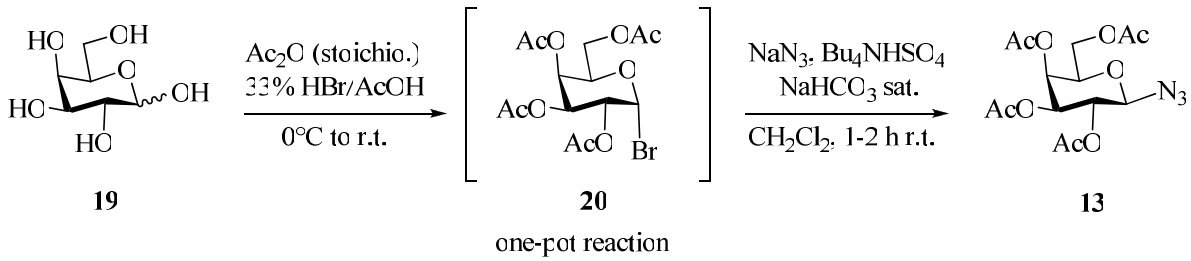
2.2.1 Glucose, Fucose and Galactose

The per-*O*-acetyl-β-glucosyl, fucosyl and galactosyl azides **1**, **13** and **14** were synthesised by treating the per-*O*-acetylated sugars **15**, **16** and **17** with trimethylsilyl azide and tin tetrachloride employing the general procedure described by Paulsen (Scheme 2).[1, 3]



Scheme 2. Stereoselective synthesis of per-*O*-acetyl-β-glycosyl azides **2**, **13** and **14** [1, 3]

Alternatively 2,3,4,6-tetra-*O*-acetyl-β-galactosyl azide **13** has also been prepared directly from the free sugar **19** with a one-pot phase-transfer methodology (Scheme 3).[4]



Scheme 3. Stereoselective synthesis of 2,3,4,6-tetra-*O*-acetyl-β-galactosyl azide **13** [4]

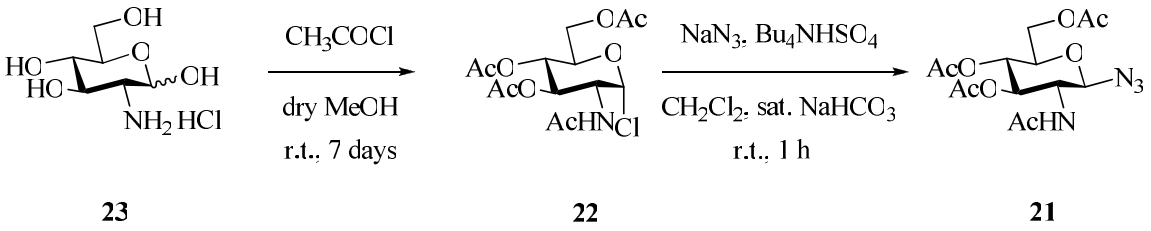
Use of a stoichiometric quantity of acetic anhydride in the presence of HBr/AcOH (30%) gives per-*O*-acetylated galactosyl bromide **20**; subsequent azidolysis of the acetyl-bromo-D-galactose formed in situ was carried out by treatment with sodium azide and tetrabutylammonium hydrogen sulphate under phase-transfer catalysis conditions in CH_2Cl_2 at to room temperature and afforded 2,3,4,6-tetra-*O*-acetyl-β-galactosyl azide **13** in excellent yield without further purification.

2.2.2 Glucosamine

The β-azide of the peracetylated glucosamine **21** cannot be synthesised by simple treatment of the

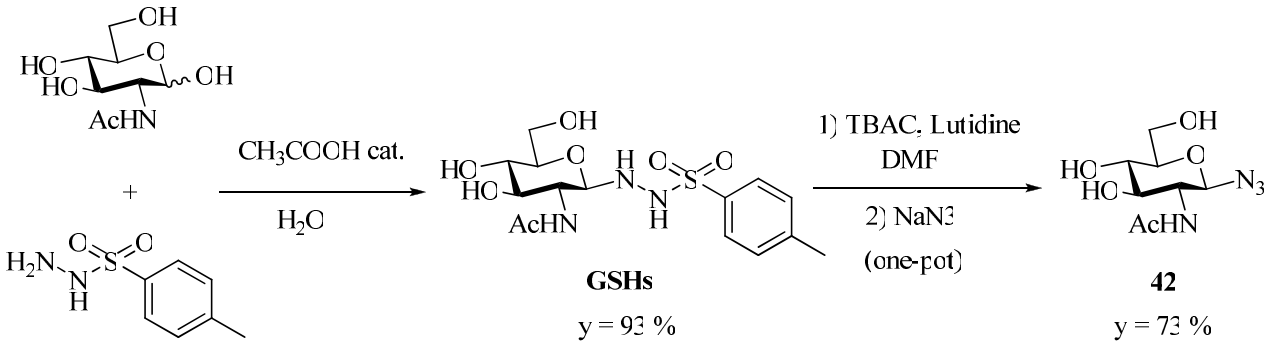
corresponding anomeric acetate with trimethylsilyl azide and tin tetrachloride as in the case of tetra-*O*-acetyl glucose.

Thus, the β-azide was prepared from the corresponding α-chloride **22** with sodium azide and tetrabutylammonium hydrogensulfate in CH_2Cl_2 and saturated $NaHCO_3$.[9] The α-chloride **22** was previously obtained from commercial *N*-acetylglucosamine hydrochloride in a one-pot procedure which employs acetyl chloride and a catalytic amount of dry MeOH (Scheme 4).[2]



Scheme 4. Stereoselective synthesis 3,4,6-tri-*O*-acetyl -2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl azide **21** [2, 9]

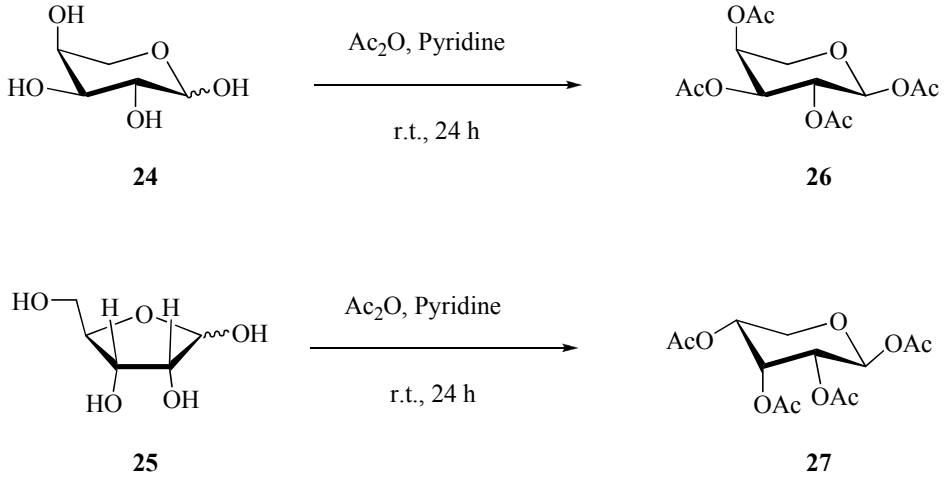
Nitz and co-worker[10] showed (Scheme 5) that unprotected *N*-acetylglucosamine condensed with *p*-toluenesulfonylhydrazine give *N*'-glucosyltoluenesulfohydrazides (GSHs), which can be activated with tetrabutylamoniumchloride, lutidine and then transformed to glucosyl azide **42** by treatment with NaN_3.



Scheme 5. Formation of glycosyl azide **42**

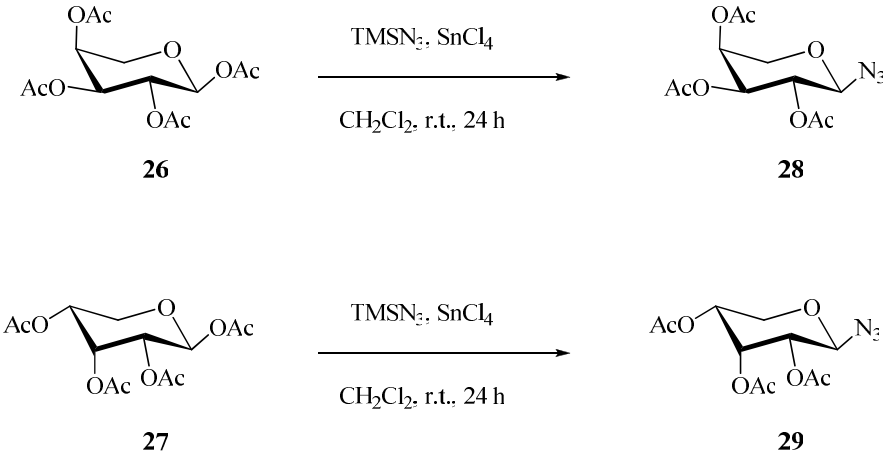
2.2.3 Arabinopyranose and Ribopyranose

Treatments of commercial available free L-arabinopyranose **24** and D-ribofuranose **25** with excess of acetic anhydride and pyridine at room temperature for 24 h afforded the conversion of the free sugar into the corresponding 1,2,3,5-tetra-*O*-acetyl-β-L-arabinopyranose **26** [11] and 1,2,3,5-tetra-*O*-acetyl-β-D-ribopyranose **27**[11, 12] as the predominant products (Scheme 6).



Scheme 6. Synthesis of 1,2,3,5-tetra-*O*-acetyl-β-L-arabinopyranose **26** [11] and 1,2,3,5-tetra-*O*-acetyl-β-D-ribopyranose **27** [11, 12]

The corresponding 1,2-*trans* glycosyl azides were prepared following the procedure of Paulsen.[1, 3] Treating 1,2,3,5-tetra-*O*-acetyl-β-L-arabinopyranose **26** with trimethylsilyl azide and tin tetrachloride the β-azide **28** was obtained.



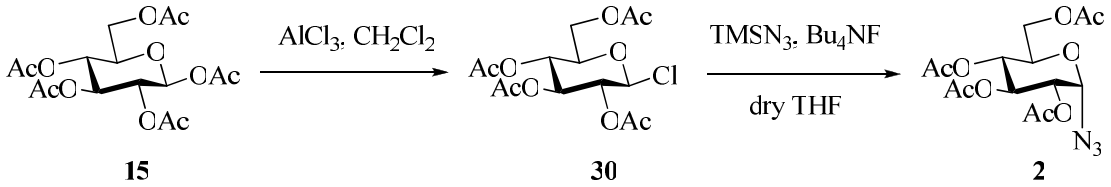
Scheme 7. Stereoselective synthesis of 1,2,3-tri-*O*-acetyl-β-L-arabinopyranosyl azide **28** and 1,2,3-tri-*O*-acetyl-β-D-ribopyranosyl azide **29**

The β configuration of **28** was supported by NOESY contact H1-H3, $J_{1,2}$ = 8 Hz and a low value of the chemical shift C4 = 67.8 ppm, which confirms the pyranose structure. Similarly 1,2,3,5-tetra-*O*-acetyl-β-D-ribopyranose **27** reacted with trimethylsilyl azide in the presence of tin tetrachloride (Scheme 7)[1, 3] to give β-azide **29**.

2.3 1,2-*cis*-per-*O*-Acetyl glycopyranosyl azides

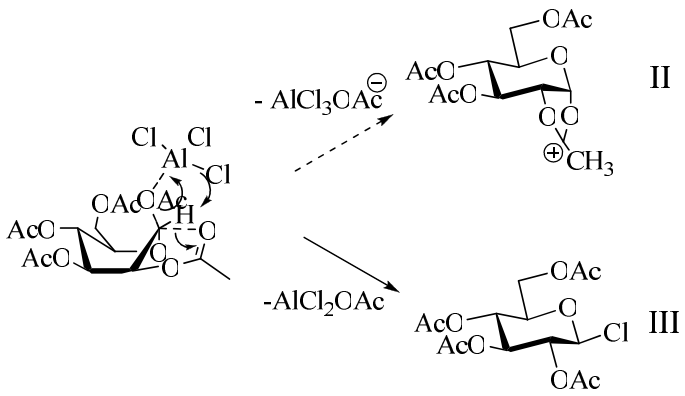
2.3.1 Glucose, Galactose and Arabinopyranose

The 2,3,4,6-tetra-*O*-acetyl α-glucosyl azide **2** was prepared from the corresponding β-chloride **30** [13] with trimethylsilyl azide and tetrabutylammonium fluoride (Scheme 8).[14,15]



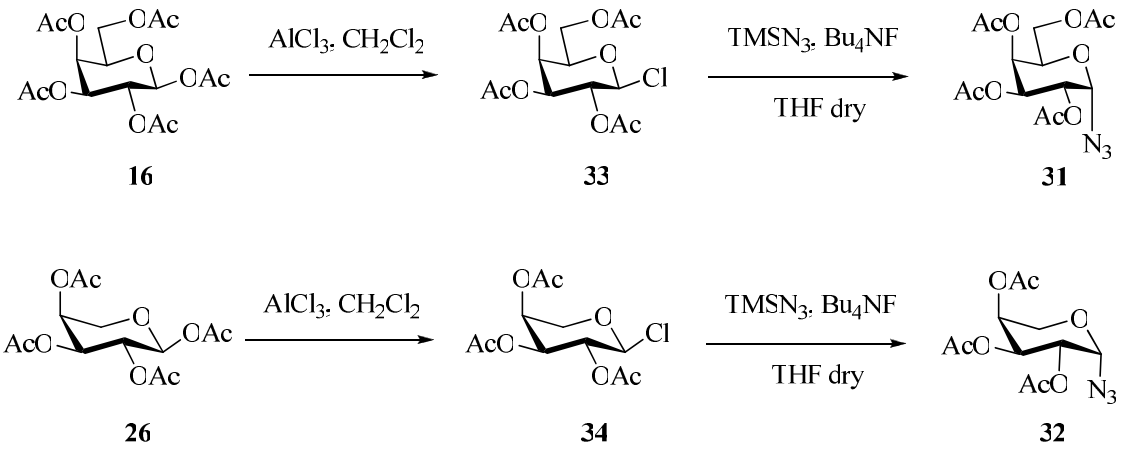
Scheme 8. Stereoselective synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl azide **2** [14]

The β-chloride **30** can be obtained because of the neighbouring group participation. The following mechanism was suggested by the authors: the electrophilic aluminium atom coordinates to the ether oxygen of the anomeric acetoxy group and the resulting change in polarity, assisted by electron displacement from the acetoxy group in position C2, cause the transfer of this group from the anomeric position to the aluminium. Complete separation of $AlCl_3OAc^-$ to afford the free ion **II** (which might exist in mesomeric forms) is unlikely, although an ion pair may be transiently formed. More probably, transfer of the chlorine from aluminium to the anomeric position within the reaction complex **I** is practically synchronous with fission of C1-acetoxy bond. The primary products are the β-chloride **III** and aluminium dichloride acetate (Scheme 9).



Scheme 9. Mechanism of the formation of the β-chloride **30** [13]

The same procedure can also been used for the synthesis of the 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl azide **31**[16] from **16** (Scheme 10) and of the 2,3,5-tri-*O*-acetyl-α-L-arabinopyranosyl azide **32** from **26** (Scheme 10).



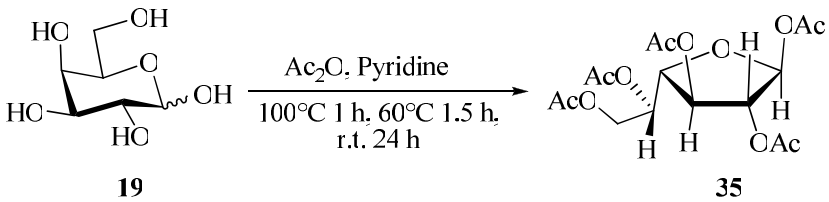
Scheme 10. Stereoselective synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl azide **31** and 2,3,5-tri-*O*-acetyl-α-L-arabinopyranosyl azide **32** [14]

The structure of **32** was established on the basis of the chemical shift of the C4 carbon at 68.7 ppm, which is consistent with the pyranose form and of the $J_{1,2}$ = 3.6 Hz consistent with a cis configuration of C1-C2.

2.4 1,2-*trans*-per-*O*-Acetyl glycofuranosyl azides

2.4.1 Galactofuranose

The conversion of free galactopyranose **19** into 1,2,3,5,6-penta-*O*-acetyl-β-galactofuranose **35** was obtained by treating the free hexose sugar with excess of acetic anhydride and pyridine at high temperature (100°C), for the first step of the reaction (Scheme 11).[17] Most probably, at high temperature pyranose-furanose equilibration is facilitated and Ac_2O treatment traps the furanose form. Isolation of 1,2,3,5,6-penta-*O*-acetyl-β-galactofuranose **35** from the reaction mixture, containing the other three epimers of galactose, required several steps of crystallization (Figure 1).



Scheme 11. Stereoselective synthesis of 1,2,3,5,6-penta-*O*-acetyl-β-galactofuranose **35** [17]

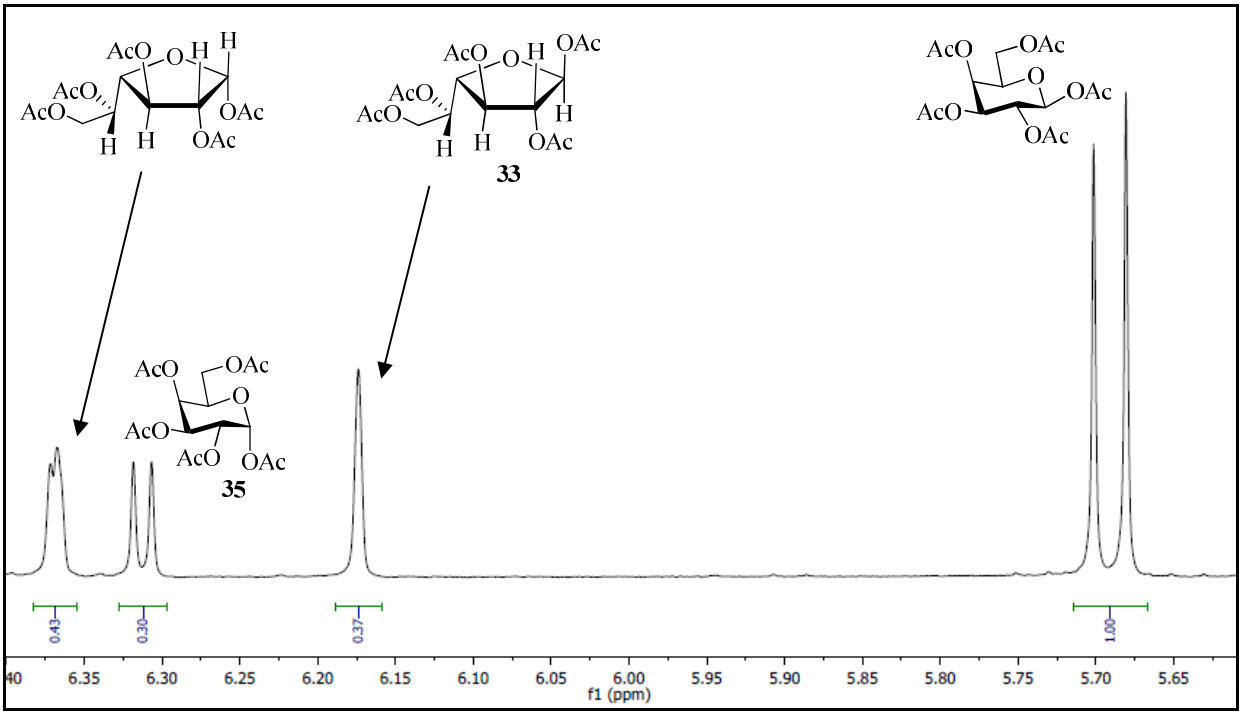


Figure 1. ^{1}H-NMR ($CDCl_3$) spectra of anomeric proton of four epimers of peracetyl-galactose.

The process was assisted by ^{1}H-NMR analysis ($CDCl_3$), which allowed clear identification of the four isomers formed (Figure 1).

When the reaction mixture was quenched into ice-water, we observed the precipitation of penta-*O*-acetyl-β-D-galactopyranose **16** characterized in the ^{1}H-NMR spectra ($CDCl_3$) by a doublet at 5.68 ppm ($J_{1\text{-}2}$ = 8.4 Hz). Then the solution was extracted with AcOEt, to give an orange oil. A second portion of penta-*O*-acetyl-β-D-galactopyranose **16** was obtained from the oil, after dissolution in MeOH and water at low temperature (4°C overnight). Finally, treatment of the residue with isopropanol and water at room temperature afforded pure penta-*O*-acetyl-β-D-galactofuranose **35** in 37 % yield (Figure 2). [17]

The compound is characterized in the 1H-NMR (Figure 1) by a broad singlet of the anomeric proton at 6.17 ppm and its 1H-NMR spectra (Figure 2) coincided with the literature data for **33**.[18]

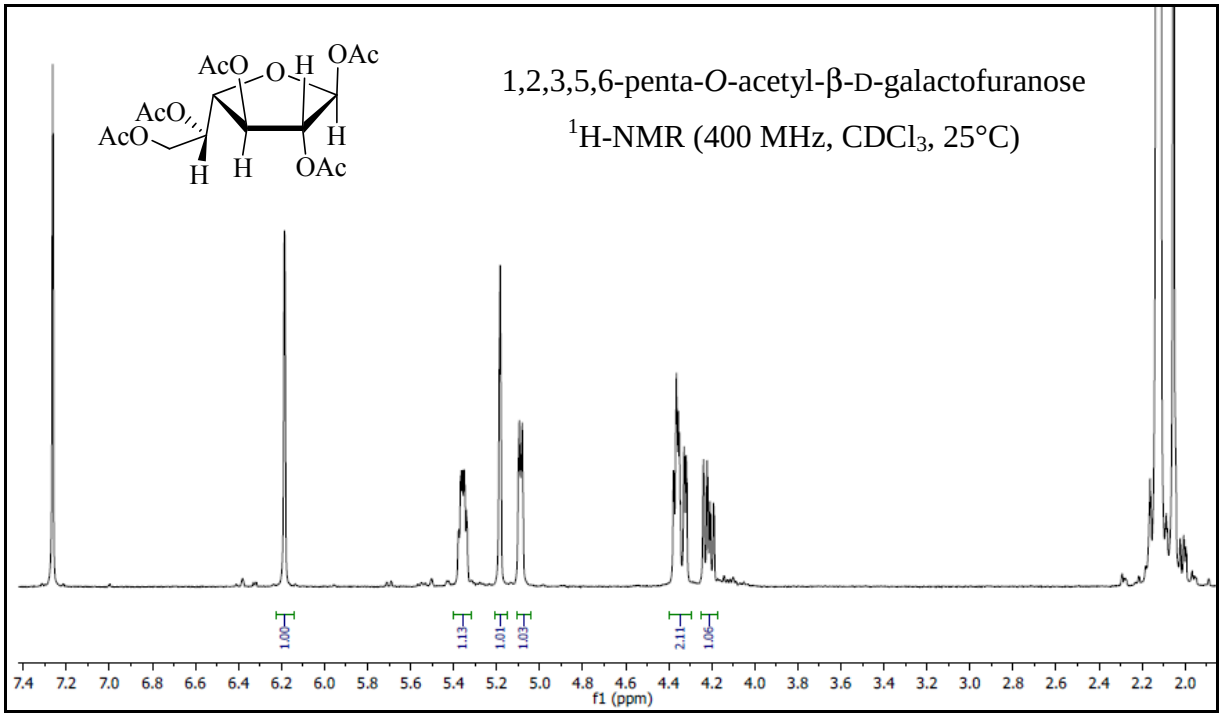
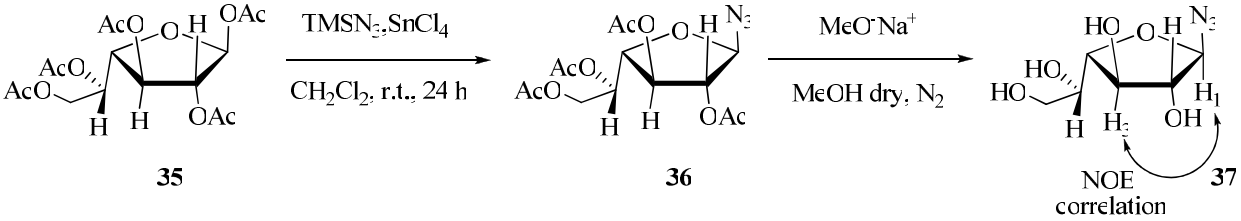


Figure 2. ^{1}H-NMR ($CDCl_3$) spectra of 1,2,3,5,6-penta-*O*-acetyl-β-D-galactofuranose **33**

The 2,3,5,6-tetra-*O*-acetyl-β-galactofuranosyl azide **36** was then synthesised with good yield and total stereocontrol using trimethylsilyl azide and thin tetrachloride employing the general procedure described by Paulsen (Scheme 12).[1, 3]



Scheme 12. Stereoselective synthesis of 2,3,5,6-tetra-*O*-acetyl-β-galactofuranosyl azide **36** and of β-galactofuranosyl azides **37**

Zemplen's deacetylation (Scheme 12), which afforded the unprotected galactofuranosyl azide **37**. The anomeric configuration of **36** was established after NOE difference experiments on **37** showed a clear correlation between protons H1 and H3 (Figure 3), and also the chemical shift of C4 in **37** at 84.2 ppm is diagnostic for the furanose form.

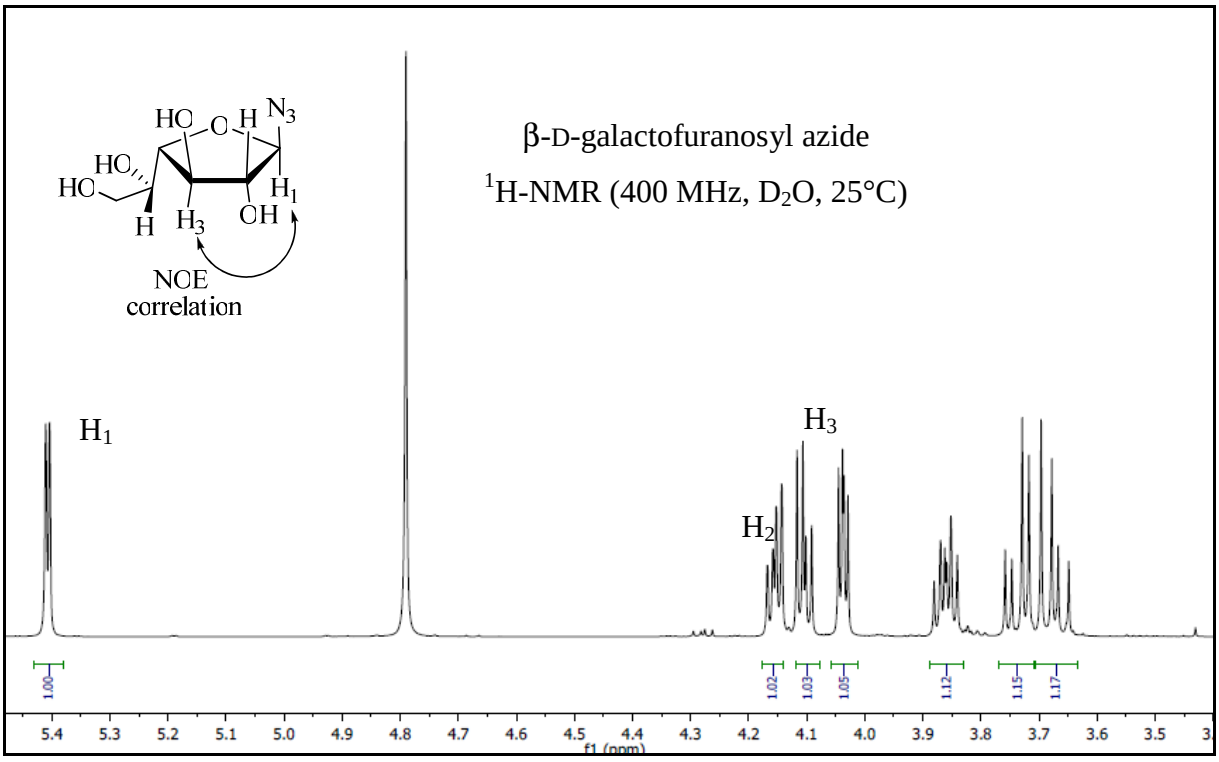
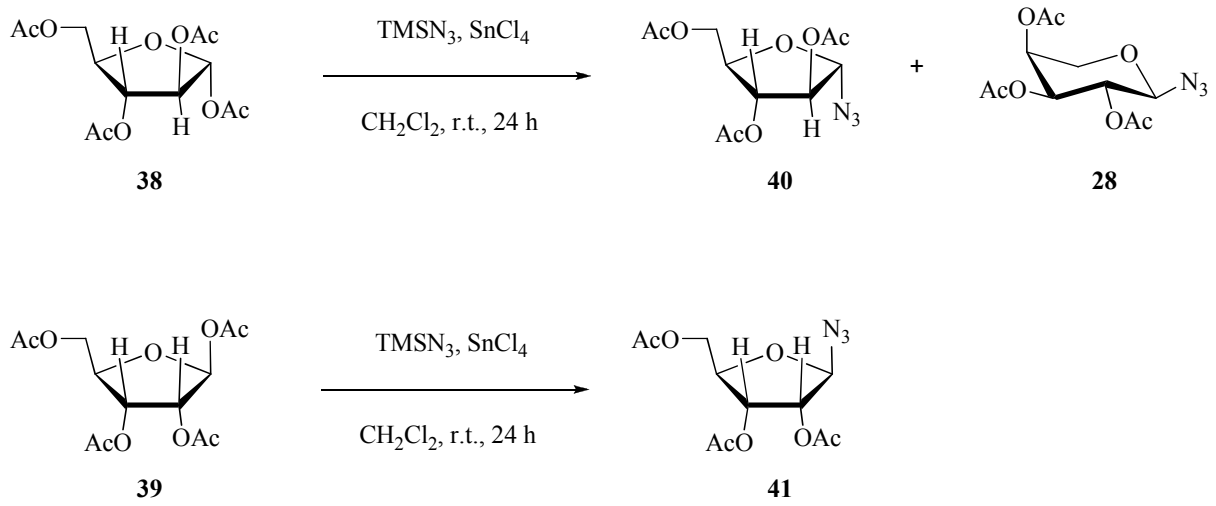


Figure 3. ^{1}H-NMR (D_2O) spectra of β-D-galactofuranosyl azide **37**

2.4.2 Arabinofuranose and Ribofuranose

The synthesis of **40** and **41**, from the commercial available **38** [19] and **39** was performed using the procedure described by Stimac and Kobe [20] under Paulsen's conditions. The reaction afforded high yields and an optimal stereoselectivity for the synthesis of β-D-ribofuranosyl azide **41** [20] from **39**. The structure of **41** was confirmed by NOESY correlation between H1-H4 and high value of C4 79.2 ppm. The 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl azide **40** was obtained from **38** in high yield, but required an additional purification step by flash chromatography or re-crystallization from hexane, to eliminate the little amount of 2,3,5-tri-*O*-acetyl-β-L-arabinofuranose **28** formed during the reaction. The anomeric configuration of **40** was characterized by NOESY correlation between H1-H5.

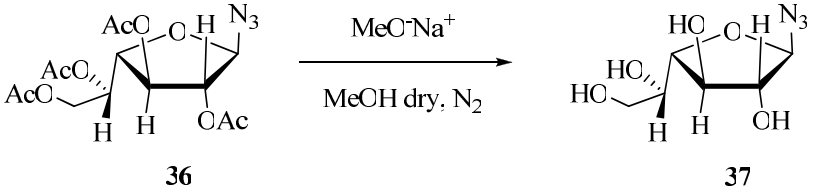


Scheme 13. Synthesis of 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl azide **40** and 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl azide **41** [20]

2.5 Unprotected glycosyl azides

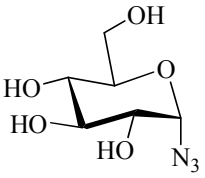
2.5.1 General procedure for the deprotection of peracetylated glycosyl azides

The deprotection of all acetylated glycosyl azides was performed with the Zemplen's procedure (a solution of NaOMe in dry methanol was added, at room temperature and under nitrogen, to a solution of peracetylated glycosyl azide in dry MeOH). This method afforded unprotected glycosyl azides without further purifications (Scheme 13).



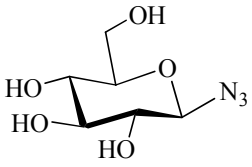
Scheme 13. Deacetylation of per-acetylated glycosyl azides

In the same way all the azides collected in Figure 4 were prepared and used without further purification. The synthesis of the a-galactofuranosyl azides **46** is described in Chapter 6. Unless otherwise stated all the sugar belong to the D-series.



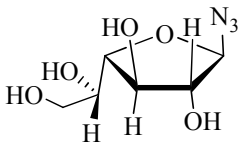
7

α-glucopyranosyl azide



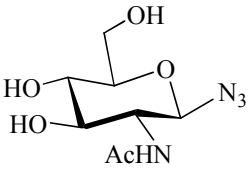
10

β-glucopyranosyl azide



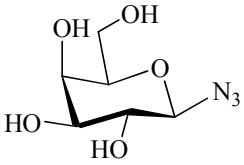
37

β-galactofuranosyl azide



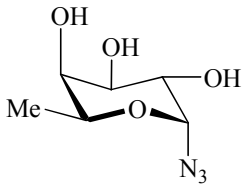
42

2-*N*-acetyl-2-deoxy-β-glucosyl azide



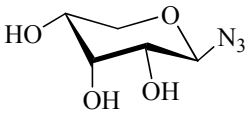
43

β-galactopyranosyl azide



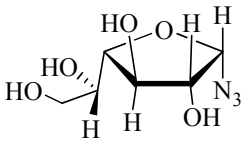
44

α-L-fucopyranosyl azide



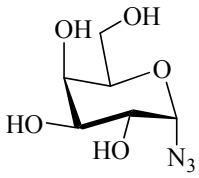
45

β-ribopyranosyl azide



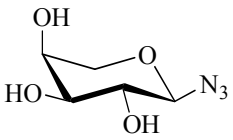
46

α-galactofuranosyl azide



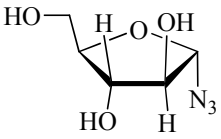
47

α-galactopyranosyl azide



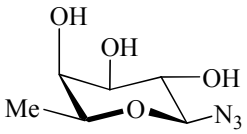
48

β-L-arabinopyranosyl azide



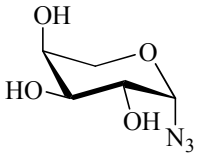
49

α-L-arabinofuranosyl azide



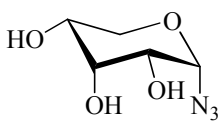
50

β-L-fucopyranosyl azide



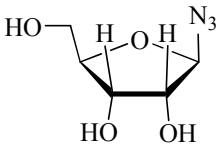
51

α-L-arabinopyranosyl azide



52

α-ribopyranosyl azide



53

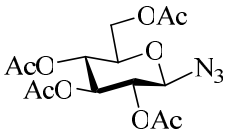
β-ribofuranosyl azide

Figure 4. Unprotected glycosyl azides used in this thesis

2.6 Experimental Section

Solvents were dried by standard procedures: dichloromethane, and methanol were dried over calcium hydride; hexane and tetrahydrofuran were dried over sodium; pyridine were dried over activated molecular sieves. Reactions requiring anhydrous conditions were performed under nitrogen. ^{1}H and ^{13}C-NMR spectra were recorded at 400 MHz on a Bruker AVANCE-400 instrument. Chemical shifts δ for ^{1}H and ^{13}C are expressed in ppm relative to internal Me_4Si as standard. Signals were abbreviated as s, singlet; bs broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained with a Bruker ion-trap Esquire 3000 apparatus (ESI ionization). Optical rotations $[\alpha]_D$ were measured in a cell of 1 dm pathlength and 1 ml capacity with a Perkin-Elmer 241 polarimeter. Thin layer chromatography (TLC) was carried out with precoated Merck F_{254} silica gel plates. Flash chromatography (FC) was carried out with Macherey-Nagel silica gel 60 (230-400 mesh).

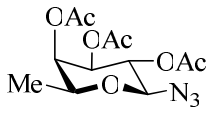
Synthesis of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (1)



Trimethylsilyl azide (238 μL, 1.79 mmol, 1.4 eq) and tin tetrachloride (45 μL, 0.384 mmol, 0.3 eq) were added, at room temperature and under nitrogen, to a solution of 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose **15** (500 mg, 1.28 mmol, 1 eq) in dry CH_2Cl_2 (2.56 mL, 0.5 M). The reaction mixture was stirred at room temperature and the reaction was monitored by TLC (60:40 hexane/AcOEt). After 24 h CH_2Cl_2 was added and the solution was washed with saturated Na_2CO_3 and then with water. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The product was purified by flash chromatography using 60:40 hexane/AcOEt as the eluent. Quantitative yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.20 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.08 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1H, H-4), 4.94 (dd, $J_{1,2}$ = 8.9 Hz, $J_{2,3}$ = 9.5 Hz, 1H, H-2), 4.63 (d, $J_{1,2}$ = 8.9 Hz, 1H, H-1), 4.26 (dd, $J_{5,6}$ = 4.7 Hz, $J_{6,6'}$ = 12.5 Hz, 1H, H-6), 4.15 (dd, $J_{5,6'}$ = 2.3 Hz, $J_{6,6'}$ = 12.5 Hz, 1H, H-6'), 3.78 (m, 1H, H-5), 2.08, 2.06, 2.01, 1.99 (4s, 12H, 4xOAc).

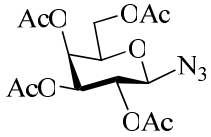
Synthesis of 2,3,4-tri-*O*-acetyl-β-D-fucosyl azide (14)



Trimethylsilyl azide (1.4 eq) and tin tetrachloride (0.3 eq) were added, at room temperature and under nitrogen, to a solution of 1,2,3,4-tetra-*O*-acetyl-D-fucose **17** (1 eq) in dry CH_2Cl_2 (0.5 M). The reaction mixture was stirred at room temperature and the reaction was monitored by TLC (60:40 hexane/AcOEt). After 24 h CH_2Cl_2 was added and the solution was washed with saturated Na_2CO_3 and then with water. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The product was purified by flash chromatography using 60:40 hexane/AcOEt as the eluent. (α/β ratio 10:90).Yield = 85 %

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.30 (dd, $J_{3,4}$ = 3.6 Hz, $J_{4,5}$ = 0.8 Hz, 1H, H-4), 5.18 (t, $J_{1,2}$ = 8.8 Hz, $J_{2,3}$ = 10.4 Hz, 1H, H-2), 5.06 (dd, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 4.60 (d, $J_{1,2}$ = 8.8 Hz, 1H, H-1), 3.94 (dq, $J_{4,5}$ = 0.8 Hz, $J_{5,6}$ = 6.4 Hz, 1H, H-5), 2.22, 2.12, 2.02 (3s, 9H, 3xOAc), 1.28 (d, $J_{5,6}$ = 6.4 Hz, 3H, H-6).

Synthesis of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl azide (13)



Procedure A

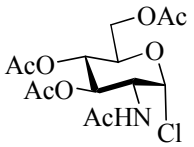
Trimethylsilyl azide (472 μL, 3.58 mmol, 1.4 eq) and tin tetrachloride (90 μL, 0.768 mmol, 0.3 eq) were added, at room temperature and under nitrogen, to a solution of 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose **16** (1 g, 2.56 mmol, 1 eq) in dry CH_2Cl_2 (6.12 mL, 0.5 M). The reaction mixture was stirred at room temperature and the reaction was monitored by TLC (60:40 hexane/AcOEt). After 24 h CH_2Cl_2 was added and the solution was washed with saturated Na_2CO_3 and then with water. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The product was purified by flash chromatography using 60:40 hexane/AcOEt as the eluent. Quantitative yield.

Procedure B

A suspension of D-galactose (1.8 g, 10.0 mmol, 1 eq) in acetic anhydride (4.82 mL, 51.0 mmol, 1.02 eq) was placed in an ice bath with continuous stirring. HBr/AcOH (30%, 2.7 mL, 10.0 mmol, 1 eq) was added in one portion to the cold suspension of the reaction mixture. An exothermic reaction started immediately and the reaction mixture was allowed to stir at room temperature until a clear solution was obtained (approx. 15 min.). The reaction mixture was cooled to 0°C, additional HBr/AcOH (30%, 5.4 mL, 20.0 mmol, 2 eq) was added slowly, and stirring was continued for 2 h at room temperature. After completion of the reaction (monitored by TLC; hexane/AcOEt 50:50), solvents were removed under reduced pressure and coevaporated with toluene. Sodium azide (1.3g, 20 mmol), tetrabutylammonium hydrogen sulfate (TBAHS) (510 mg, 1.5 mmol) and aq. Na_2CO_3 (1 M, 70 mL) were added successively to a solution of the crude mass in CH_2Cl_2 (50mL) and the two phase reaction mixture was allowed to stir vigorously for another 1.5 h. The reaction mixture was diluted with CH_2Cl_2 (50mL). The organic layer was separated and washed with water, dried (Na_2SO_4), and concentrated under reduced pressure. The product was purified by flash chromatography using 65:35 hexane/AcOEt as the eluent. Yield.= 85 %

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.42 (dd, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 1.2 Hz, 1H, H-4), 5.16 (q, $J_{1,2}$ = 8.4 Hz, $J_{2,3}$ = 10.4 Hz, 1H, H-2), 5.03 (dd, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 4.59 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.20-4.12 (m, 2H, H-6, H-6'), 4.01 (dt, $J_{4,5}$ = 1.2 Hz, 1H, H-5), 2.16, 2.10, 2.07, 1.98 (4s, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.6, 170.3, 170.2, 169.6, 88.5 (C-1), 73.1 (C-5), 70.9 (C-3), 68.3 (C-2), 67.1 (C-4), 61.4 (C-6), 20.9-20.7 (4xOAc).

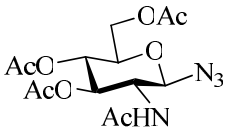
Synthesis of 3,4,6-tri-*O*-acetyl-2-*N*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (22)



A suspension of *N*-acetylglucosamine hydrochloride **23** (510 mg, 2.3 mmol, 1 eq) in acetyl chloride (37.7 ml) was prepared at room temperature. The solution was cooled to 0 °C and dry MeOH (500 µL) was added. After 24 h the starting material was completely consumed. The solution was stirred at room temperature for 20 days (TLC: AcOEt). The solvent was evaporated under reduced pressure. The crude product was used without further purification.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 6.20 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.89 (d, $J_{2\text{-NH}}$ = 7.3 Hz, 1H, NH), 5.34 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.23 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1H, H-4), 4.55 (m, 1H, H-2), 4.32-4.25 (m, 2H, H-5, H-6), 4.14 (m, 1H, H-6'), 2.11, 2.06 (2), 1.99 (4s, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.4, 170.5, 170.1, 169.1, 93.6, 70.9, 70.1, 66.9, 61.2, 53.5, 23.1, 20.7, 20.5.

Synthesis of 3,4,6-tri-*O*-acetyl -2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl azide (21)



Procedure A

A solution of 3,4,6-tri-*O*-acetyl-2-*N*-acetyl-2-deoxy-α-D-glucopyranosyl chloride **22** (25.6 mg, 0.07 mmol, 1 eq) containing sodium azide (13.4 mg, 0.21 mmol, 3 eq) and tetrabutylammonium hydrogensulfate (23.8 mg, 0.07 mmol, 1 eq) in CH_2Cl_2 (256 µL) was prepared at room temperature. Saturated $NaHCO_3$ (256 µL) was added and the mixture was stirred vigorously for 1 h; the reaction was monitored by TLC (AcOEt). The mixture was diluted with AcOEt and washed with water, saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. Yield.= 91 %

Procedure B

At room temperature and under nitrogen, a solution of 3,4,6-tri-*O*-acetyl-2-*N*-acetyl-2-deoxy-α-D-glucopyranosyl chloride **22** (18.7 mg, 0.051 mmol, 1 eq) containing sodium azide (10.0 mg, 0.153 mmol, 3 eq) in dry dimethylformamide (510 µL) was prepared. The mixture was heated to 70 °C and stirred for 3 h. The reaction was monitored by TLC (AcOEt). The solvent was then evaporated under reduced pressure. The residue was taken up with AcOEt and washed with water. The organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. Yield = 90 %

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.86 (d, $J_{2\text{-NH}}$ = 8.8 Hz, 1H, NH), 5.27 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, 1H, H-3), 5.11 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1H, H-4), 4.79 (d, $J_{1,2}$ = 9.2 Hz, 1H, H-1), 4.28

(dd, $J_{5,6}$ = 4.7 Hz, $J_{6,6'}$ = 12.4 Hz, 1H, H-6), 3.75 (dd, $J_{5,6'}$ = 1.5 Hz, $J_{6,6'}$ = 12.4 Hz, 1H, H-6'), 3.94 (m, 1H, H-2), 4.32-3.82 (m, 1H, H-5), 2.11, 2.05, 2.04, 1.99 (4s, 12H, 4xOAc).
^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.9, 170.7, 170.5, 169.3, 88.4, 73.9, 72.2, 68.1, 61.9, 54.2, 23.2, 20.7, 20.6, 20.5.

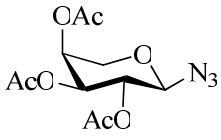
General procedure for the synthesis of 1,2,3,5-tetra-*O*-acetyl-β-L-arabinopyranose (26) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribopyranose (27)

Acetic anhydride (10 eq) and a catalytic amount of *N*,*N*-dimethylaminopyridine were added, at room temperature, to a solution of substrate (1 eq) in pyridine dried on molecular sieves (0.1 M). The solution was stirred for 24 h and then was concentrated in vacuo. The residue was dissolved in AcOEt and washed with aqueous 5 % HCl, aqueous 5 % $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated to give the product in quantitative yield. The crude product was used without further purification.

General procedure for the synthesis of 2,3,5-tri-*O*-acetyl-β-L-arabinopyranosyl azide (28) and 2,3,5-tri-*O*-acetyl-β-D-ribopyranosyl azide (29)

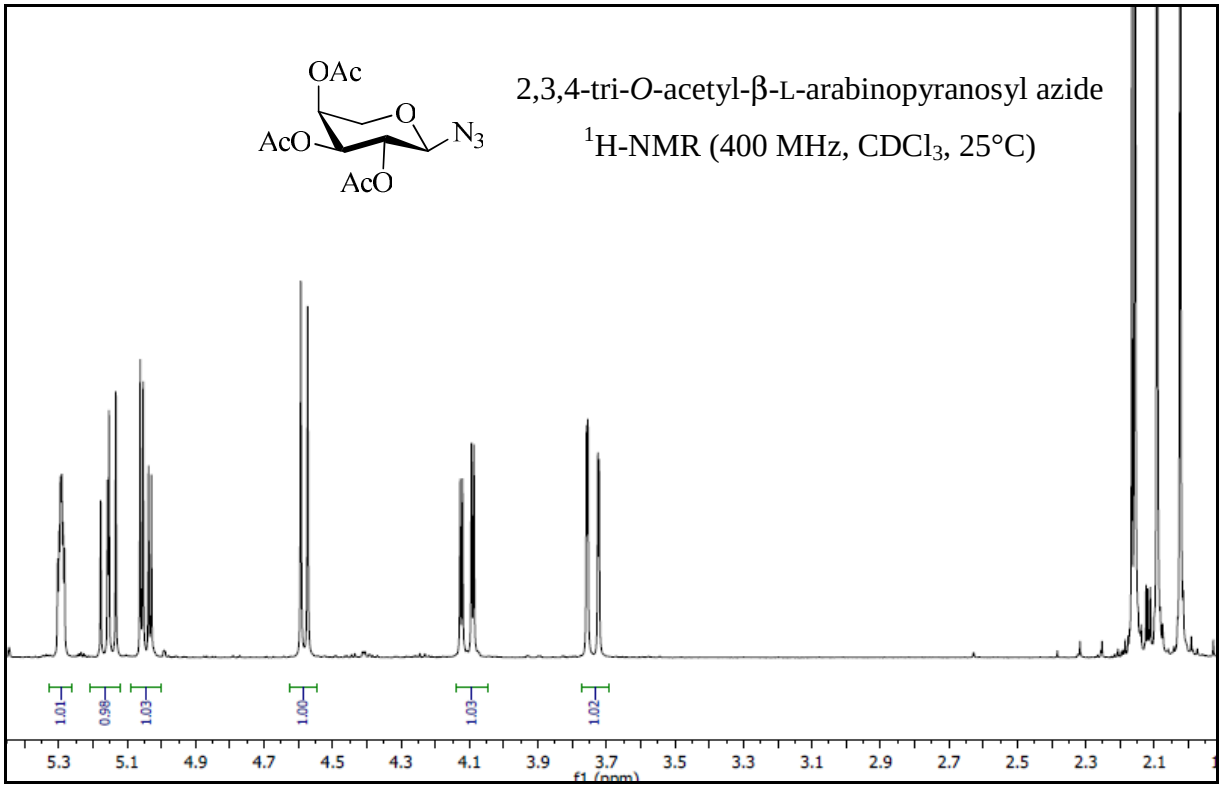
Trimethylsilyl azide (1.4 eq) and tin tetrachloride (0.3 eq) were added, at room temperature and under nitrogen, to a solution of substrate **26** or **27** (1 eq) in dry CH_2Cl_2 (0.5 M). The reaction mixture was stirred at room temperature and the reaction was monitored by TLC (60:40 hexane/AcOEt). After 24 h CH_2Cl_2 was added and the solution was washed with saturated Na_2CO_3 and then with water. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure.

2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl azide (28)

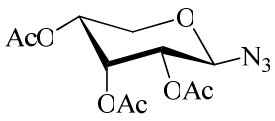


The compound was purified by flash chromatography (hexane/acetone 80:20) yield = 75 %.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.29 (ddd, $J_{3,4}$ = 3.6 Hz, $J_{4,5}$ = 1.6 Hz, $J_{4,5'}$ = 2.8 Hz, 1H, H-4), 5.15 (dd, $J_{1,2}$= 8 Hz, $J_{2,3}$ = 9.6 Hz, 1H, H-2), 5.05 (dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 3.6 Hz, 1H, H-3),

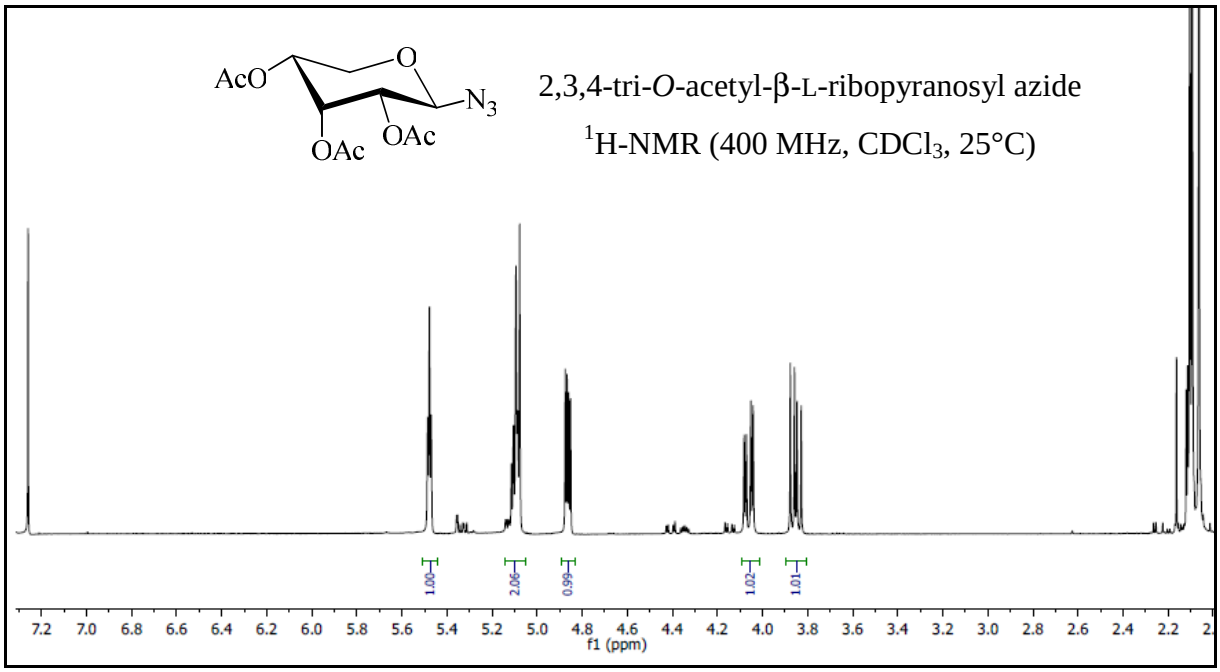
4.58 (d, $J_{1,2}$= 8 Hz, 1H, H-1), 4.11 (dd, $J_{4,5}$ = 2.8 Hz, $J_{5,5'}$ = 13.2 Hz, 1H, H-5), 3.73 (dd, $J_{4,5'}$ = 1.6 Hz, $J_{5,5'}$ = 13.2 Hz, 1H, H-5'), 2.16, 2.09, 2.02 (3s, 9H, 3xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.4, 170.2, 169.6, 88.7 (C-1), 70.4 (C-3), 68.6 (C-2), 67.8 (C-4), 65.8 (C-5), 21.1-20.8 (3xOAc). NOESY (400 MHz, $CDCl_3$, 25°C): contact between H-1/H-3. ESI-MS: *m/z* 324.1 (M+Na).



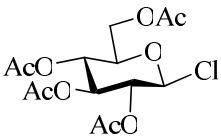
2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl azide (29)



The compound was purified by flash chromatography (hexane/acetone 80:20) yield = 65 %. ^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.48 (t, $J_{2,3}$ = 3.2 Hz, 1H, H-3), 5.15-5.06 (m, 2H, H-1, H-4), 4.86 (dd, $J_{1,2}$ = 6.4 Hz, $J_{2,3}$ = 3.2 Hz, 1H, H-2), 4.07 (dd, $J_{4,5'}$ = 4 Hz, $J_{5,5'}$ = 12 Hz,1H, H-5), 3.85 (dd, $J_{4,5'}$ = 3.2 Hz, $J_{5,5'}$ = 12 Hz, 1H, H-5'), 2.11, 2.08, 2.06 (3s, 9H, 3xOAc). ESI-MS: *m/z* 324.1 (M+Na).

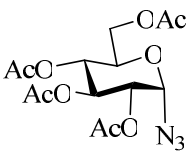


Synthesis of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride (30)



Aluminium trichloride (85.4 mg, 0.64 mmol, 0.5 eq) was added, at room temperature and under argon, to a solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose **15** (500 mg, 1.28 mmol, 1 eq) in dry CH_2Cl_2 (2.56 mL, 0.5 M). Aluminium trichloride gradually disappeared and was replaced by a fine white precipitate. After 2 h, the mixture was filtered into a large volume (50 mL) of dry hexane. The resulting white precipitate was filtered on celite and washed with dry CH_2Cl_2 (2.5 mL) and 60:40 hexane/AcOEt (50 mL). The solvent was evaporated under reduced pressure and the crude product was used without further purification.

Synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl azide (2)



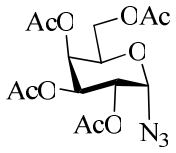
At room temperature and under nitrogen, trimethylsilyl azide (108 μL, 0.820 mmol, 1.4 eq) and tetrabutylammonium fluoride (1 M in THF, 820 μL, 1.4 eq) were added to a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride **30** (215 mg, 0.586 mmol, 1 eq) in dry THF (8.2 mL, 0.1 M). The solution was heated to 65 °C and stirred for 30 h. The reaction was monitored by TLC (60:40 hexane/AcOEt). The solvent was evaporated under reduced pressure and the crude was purified by flash chromatography using 65:35 hexane/AcOEt as the eluent.

Yield = 69 % (over 2 steps), α/β ratio 89:11

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.62 (d, $J_{1,2}$ = 4.3 Hz, 1H, H-1), 5.40 (dd, $J_{2,3}$ = 10 Hz, $J_{3,4}$ = 9.8 Hz, 1H, H-3), 5.06 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1H, H-4), 4.96 (dd, $J_{1,2}$ = 4.3 Hz, $J_{2,3}$ = 10 Hz, 1H, H-2), 4.29 (dd, $J_{5,6}$ = 4.7 Hz, $J_{6,6'}$ = 12.4 Hz, 1H, H-6), 4.19-4.12 (m, 2H, H-5 and H-6'), 2.11, 2.10, 2.05, 2.03 (4s, 12H, 4xOAc).

^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.5, 169.9, 169.4, 86.2 (C-1), 70.1 (C-5), 69.6 (C-4), 69.5 (C-2), 67.9 (C-3), 61.5 (C-6), 20.6, 20.6, 20.5, 20.5 (4xOAc).

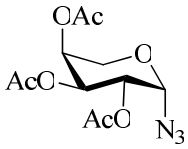
Synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl azide (31)



See procedure for the synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl azide **2**. The compound was purified by flash chromatography (hexane/AcOEt 60:40) Yield = 62 % (over 2 steps), α/β ratio 75:25

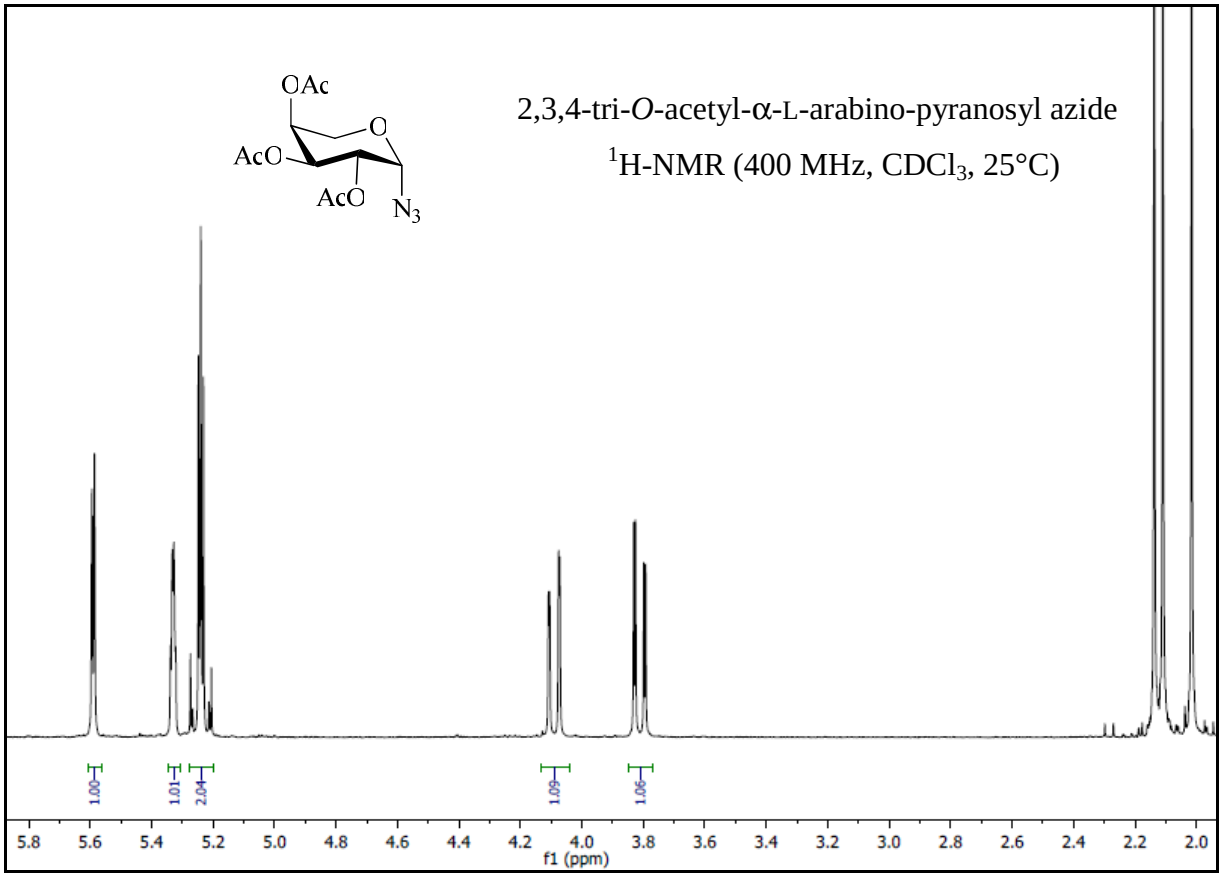
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.65 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.45 (dd, $J_{3,4}$ = 3 Hz, $J_{4,5}$ = 1.2 Hz, 1H, H-4), 5.24 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3 Hz, 1H, H-3), 5.19 (dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.8 Hz, 1H, H-2), 4.35 (dt, $J_{4,5}$ = 1.2 Hz, 1H, H-5), 4.15-4.07 (m, 2H, H-6, H-6'), 2.14, 2.10, 2.06, 1.99 (4s, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.5, 170.3, 170.1, 169.9, 86.9 (C-1), 68.7 (C-5), 67.8 (C-4), 67.5 (C-2), 67.4 (C-3), 61.6 (C-6), 21.2, 20.8, 20.7 (4xOAc).

2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl azide (32)

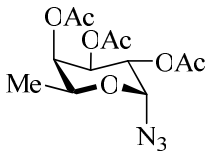


See procedure for the synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl azide **2**. The compound was purified by flash chromatography (hexane/AcOEt 70:30).

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.59 (d, $J_{1,2}$ = 3.2 Hz, 1H, H-1), 5.33 (q, $J_{4,5}$ = 1.4 Hz, $J_{4,5'}$ = 2.4 Hz, 1H, H-4), 5.27-5.20 (m, 2H, H-2, H-3), 4.08 (dd, $J_{4,5}$ = 1.4 Hz, $J_{5,5'}$ = 13 Hz, 1H, H-5), 3.80 (dd, $J_{4,5'}$ = 2.4 Hz, $J_{5,5'}$ = 13 Hz, 1H, H-5'), 2.14, 2.11, 2.02 (3xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.4, 170.3, 170, 87.4 (C-1), 68.7 (C-4), 68, 67.1, 62.7 (C-5), 21.1-20.8 (3xOAc).

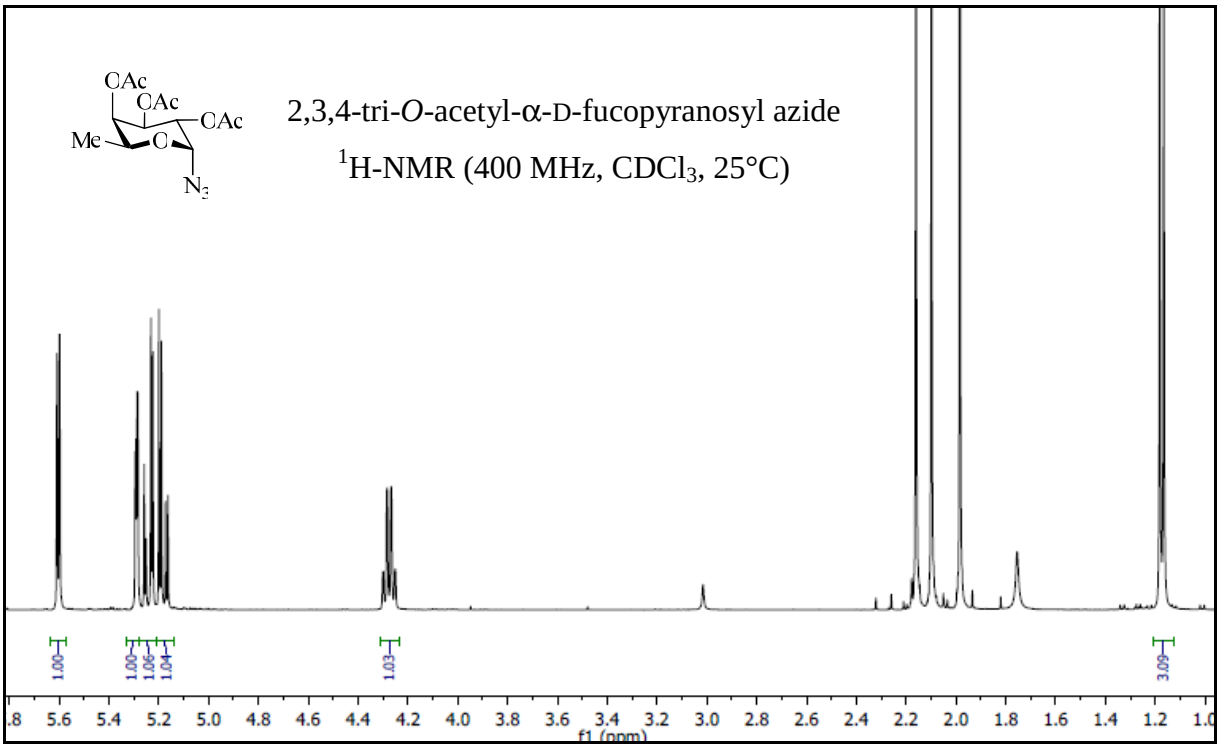


2,3,4-tri-*O*-acetyl-α-D-fucosyl azide (18)

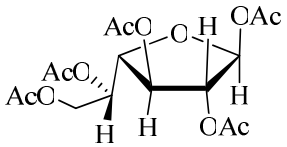


Obtained as by-prodouct from the synthesis of the β-fucosyl azide. Yield = 10%

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.59 (d, $J_{1,2}$ = 4 Hz, 1H, H-1), 5.29 (dd, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 1.2 Hz, 1H, H-4), 5.24 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 5.18 (dd, $J_{1,2}$ = 4 Hz, $J_{2,3}$ = 10.8 Hz, 1H, H-2), 4.27 (q, $J_{5,6}$ = 6.4 Hz, 1H, H-5), 2.15, 2.01, 1.98 (3s, 9H, 3xOAc), 1.17 (d, $J_{5,6}$ = 6.4 Hz, 3H, H-6). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.6, 170.4, 170, 87.2 (C-1), 70.8 (C-4), 67.8, 67.7, 67.1 (C-5), 20.9-20.8 (3xOAc), 16.1 (C-6).

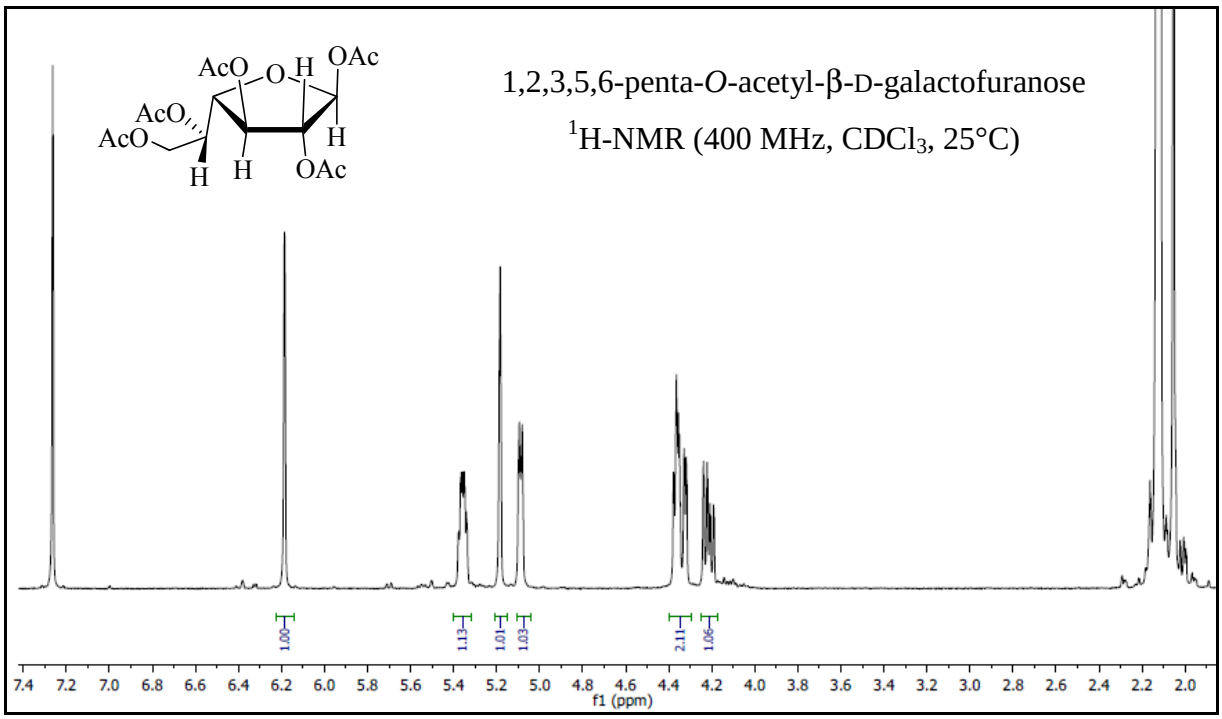


Synthesis of 1,2,3,5,6-penta-*O*-acetyl-β-D-galactofuranose (35).

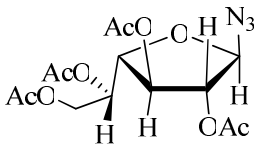


D-Galactose (10g, 5,5 mmol, 1 eq) was heated with pyridine (150 mL) for 1 h at 100°C. The temperature was lowered to 60°C, and acetic anhydride (33.12 mL, 35.2 mmol, 6.4 eq) was added dropwise. The mixture was kept for 1.5 h at 60°C, and, after 24 h at room temperature, it was quenched into ice-water (precipitation of penta-*O*-acetyl-β-D-galactopyranose). The solution was extracted with AcOEt (2x250 mL) and washed with aqueous 5 % HCl, aqueous 5 % $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4, filtered and evaporate under reduced pressure to give an orange oil. The oil was dissolved in MeOH, then water was added until the cloud point was reached. The solution was cooling at 4°C overnight, and the resulting white solid was filtered (penta-*O*-acetyl-β-D-galactopyranose); the solvent was evaporated under reduced pressure, the oil was dissolved in isopropanol, then water was added until the cloud point was reached, and the resulting white solid was filtered: yield = 36 %

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 6.18 (s, 1H, H-1), 5.36 (m, 1H, H-5), 5.18 (s, 1H, H-4), 5.08 (d, J = 5.6 Hz, 1H), 4.38-4.31 (m, 2H, H-6), 4.24-4.19 (m, 1H, H-6'), 2.12-2.03 (m, 15H, 5xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.7, 170.2, 169.9, 169.6, 169.2, 99.3 (C-1), 82.3, 80.7 (C-4), 76.5, 69.4 (C-5), 62.7 (C-6), 21.1-20.8 (5xOAc).

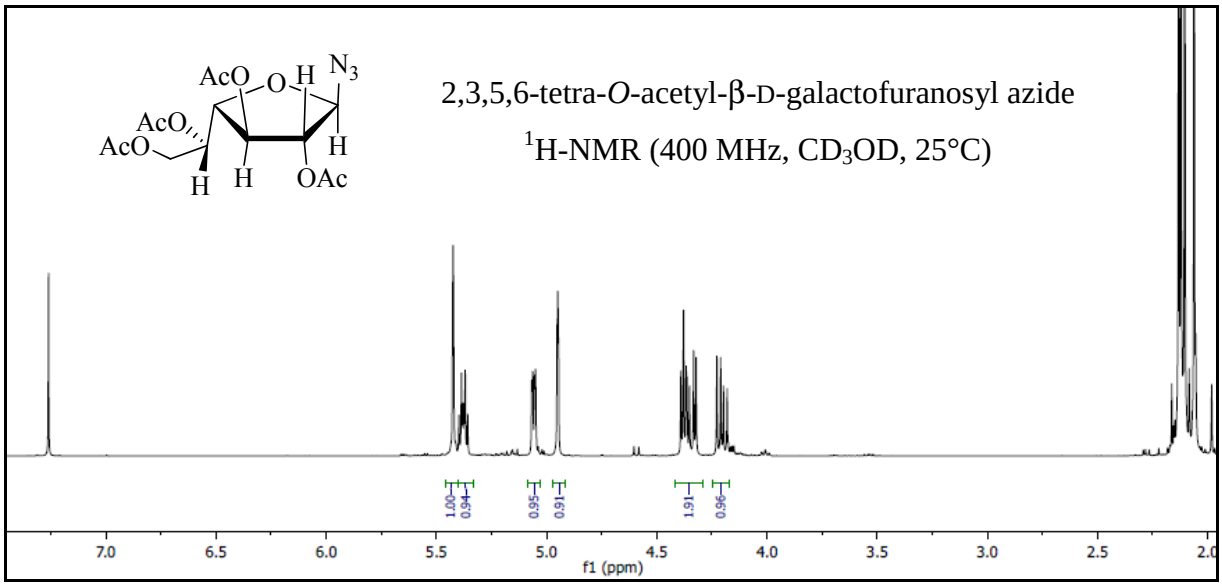


Synthesis of 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl azide (36).

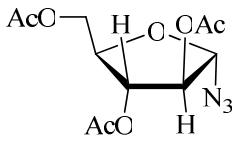


Trimethylsilyl azide (154 µL, 1.16 mmol, 1.4 eq) and tin tetrachloride (29.1 µL, 0.25 mmol, 0.3 eq) were added, at room temperature and under nitrogen, to a solution of 1,2,3,4,6-penta-*O*-acetyl-D-galactofuranose **33** (324 mg, 0.83 mmol, 1 eq) in dry CH_2Cl_2 (3.3 mL, 0.5 M). The reaction mixture was stirred at room temperature and the reaction was monitored by TLC (60:40 hexane/AcOEt). After 24 h CH_2Cl_2 was added and the solution was washed with saturated Na_2CO_3 and then with water. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The product was purified by flash chromatography using 60:40 hexane/AcOEt as the eluent. Yield = 90 %

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.42 (s, 1H, H-1), 5.40-5.35 (m, 1H, H-5), 5.05 (dd, $J_{2,3}$ = 5.2 Hz, $J_{3,4}$ = 2.8 Hz, 1H, H-3), 4.95 (t, $J_{3,4}$ = 2.8 Hz, 1H, H-4), 4.39-4.32 (m, 2H, H-2, H-6), 4.19 (dd, $J_{5,6'}$ = 6.8 Hz, $J_{6,6'}$ = 11.6 Hz, 1H, H-6'), 2.16, 2.14, 2.12, 2.06 (4s, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.6, 170.1, 169.9, 169.7, 94.3 (C-1), 82.2 (C-2), 81.1 (C-4), 76.5 (C-3), 69.4 (C-5), 62.5 (C-6), 20.9-20.7 (4xOAc). ESI-MS: *m/z* 396.2 (M+Na).

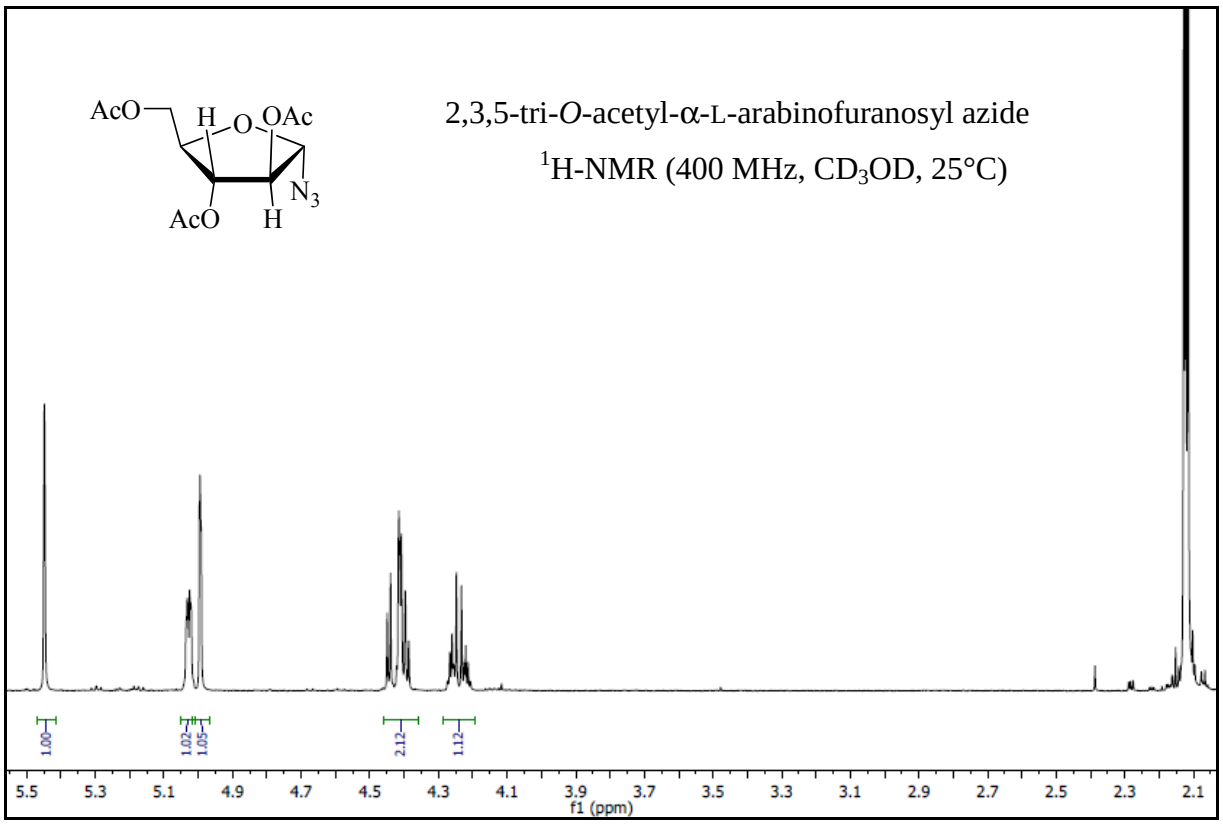


2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl azide (40)

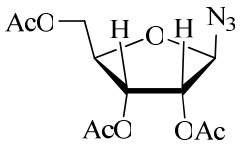


See procedure for the synthesis of 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl azide **36**. The compound was purified by flash chromatography (hexane/acetone 80:20 or crystallization in hexane) Yield = 80 %

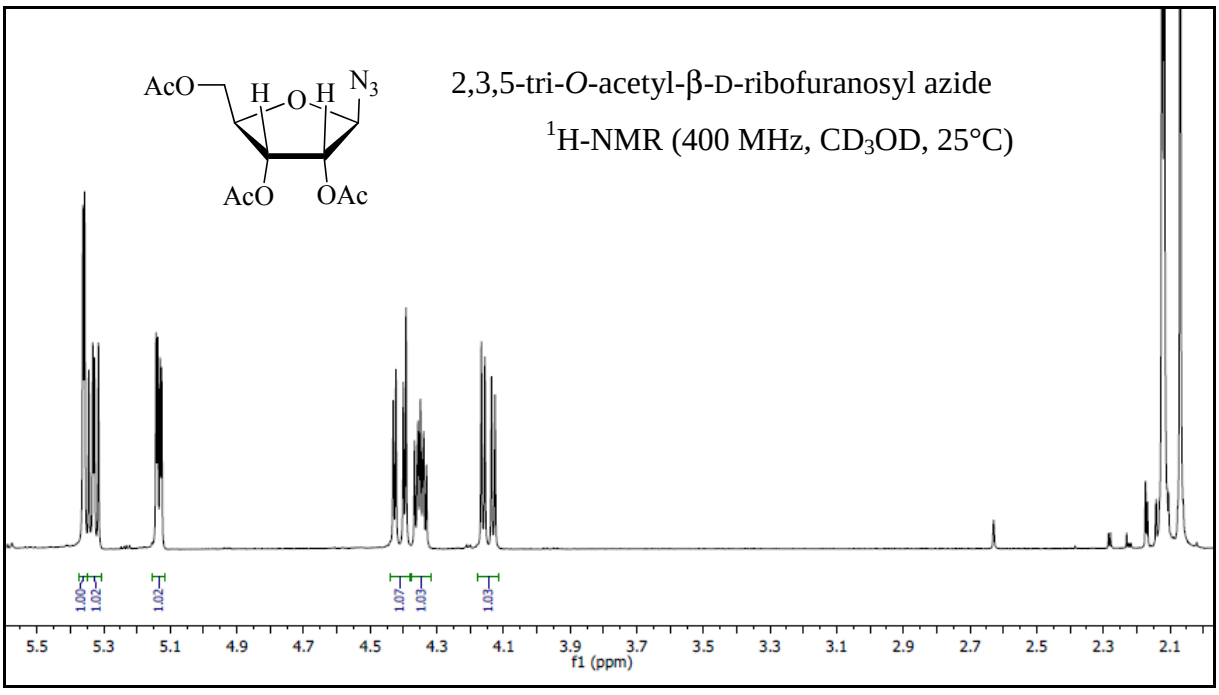
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.45 (s, 1H, H-1), 5.02 (dd, J = 2.8 Hz, J = 1.2 Hz, 1H, H-3), 4.99 (s, 1H, H-2), 4.45-4.38 (m, 2H, H-4, H-5), 4.27-4.20 (m, 1H, H-5'), 2.13, 2.12, 2.10 (3s, 9H, 3xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.7, 170.2, 169.7, 94.5 (C-1), 82.6 (C-4), 78.3 (C-3), 81.1 (C-2), 77.1 (C-3), 63.2 (C-5), 21-20.9 (3xOAc).



Synthesis of 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl azide (41)



See procedure for the synthesis of 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl azide **36**. The compound was purified by flash chromatography (hexane/acetone 80:20) Yield = 94 %
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.35 (d, $J_{1,2}$ = 2.0 Hz, 1H, H-1), 5.32 (dd, $J_{2,3}$ = 4.8 Hz, $J_{3,4}$ = 6.8 Hz, 1H, H-3), 5.13 (dd, $J_{1,2}$ = 2 Hz, $J_{2,3}$ = 4.8 Hz, 1H, H-2), 4.41 (dd, $J_{4,5}$ = 3.2 Hz, $J_{5,5'}$ = 12 Hz, 1H, H-5), 4.35 (ddd, $J_{3,4}$ = 6.8, Hz, $J_{4,5}$ = 3.2 Hz, $J_{4,5'}$ = 4.4 Hz, 1H, H-4), 4.14 (dd, $J_{4,5'}$ = 4.4 Hz, $J_{5,5'}$ = 12 Hz, 1H, H-5'),2.12, 2.11, 2.07 (3s, 9H, 3xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.7, 169.7, 169.5, 92.8 (C-1), 79.5 (C-4), 74.6 (C-2), 70.6 (C-3), 63.2 (C-5), 20.8, 20.7, 20.6 (3xOAc). NOESY (400 MHz, $CDCl_3$, 25°C): contact between H-1/H-4.

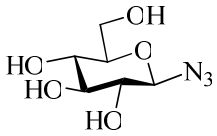


General procedure for the deprotection of peracetylated glycosyl azides.

A solution of NaOMe 0.1M in dry methanol (0.5 eq) was added, at room temperature and under nitrogen, to a solution of peracetylated glycosyl azides (1eq) in dry MeOH (0.1 M). The mixture

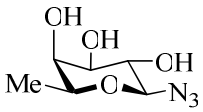
was stirred at room temperature. After 45 minutes TLC monitoring (eluents: hexane/AcOEt 50:50 and $CHCl_3$:MeOH 80:20) showed total consumption of the starting material, the acid resin Amberlyst IRA 120 H^+ was added. The mixture was stirred for 30 minutes (pH = 3). The resin was filtered and washed with MeOH, the solvent was removed under reduced pressure. The product, isolated in quantitative yield, was used without further purification.

β-D-glucopyranosyl azide (10)



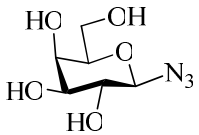
^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 4.48 (d, $J_{1,2}$ = 8.8 Hz, 1H, H-1), 3.87 (dd, $J_{5,6}$ = 2 Hz, $J_{6,6'}$ = 12 Hz, 1H, H-6), 3.67 (dd, $J_{5,6'}$ = 5.6 Hz, $J_{6,6'}$ = 12 Hz, 1H, H-6'), 3.38-3.31 (m, 2H, H-3, H-5), 3.30-3.25 (m, 1H, H-4), 3.13 (t, $J_{1,2}$ = 8.8 Hz, 1H, H-2). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 92.2 (C-1), 80.3 (C-5), 78.3 (C-3), 74.9 (C-2), 71.3 (C-4), 62.7 (C-6).

β-D-fucosyl azide (50)



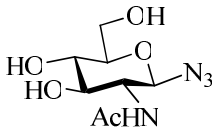
^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 4.41 (d, $J_{1,2}$ = 8 Hz, 1H, H-1), 3.73 (q, $J_{5,6}$ = 6.4 Hz, 1H, H-5), 3.64 (d, $J_{4,5}$ = 1.6 Hz, 1H, H-4), 3.47-3.41 (m, 2H, H-2, H-3), 1.29 (d, $J_{5,6}$ = 6.4 Hz, 3H, H-6). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 92.5 (C-1), 75.3, 74.4 (C-5), 73 (C-4), 71.9, 16.9 (C-6).

β-D-galactopyranosyl azide (43)

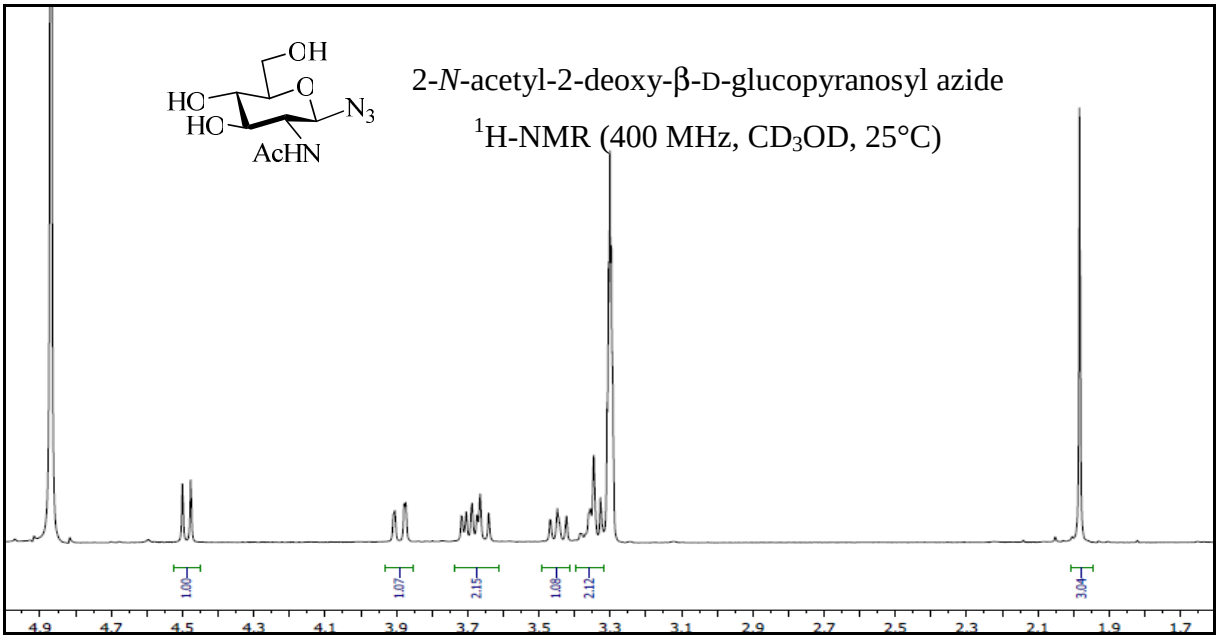


^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 4.42 (d, $J_{1,2}$ = 8 Hz, 1H, H-1), 3.86 (dd, $J_{4,5}$ = 2 Hz, 1H, H-4), 3.77 (dd, $J_{5,6}$ = 6.4 Hz, $J_{6,6'}$ = 11.6 Hz, 1H, H-6), 3.70 (dd, $J_{5,6'}$ = 5.2 Hz, $J_{6,6'}$ = 11.6 Hz, 1H, H-6'), 3.61 (dt, $J_{4,5}$ = 2 Hz, $J_{5,6}$ = 6.4 Hz, $J_{5,6'}$ = 5.2 Hz, 1H, H-5), 3.52-3.46 (m, 2H, H-2, H-3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 92.8 (C-1), 79 (C-5), 75.1, 72.1, 70.4 (C-4), 62.6 (C-6).

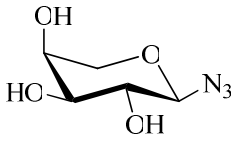
2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl azide (42)



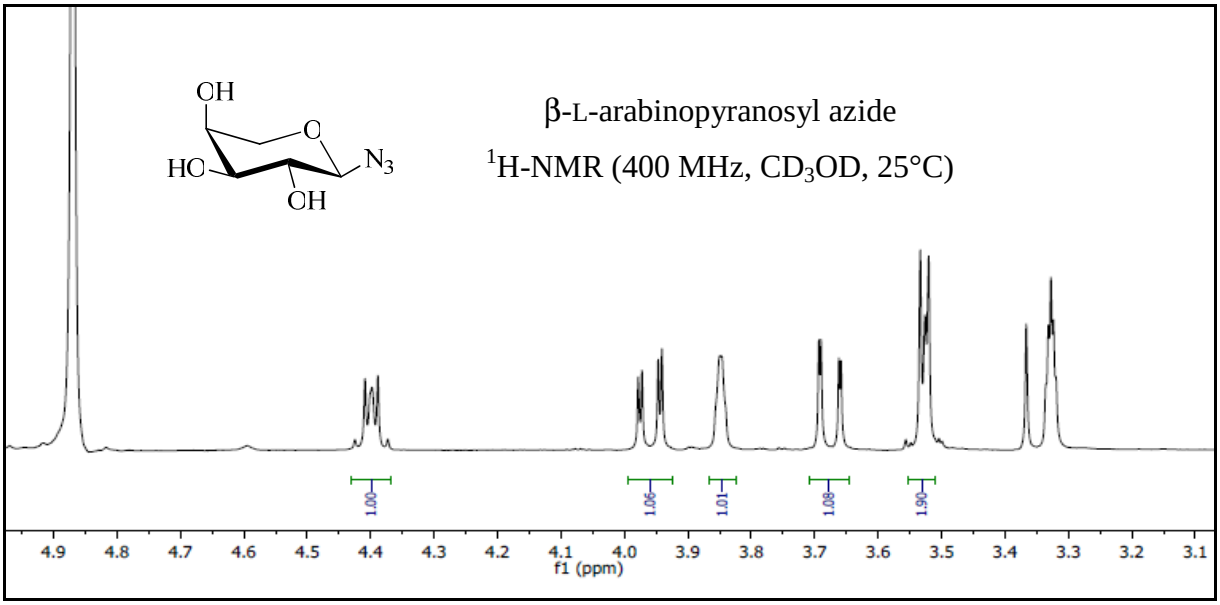
^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 4.48 (d, $J_{1,2}$ = 9.2 Hz, 1H, H-1), 3.88 (dd, $J_{5,6}$ = 1.6 Hz, $J_{6,6'}$ = 12 Hz, 1H, H-6), 3.72-3.64 (m, 2H, H-2, H-6), 3.45 (t, J = 10 Hz, 1H, H-3), 3.37-3.32 (m, 2H, H-4, H-5), 1.98 (s, 3H, OAc). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 173.9, 90.3 (C-1), 80.5 (C-5), 75.9 (C-3), 71.8 (C-4), 62.8 (C-6), 56.9 (C-2), 23 (CH_3).



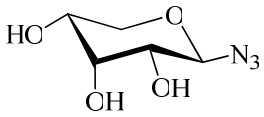
β-L-arabinopyranosyl azide (48)



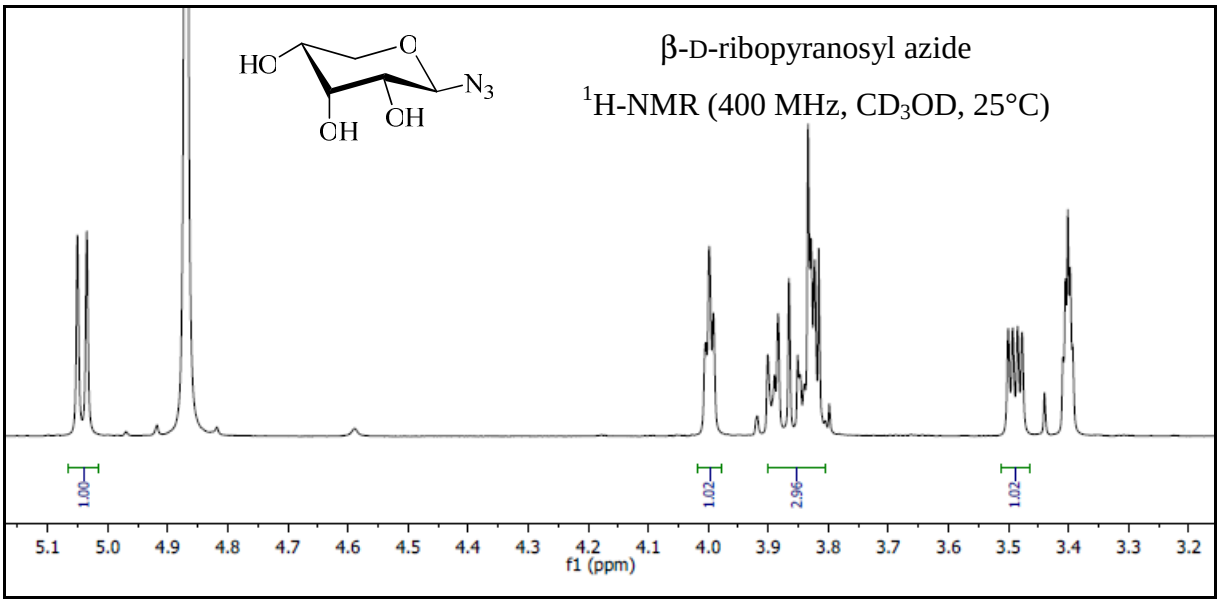
^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 4.44 (t, $J_{1,2}$ = 8 Hz, 1H, H-1), 3.95 (dd, $J_{4,5}$ = 2.4 Hz, $J_{5,5'}$ = 12.4 Hz, 1H, H-5), 3.88 (d, $J_{4,5'}$ = 1.6 Hz, 1H, H-4), 3.67 (dd, $J_{4,5'}$ = 1.6 Hz, $J_{5,5'}$ = 12.4 Hz, 1H, H-5'), 3.59-3.54 (m, 2H, H-2, H-3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 92.8 (C-1), 74.5, 72.1, 70 (C-4), 69.7 (C-5).



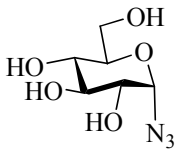
β-D-ribopyranosyl azide (45)



^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.04 (d, $J_{1,2}$ = 6 Hz, 1H, H-1), 3.99 (t, J = 2.4 Hz, 1H, H-4), 3.93-3.79 (m, 3H, H-3, H-5, H-5'), 3.48 (dd, $J_{1,2}$ = 6 Hz, J = 2.8 Hz, 1H, H-2). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 90.8 (C-1), 72.2 (C-2), 70 (C-4), 69.4 (C-3), 66.5 (C-5).

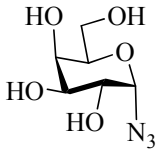


α-D-glucopyranosyl azide (7)



^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 5.46 (d, $J_{1,2}$ = 4 Hz, 1H, H-1), 3.81 (dd, $J_{6,6'}$ = 12 Hz, $J_{5,6}$ = 5.5 Hz, 1H, H-6), 3.72 (m, 1H, H-6'), 3.68 (t, J = 5.2 Hz, $J_{3,4}$ = 12 Hz, $J_{4,5}$ = 9.6 Hz, 1H, H-4), 3.58 (dd, J = 4 Hz, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 5.5 Hz, 1H, H-5), 3.51 (t, $J_{1,2}$ = 4 Hz, $J_{2,3}$ = 18.4 Hz, 1H, H-2), 3.33 (t, $J_{2,3}$ = 18.4 Hz, $J_{3,4}$ = 5.2 Hz, 1H, H-3).
^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 89.2 (C-1), 73.8 (C-4), 72.7 (C-5), 70.7 (C-2), 69.2 (C-3), 60.5 (C-6).

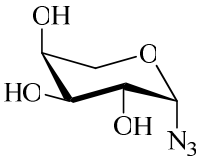
α-D-galactopyranosyl azide (47)



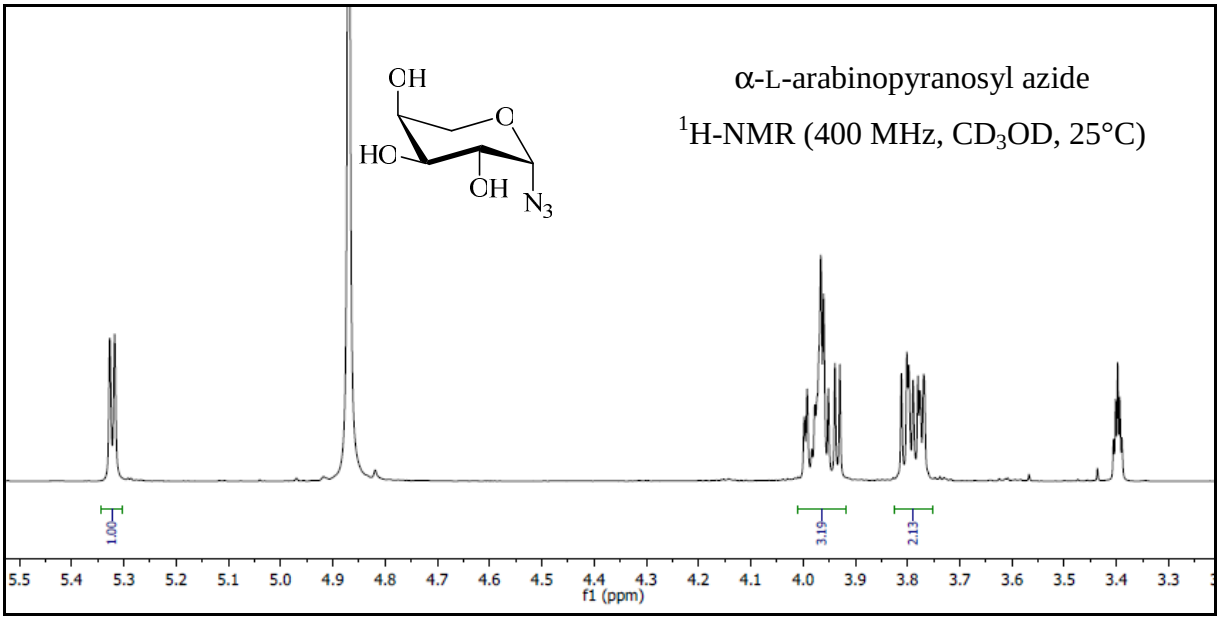
^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 5.48 (d, $J_{1,2}$ = 4.4 Hz, 1H, H-1), 3.99 (t, J = 6 Hz, 1H, H-5), 3.90 (d, J = 3.2 Hz, 1H, H-4), 3.84 (d, $J_{1,2}$ = 4.4 Hz, $J_{2,3}$ = 10.2 Hz, 1H, H-2), 3.71-3.64 (m, 3H,

H-3, H-6, H-6'). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 89.4 (C-1), 73.1 (C-5), 69.2 (C-3), 69 (C-4), 67.6 (C-2), 61.2 (C-6).

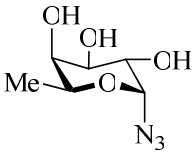
α-L-arabinopyranosyl azide (51)



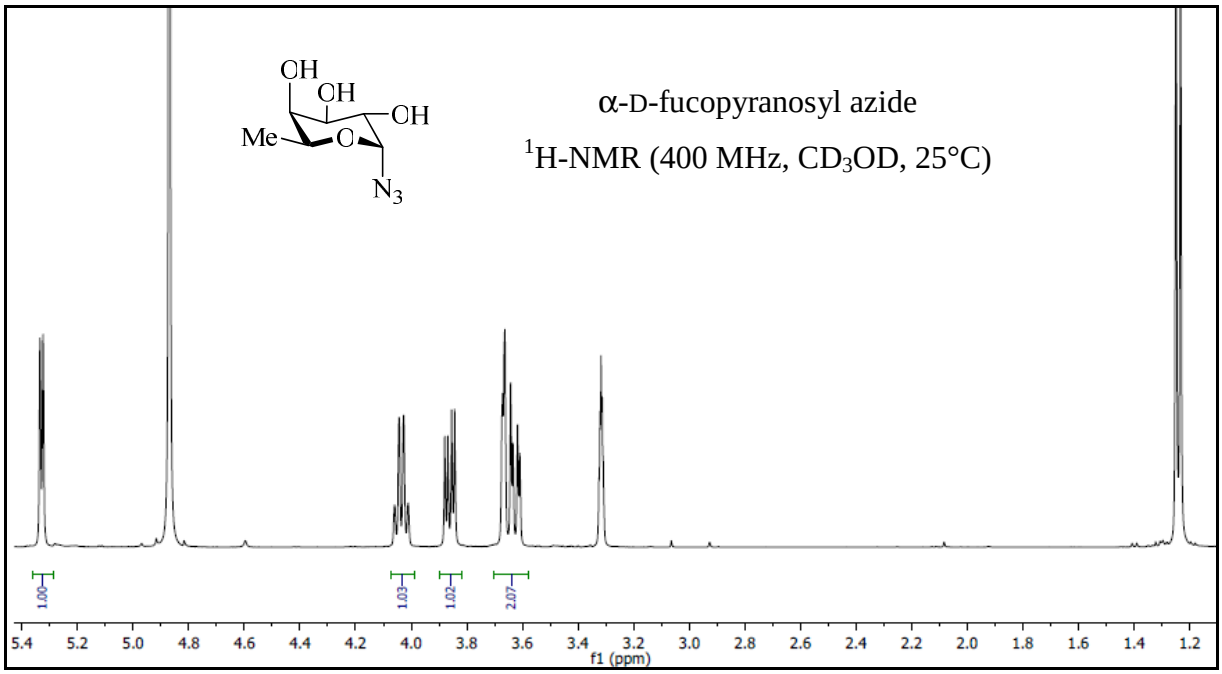
^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.32 (d, $J_{1,2}$ = 4 Hz, 1H, H-1), 4.02-3.93 (m, 3H, H-2, H-5), 3.81-3.77 (m, 2H, H-5'). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 91.5 (C-1), 70.9, 70.5, 69.5, 66.4 (C-5).



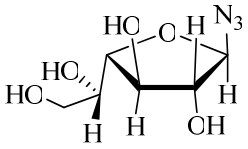
α-D-fucosyl azide (44)



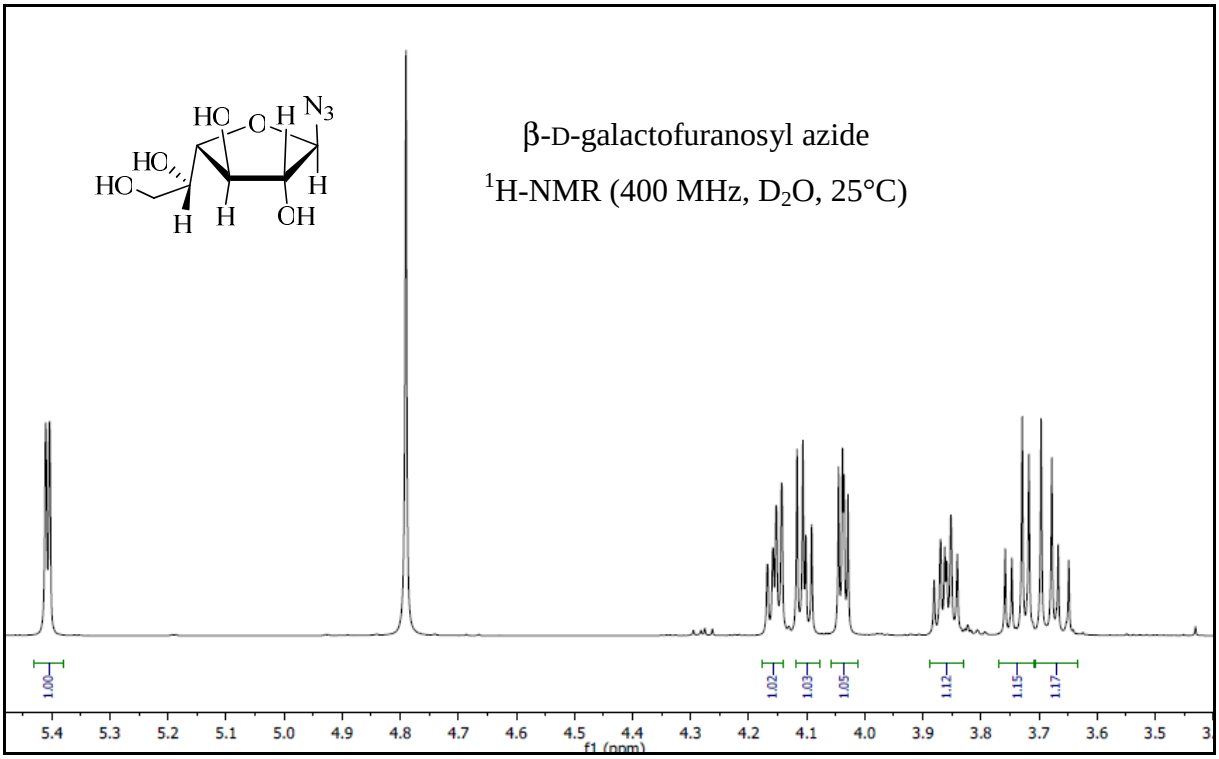
^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.32 (d, $J_{1,2}$ = 4.4 Hz, 1H, H-1), 4.03 (q, $J_{5,6}$ = 6.8 Hz, 1H, H-5), 3.86 (dd, $J_{1,2}$ = 4.4 Hz, $J_{2,3}$ = 10 Hz, 1H, H-2), 3.66 (d, $J_{3,4}$ = 3.2 Hz, 1H, H-4), 3.62 (dd, $J_{2,3}$ = 10 Hz, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 1.24 (d, $J_{5,6}$ = 6.8 Hz, 3H, H-6). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 92.4 (C-1), 73.4 (C-4), 71.5 (C-3), 70.2 (C-5), 69.5 (C-2), 16.8 (C-6).



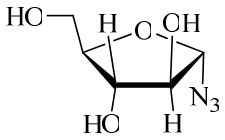
β-D-galactofuranosyl azide (37)



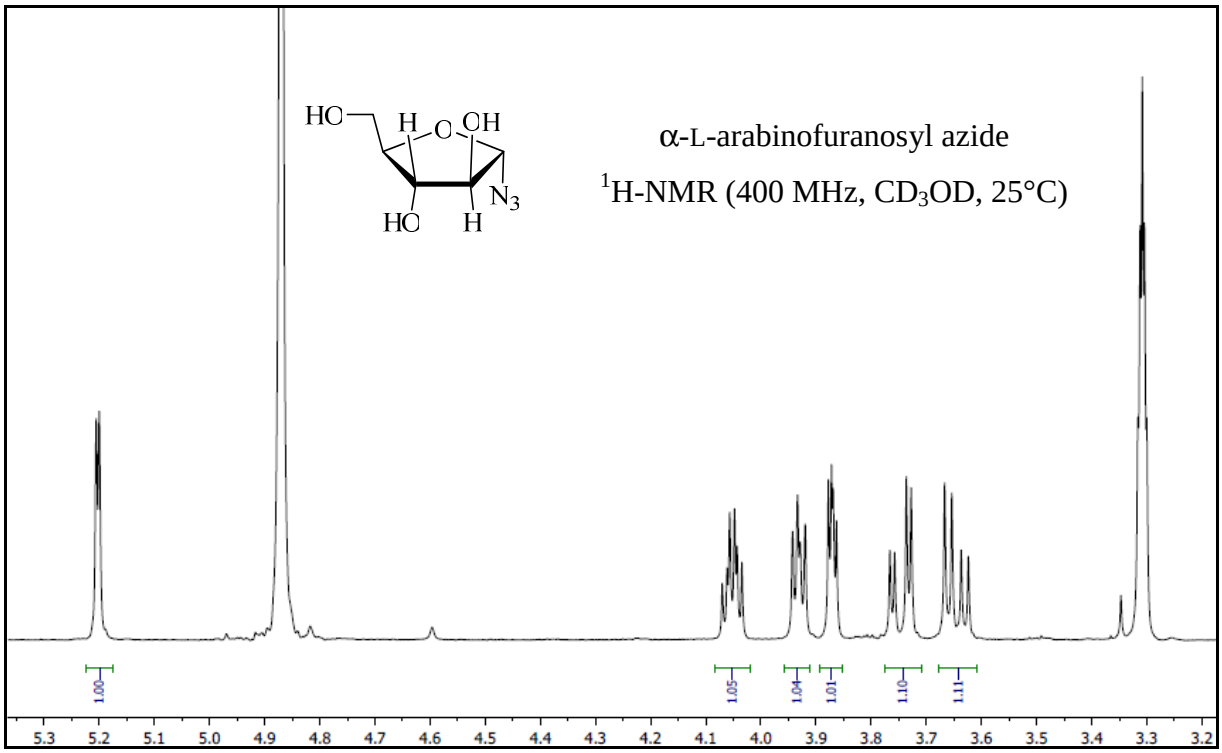
^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 5.31 (d, $J_{1,2}$ = 2.8 Hz, 1H, H-1), 4.06 (ABX system, part A dd, $J_{2,3}$ = 4 Hz, $J_{3,4}$ = 10 Hz 1H, H-3), 4.01 (ABX system, part B, dd, $J_{3,4}$ = 10.4 Hz, $J_{4,5}$ = 4.4 Hz, 1H, H-4), 3.94 (ABX system, part X, dd, $J_{1,2}$ = 2.8 Hz, $J_{2,3}$ = 3.6 Hz, 1H, H-2), 3.80-3.73 (ddd, $J_{4,5}$ = 4.4 Hz, $J_{5,6'}$ = 7.2 Hz, $J_{5,6}$ = 4.8 Hz, 1H, H-5), 3.64 (dd, $J_{5,6}$ = 4.8 Hz, $J_{6,6'}$ = 11.6 Hz, 1H, H-6), 3.57 (dd, $J_{5,6'}$ = 7.2 Hz, $J_{6,6'}$ = 11.6 Hz, 1H, H-6'). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 95.3 (C-1), 84.2 (C-4), 80.6 (C-2), 76.4 (C-3), 70.7 (C-5), 62.6 (C-6). $[\alpha]^{D}_{25}$ = -153 (c 1, MeOH). NOESY (400 MHz, D_2O, 25°C): contact between H-1/H-3.



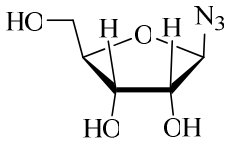
α-L-arabinofuranosyl azide (49)



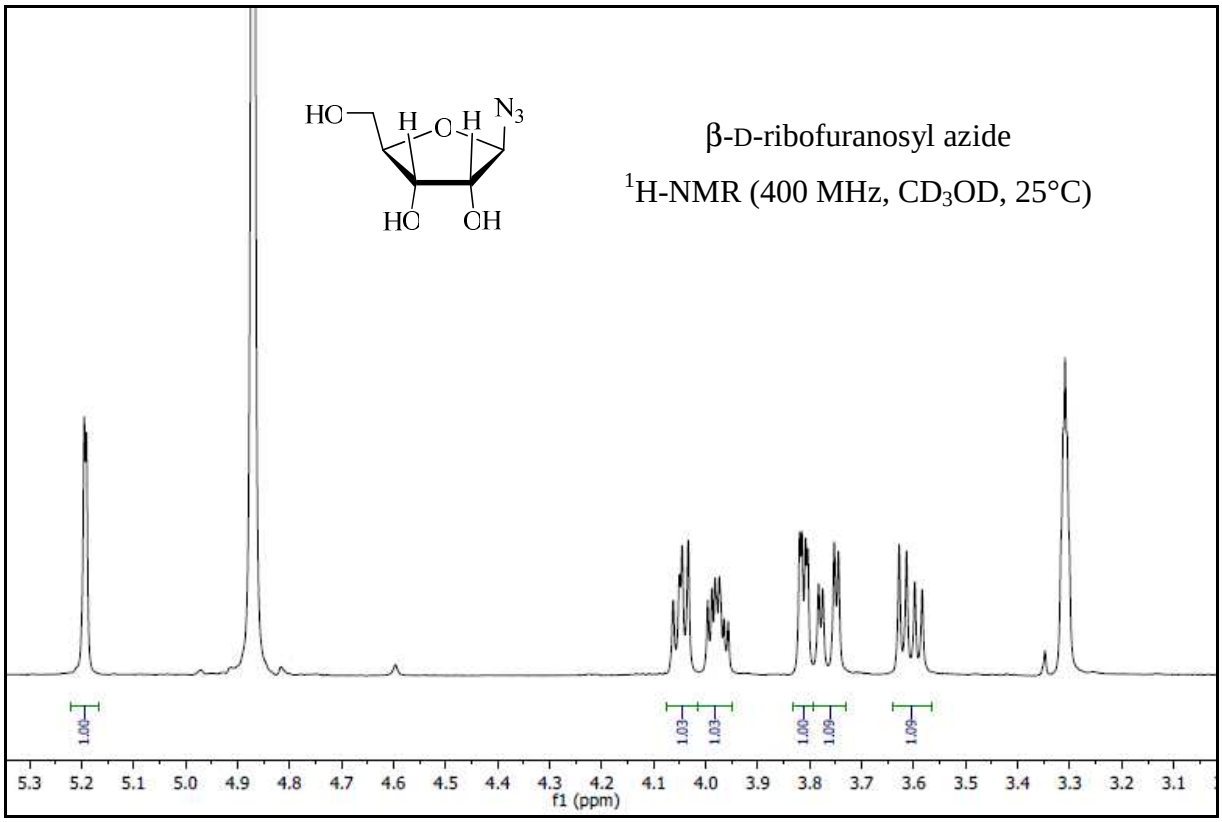
^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.25 (d, $J_{1,2}$ = 2 Hz, 1H, H-1), 4.11 (ddd, $J_{3,4}$ = 5.6 Hz, $J_{4,5}$ = 3.6 Hz, $J_{4,5'}$ = 5.2 Hz, 1H, H-4), 3.99 (dd, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 5.6 Hz, 1H, H-3), 3.93 (dd, $J_{1,2}$ = 2 Hz, $J_{2,3}$ = 3.6 Hz, 1H, H-2), 3.80 (dd, $J_{4,5}$ = 3.6 Hz, $J_{5,5'}$ = 12 Hz, 1H, H-5), 3.70 (dd, $J_{4,5'}$ = 5.2 Hz, $J_{5,5'}$ = 12 Hz, 1H, H-5'). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 97.9 (C-1), 87.8 (C-4), 83.2 (C-2), 78.4 (C-3), 63 (C-5).



β-D-ribofuranosyl azide (53)



^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.19 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 4.04 (dd, $J_{2,3}$ = 4.8 Hz, $J_{3,4}$ = 6.8 Hz, 1H, H-3), 3.97 (ddd, $J_{3,4}$ = 6.8 Hz, $J_{4,5}$ = 3.2 Hz, $J_{4,5'}$ = 6 Hz, 1H, H-4), 3.81 (dd, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 4.8 Hz, 1H, H-2), 3.76 (dd, $J_{4,5}$ = 3.2 Hz, $J_{5,5'}$ = 12 Hz, 1H, H-5), 3.60 (dd, $J_{4,5'}$ = 6 Hz, $J_{5,5'}$ = 12 Hz, 1H, H-5'). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 96.7 (C-1), 85.7 (C-4), 76.6 (C-2), 72.2 (C-3), 63.8 (C-5).


HO
H
O
H
N3
HO
OH
β-D-ribofuranosyl azide
1H-NMR (400 MHz, CD3OD, 25°C)
1.00
1.03
1.03
1.00
1.09
1.09
5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1
f1 (ppm)

2.7 Reference

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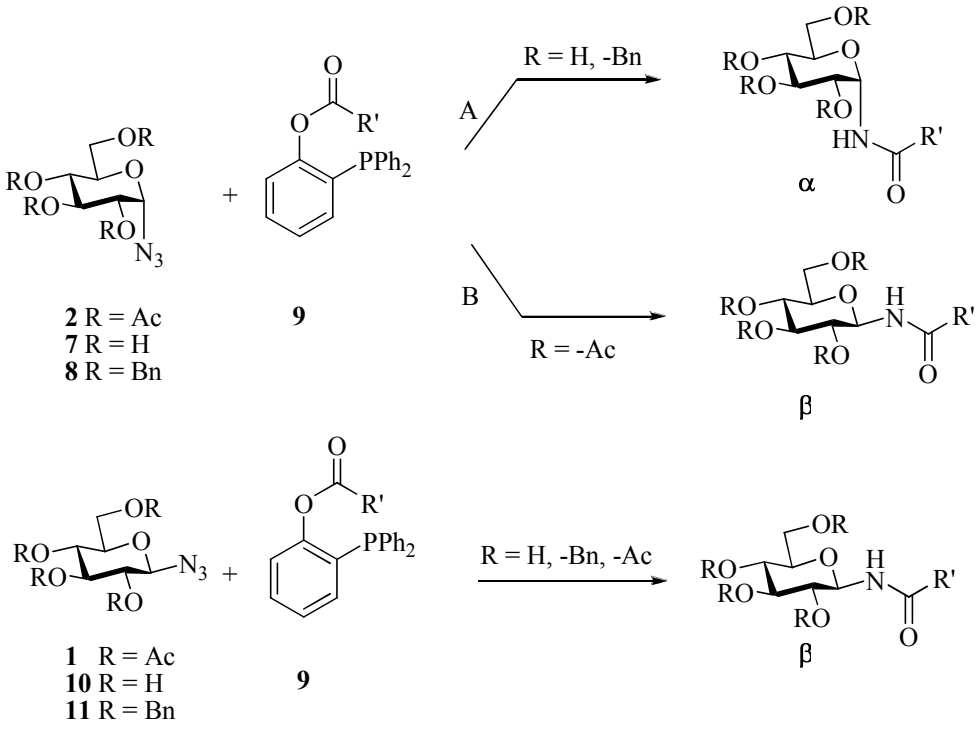
Chapter 3

Synthesis of different phosphines

In order to develop a stereoselective Staudinger ligation for the synthesis of glycosyl amides, it was decided to employ the procedure based on the Staudinger reduction-acylation process of the corresponding glycosyl azides. Our methods to achieve a stereoselective synthesis of glycosyl amides with traceless Staudinger ligation are described in Chapters 4-6. Before that, the procedures employed for the synthesis of phosphines are described in the following sections.

3.1 Synthesised phosphines

The traceless Staudinger ligation affords one approach to the synthesis of unnatural α-glycosyl amides, starting from the corresponding α-azides, because it allows for reduction of the starting material and immediate trapping of the Staudinger aza-ylide intermediate in an intramolecular fashion, thus resulting in the direct formation of an amide link. Fast, intramolecular acylation of the anomeric nitrogen can in many cases prevent epimerisation and give retention of configuration at the anomeric carbon. In 2006 [1] and 2008 [2] our laboratory has reported that the traceless Staudinger ligation of α-glycosyl azides **2**, **7** and **8** with diphenylphosphanyl-phenyl esters **9** in polar aprotic solvents yields α-glycosyl amides with good yields and selectivity (Scheme 1).



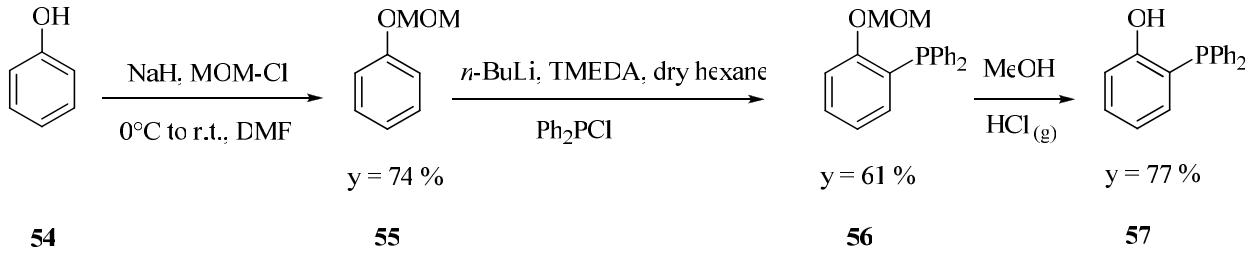
Scheme 1. Traceless Staudinger ligation of glycosyl azides with functionalized phosphine [1, 2]

The steric course of the reaction is controlled both by configuration of the starting azide and by the presence and nature of protecting groups on the pyranose ring. In fact, the *O*-acetyl azide **2** allows full inversion of the anomeric center and yields the β-amide only. The phosphines **9** employed are air stable and can be easily isolated and purified by flash chromatography.

The process described, however, left various synthetic problems unresolved. The reactivity of α-glycosyl azides was uniformely low, and the corresponding amides were obtained generally in modest yields. Moreover, the yields of the ligation appeared to depend critically also on the nature of the acyl group to be transferred and were specially disappointing for the transfer of amino acids to α-azides. Thus, in order to further explore the reactivity and stereoselectivity of ligation in the synthesis of α-glycosyl amides, different acyl phosphines were prepared, trying to vary the basicity of the P atom and the nature of the phenyl ester leaving group.

3.2 Synthesis of diphenylphosphanyl-phenyl esters

Phosphines **9** had been obtained in the course of a previous thesis[3] starting from *o*-diphenylphosphinophenol **57**. This was prepared in three steps starting from phenol and using a procedure introduced by Rauchfuss with small modifications.[4] Firstly, the hydroxyl group was protected as the methoxymethyl ether:[5] the presence of the MOM-ether both protects and activates the phenol ring. Secondly, the diphenylphosphino group was attached by ortho lithiation of the ether **55** (BuLi, hexane, TMEDA) followed by reaction with chlorodiphenylphosphine (Ph_2PCl). Finally, the hydroxyl group was deprotected with HCl in MeOH to afford **57** (Scheme 2).



Scheme 2. Synthesis of *o*-diphenylphosphinophenyl ester **57**

Interestingly Luo *et al.* found that treatment of other methoxymethyl-protected phenols bearing phosphine groups with HCl led to phosphonium salts because of the basicity of the phosphine

moiety.[6] As the authors noted, in the previously reported preparation of **57**, a base-neutralisation step was omitted but the crude product was sublimed, which is consistent with known loss of HCl from phosphonium salts at elevated temperatures. In fact, **57** in our hands exhibited a single ^{31}P resonance at δ = -26.6 ppm, whereas omission of the base-neutralisation step led to the hydrochloride salt **58** with a ^{31}P resonance at + 21.6 ppm (Figure 1).[7]

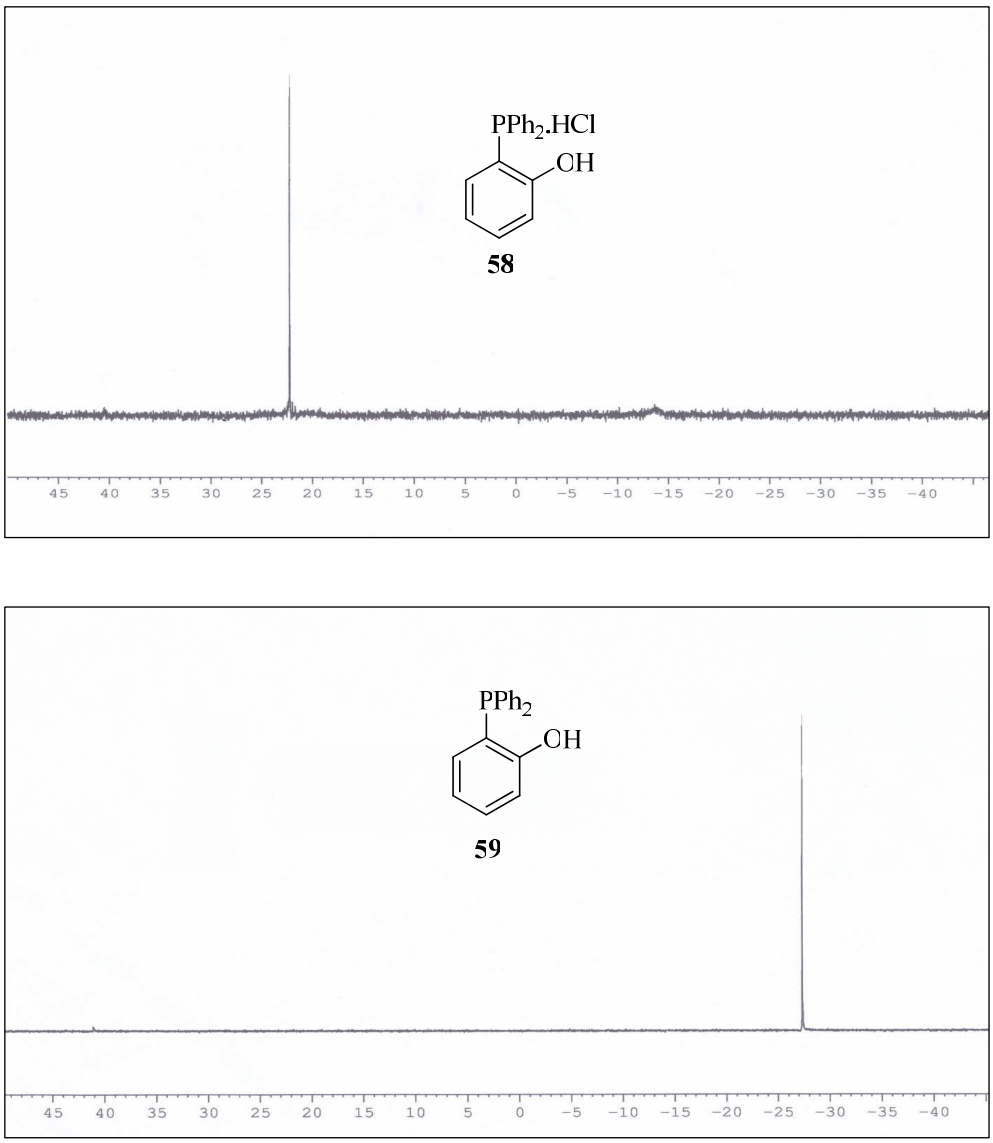


Figure 1. ^{31}P resonance of hydrochloride salt of *o*-diphenylphosphinophenol **58** (up) and *o*-diphenylphosphinophenol **57** (down)

o-Diphenylphosphinophenol **57** was then acylated to give the corresponding functionalised phosphines which were used for the Staudinger ligation reactions.

3.3 Synthesis of the diphenyl- and dialkyl-phosphanylphenols

Following a similar approach, in the course of this thesis a group of other functionalized diphenyl- and dialkyl-phosphanylphenols was prepared. This include the diphenylphosphanyl-4-methoxyphenol **59** (Figure 2),[8] diphenylphosphanyl-4-fluorophenol **60**[8] and several novel phoshines (**61**, **62**, **63** and **64** Figure 2) with various substituents at phosphorus and characterized by an electronpoor phenol ring.

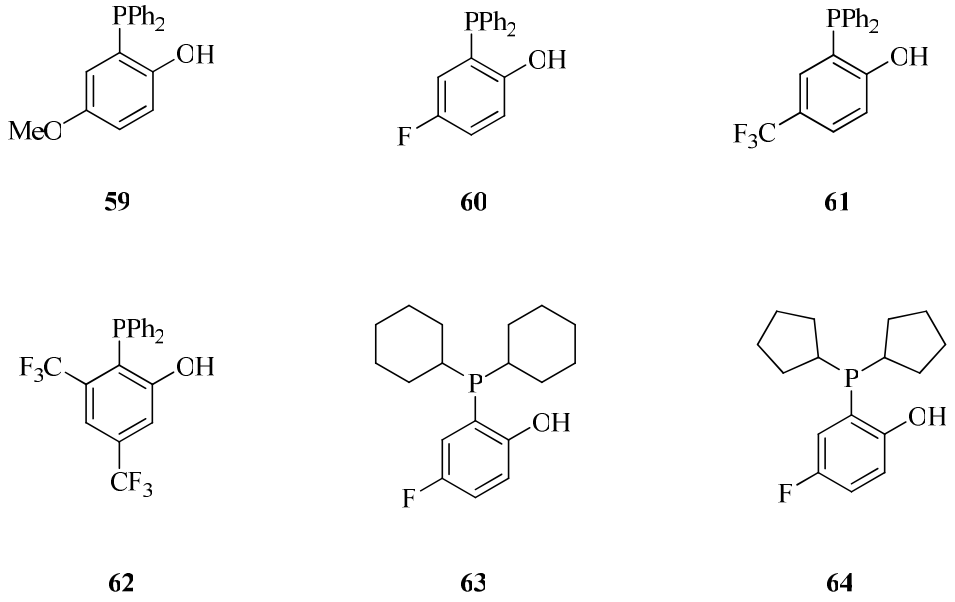
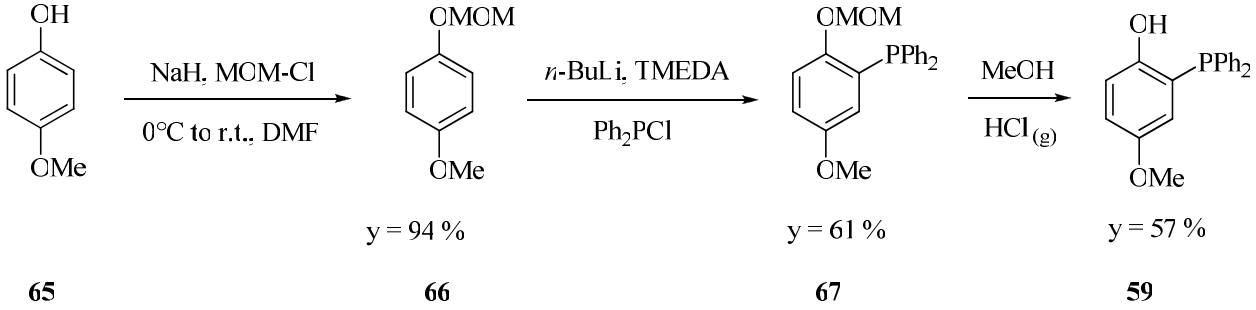


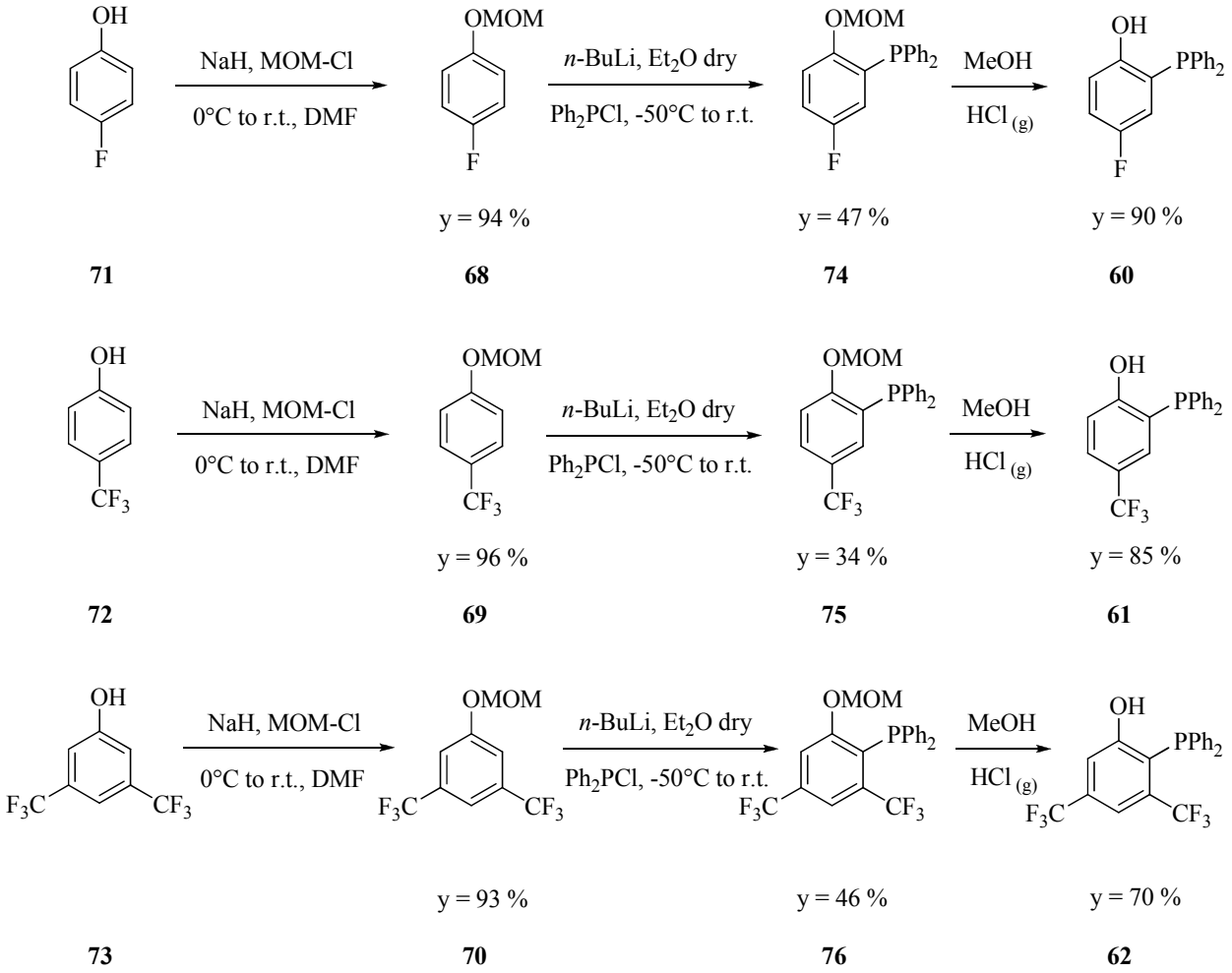
Figure 2. Set of diphenylphosphanyl-phenols (**59** to **64**) synthesized in this work

The diphenylphosphanyl-4-methoxyphenol **59** (Scheme 3) is a known compound:[8] it was prepared following exactly the same procedure described for **57** (Scheme 3) [3,4] and the analytical data were consistent with the reported structure.



Scheme 3. Synthesis of the diphenylphosphanyl-4-methoxyphenol **59**

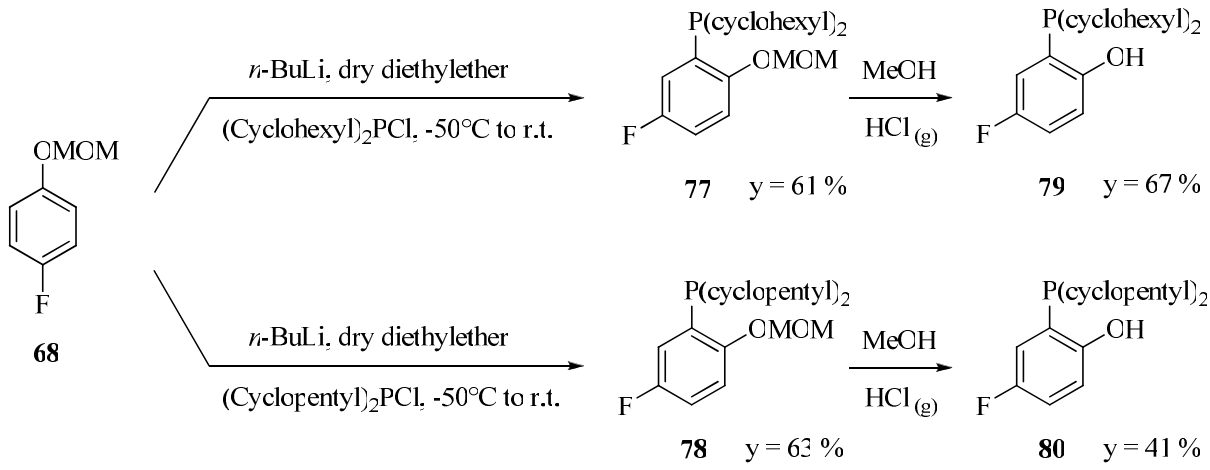
For the synthesis of **60**-**62** the required fluorinated-methoxymethyl phenyl ethers **68**-**70** (Scheme 4) were synthesised as usual by the reaction of phenols **71**-**73** with sodium hydride followed by treatment with chloromethyl methyl ether (MOM-Cl) (Scheme 4).[4] Metalation and reaction with the appropriate chlorophosphine (Scheme 4) was performed according to Fink[8], using diethyl ether rather than hexane, in the absence of TMEDA and at low temperature -50°C, probably to avoid *ortho* metalation respect to the flourus atom.[9] Finally, HCl deprotection afforded the required phosphines **60**, **61** and **62**.



Scheme 4. Synthesis of fluorinated-*o*-diphenylphosphinophenol

Similarly, Fink [8] metalation of fluorinated-methoxymethyl phenyl ether **68** followed by reaction with commercially available chlorodicyclohexylphosphine or chlorodicyclopentylphosphine, gave the methoxymethyl *o*-dicyclohexylphosphinophenyl ether **77** and methoxymethyl *o*-dicyclopentylphosphinophenyl ether **78** (Scheme 5), which were deprotected with HCl, as usual,

to yield **79** and **80**. Both these phosphines are air stable and can be easily isolated and purified by flash chromatography.



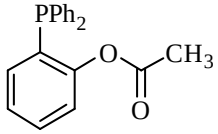
Scheme 5. Synthesis of the dialkyl-phosphanylphenols **79** and **80**

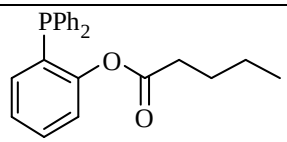
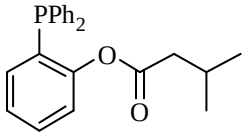
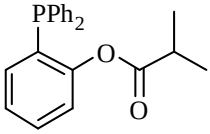
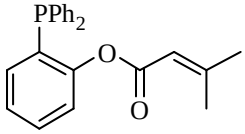
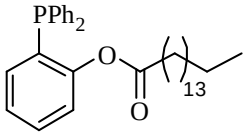
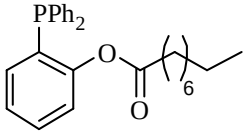
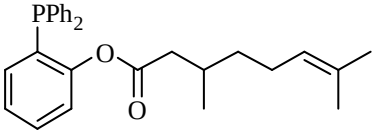
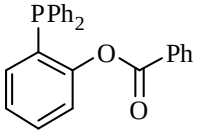
3.4 Acylation of the phosphanylphenols

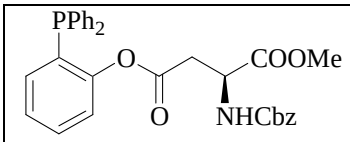
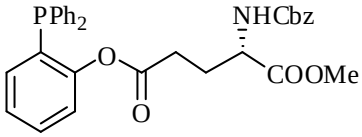
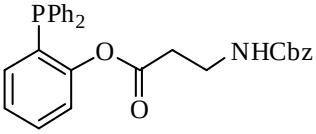
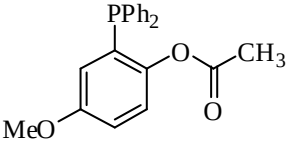
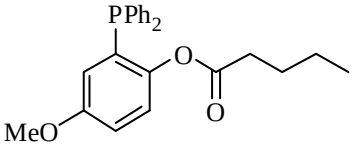
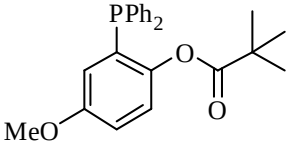
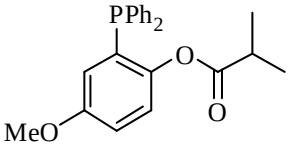
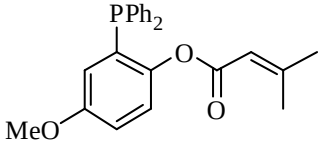
The phosphanylphenols described above were acylated with standard methods, which vary depending on the nature of the acyl chain employed. In general, the corresponding acyl chlorides were used, when commercially available. Benzoates were obtained from benzoic anhydride. Condensing agents (DCC or EDC) were employed for amino acids.

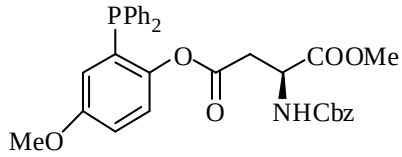
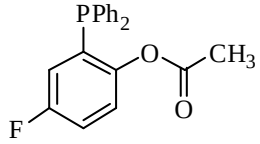
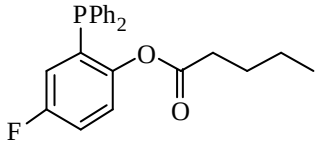
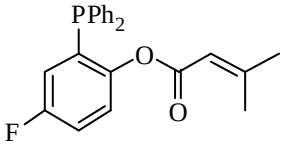
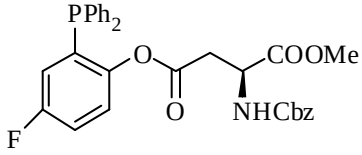
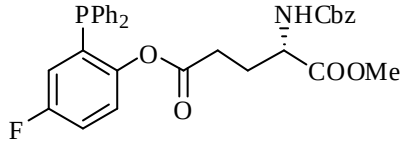
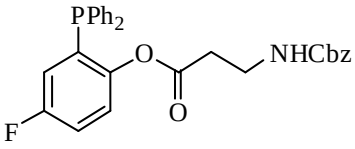
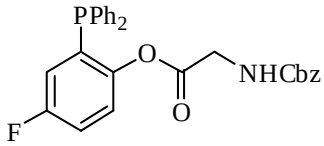
All the compounds prepared are collected in Table 1, where the method of acylation is indicated. All other details are reported in the experimental section of this chapter.

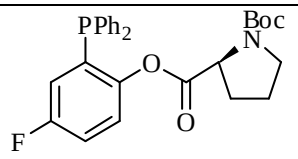
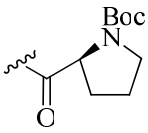
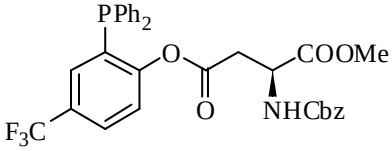
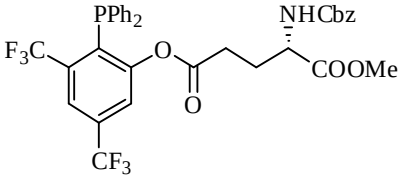
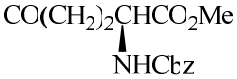
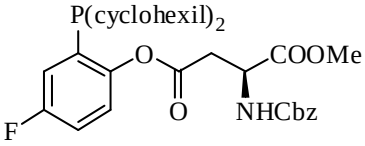
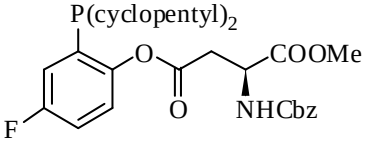
Table 1. *O*-acylphosphanylphenols prepared

Compound	**Starting phosphanylphenol**	**R-C=O**	**Acylation method**	**Y (%)**
 **81a**	**57**	CH_3CO	Ac_2O, Pyridine, DMAP	Quant

 **81b**	**57**	$CH_3(CH_2)_3CO$	RCOCl/TEA	96 %
 **81c**	**57**	$(CH_3)_2CHCH_2CO$	RCOCl/TEA	97 %
 **81d**	**57**	$(CH_3)_2CHCO$	RCOCl/TEA	87 %
 **81e**	**57**	$(CH_3)_2C=CHCO$	RCOCl/TEA	84 %
 **81f**	**57**	$CH_3(CH_2)_{14}CO$	RCOCl/TEA	85 %
 **81g**	**57**	$CH_3(CH_2)_6CO$	RCOOH/DCC DMAP	90 %
 **81h**	**57**	citronellic acid	RCOOH/DCC DMAP	75 %
 **81i**	**57**	PhCO	$(PhCO)_2O$, Pyridine, DMAP	Quant

 **81j**	**57**	$COCH_2CHCO_2Me$ NHCbz	RCOOH/EDC, DIPEA, DMAP	85 %
 **81k**	**57**	$CO(CH_2)_2CHCO_2Me$ NHCbz	RCOOH/EDC, DIPEA, DMAP	75 %
 **81l**	**57**	$CO(CH_2)_2NHCbz$	RCOOH/DCC DMAP	82 %
 **82a**	**59**	CH_3CO	Ac_2O, Pyridine, DMAP	Quant
 **82b**	**59**	$CH_3(CH_2)_3CO$	RCOCl/TEA	87 %
 **82c**	**59**	$(CH_3)_3CCO$	RCOCl/TEA	83 %
 **82d**	**59**	$(CH_3)_2CHCO$	RCOCl/TEA	82 %
 **82e**	**59**	$(CH_3)_2C=CHCO$	RCOCl/TEA	84 %

 **82j**	**59**	$COCH_2CHCO_2Me$ NHCbz	RCOOH/EDC, DIPEA, DMAP	75 %
 **83a**	**60**	CH_3CO	Ac_2O, Pyridine, DMAP	Quant
 **83b**	**60**	$CH_3(CH_2)_3CO$	RCOCl/TEA	95 %
 **83e**	**60**	$(CH_3)_2C=CHCO$	RCOCl/TEA	91 %
 **83j**	**60**	$COCH_2CHCO_2Me$ NHCbz	RCOOH/EDC, DIPEA, DMAP	85 %
 **83k**	**60**	$CO(CH_2)_2CHCO_2Me$ NHCbz	RCOOH/EDC, DIPEA, DMAP	85 %
 **83l**	**60**	$CO(CH_2)_2NHCbz$	RCOOH/DCC DMAP	92 %
 **83m**	**60**	$COCH_2NHCbz$	RCOOH/DCC DMAP	90 %

 **83l**	**60**		RCOOH/DCC DMAP	94 %
 **84j**	**61**		RCOOH/EDC, DIPEA, DMAP	90 %
 **85k**	**62**		RCOOH/EDC, DIPEA, DMAP	95 %
 **86j**	**79**		RCOOH/EDC, DIPEA, DMAP	80 %
 **87j**	**80**		RCOOH/EDC, DIPEA, DMAP	80 %

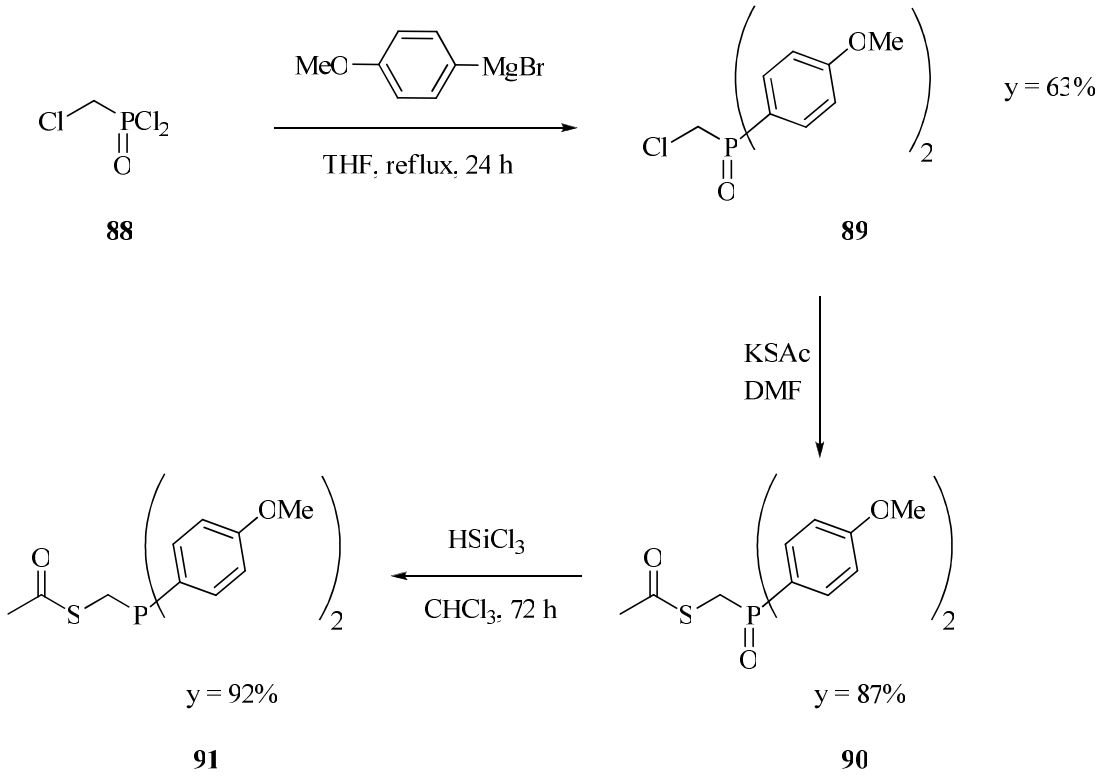
3.5 Synthesis of diphenylphosphinothiol ester

The Staudinger ligation has also been proposed for the ligation of peptides en route to the total chemical synthesis of proteins.[10] (Diphenylphosphino)methanethiol is the most efficacious of known reagents, in this case providing high isolated yields for equimolar couplings in which a glycine residue is at the nascent junction.

This reagent has been used in the orthogonal assembly of a protein,[11] site-specific immobilization

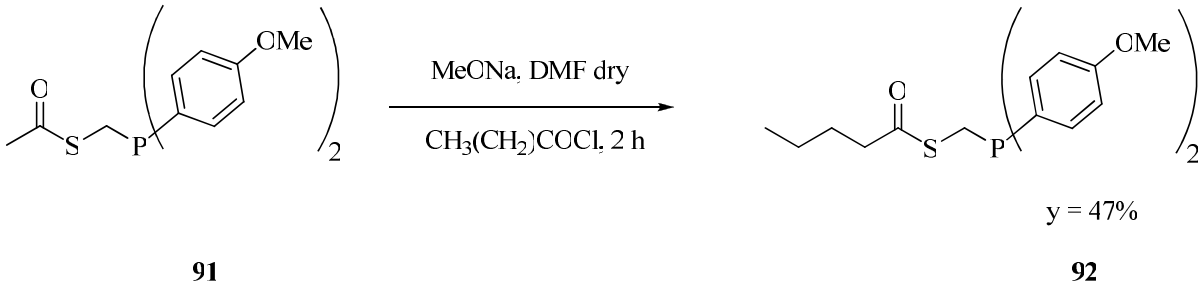
of peptides and proteins to a surface,[12, 13] and synthesis of glycopeptides.[14] We prepared it to test it in the ligation of α-glycosyl azides. The diphenylphosphinothiol ester **91** was synthesised by treating the chloromethylphosphonic dichloride **88** with 4-methoxyphenylmagnesium bromide

and potassium thioacetate to give phosphine oxide **88**. Thus the desired compound **91** was prepared for reduction with trichlorosilane from corresponding oxide **89** (Scheme 6).[10]



Scheme 6. Synthesis of phosphinothioester **91**

Also in this case, the diphenylphosphinothiol ester **91** was functionalized with valeroyl chloride to give phosphine **92** (Scheme 7).

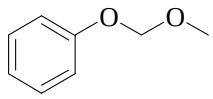


Scheme 7. Synthesis of phosphine **92**

3.6 Experimental Section

Solvents were dried by standard procedures: dichloromethane, methanol, *N*,*N*-diisopropylethylamine and triethylamine were dried over calcium hydride; *N*,*N*-dimethylformamide, *N*,*N*,*N'*,*N'*-tetramethylethylenediamine, diethyl ether, chloroform and pyridine were dried over activated molecular sieves. Reactions requiring anhydrous conditions were performed under nitrogen. ^{1}H, ^{13}C and ^{31}P-NMR spectra were recorded at 400 MHz on a Bruker AVANCE-400 instrument. Chemical shifts (δ) for ^{1}H and ^{13}C spectra are expressed in ppm relative to internal Me_4Si as standard. Chemical shifts (δ) for ^{31}P are expressed in ppm relative to internal H_3PO_4 as standard. Signals were abbreviated as s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained with a Bruker ion-trap Esquire 3000 apparatus (ESI ionization). Thin layer chromatography (TLC) was carried out with pre-coated Merck F_{254} silica gel plates. Flash chromatography (FC) was carried out with Macherey-Nagel silica gel 60 (230-400 mesh).

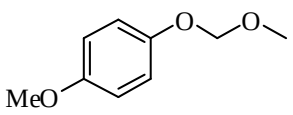
Synthesis of methoxymethyl phenyl ether (55)



At 0 °C and with stirring, phenol **54** (5 g, 53.1 mmol, 1 eq) was added to a slurry of NaH (3.19 g, 79.7 mmol, 1.5 eq 60 % in oil) in dimethylformamide (26.5 mL, 2 M). After 30 minutes, chloromethyl methyl ether (6.05 mL, 79.7 mmol, 1.5 eq) was added, then the temperature was allowed to warm up to room temperature. The mixture was stirred and the reaction was monitored by TLC (8:2 hexane/AcOEt). After 30 minutes, water (106 mL) was added. The product was exctracted with hexane (3 x 52 mL). The organic layer was dried over Na_2SO_4 and concentrated. Yield = 74 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.38-7.31 (m, 11H, Ph), 7.13 (m, 1H), 6.93 (m, 1H), 6.72 (m, 1H), 5.19 (s, 2H, CH_2O), 3.49 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 158.6, 136.9, 134.4, 134.0, 133.7, 130.4, 128.9, 128.7, 128.5, 122.2, 113.6, 113.6, 94.3, 56.1.

Synthesis of methoxymethyl-4-methoxyphenyl ether (66)

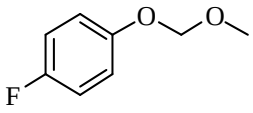


At 0 °C and with stirring, phenol **65** (2.98 g, 24 mmol, 1 eq) was added to a slurry of NaH (90.9 mg, 36 mmol, 1.5 eq) in dimethylformamide (12 mL, 2 M). After 30 minutes, chloromethyl methyl ether (2.72 mL, 36 mmol, 1.5 eq) was added, then the temperature was allowed to warm up to room temperature. The mixture was stirred and the reaction was monitored by TLC (80:20 hexane/AcOEt). After 30 minutes, water (60 mL) was added. The product was exctracted with hexane (3 x 25 mL). The organic layer was dried over Na_2SO_4 and concentrated. yield = 94 %.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.01 (dd, J = 2.4 Hz, J = 7.2 Hz, 2H, ArH), 6.85 (dd, J = 2 Hz, J = 6.8 Hz, 2H, ArH), 5.13 (s, 2H, CH_2O), 3.78 (s, 3H, OCH_3), 3.49 (s, 3H, CH_3).

General procedure for the synthesis of the methoxymethyl-fluorinated phenyl ether

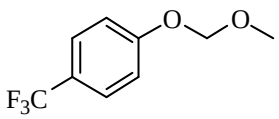
At 0 °C and with stirring, fluorinated phenol **71**, **72** and **73** (1 eq) was added to a slurry of NaH (1.5 eq) in dimethylformamide (2 M). After 30 minutes, chloromethyl methyl ether (1.5 eq) was added, then the temperature was allowed to warm up to room temperature. The mixture was stirred and the reaction was monitored by TLC (80:20 hexane/AcOEt). After 30 minutes, water was added. The product was exctracted with hexane (3 x 50 mL). The organic layer was dried over Na_2SO_4 and concentrated.

4-fluorophenyl methoxymethyl ether (68)



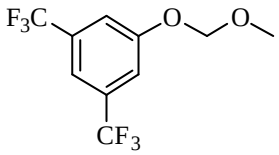
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.04-6.98 (m, 4H, Ph), 5.16 (s, 2H, CH_2O), 3.51 (s, 3H, OCH_3). Yield = 94 %.

4-trifuoromethylphenyl methoxymethyl ether (69)



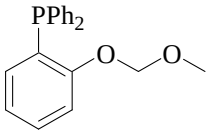
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.54 (d, J = 8 Hz, 2H, ArH), 7.10 (d, J = 8 Hz, 2H, ArH), 5.22 (s, 2H, CH_2O), 3.48 (s, 3H, OCH_3). Yield = 96 %.

3,5-ditrifuoromethylphenyl methoxymethyl ether (70)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.52 (s, 1H, ArH), 7.49 (s, 2H, ArH), 5.26 (s, 2H, CH_2O), 3.50 (s, 3H, OCH_3). Yield = 93 %.

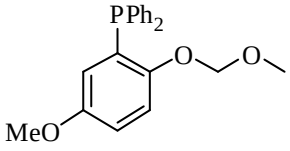
Synthesis of methoxymethyl *o*-diphenylphosphinophenyl ether (56)



A solution containing 1.6 M *n*-BuLi (10 mL, 16 mmol, 1.1 eq) and *N*,*N*,*N*',*N*'-tetramethylendiamine (2.24 mL, 14.9 mmol, 1.03 eq) in dry hexane was added, under nitrogen, to an ice-cooled solution of methoxymethyl phenyl ether **55** (2 g, 14.5 mmol, 1 eq) in dry hexane (final concentration: 0.5 M). The temperature was allowed to warm up to room temperature. The solution became yellow and a pale precipitate formed after 1.5 h. The solution was cooled in ice and chlorodiphenylphosphine (2.74 mL, 14.5 mmol eq) was added slowly. The resultant solution was stirred for 16 h at room temperature and the solvent evaporated *in vacuo*. The residue was diluted with Et_2O and washed with 1 M aqueous Na_2HPO_4. The organic layer was dried over Na_2SO_4 and concentrated to give a white solid. The solid was dissolved in MeOH. After cooling for 30 minutes, the crystals were collected and recrystallized from CH_2Cl_2-MeOH; yield = 61 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.38-7.31 (m, 11H, Ph), 7.13 (m, 1H), 6.93 (m, 1H), 6.72 (m, 1H), 5.06 (s, 2H, CH_2O), 3.16 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 158.6, 136.9, 134.4, 134.0, 133.7, 130.4, 128.9, 128.7, 128.5, 122.2, 113.6, 113.6, 94.3, 56.1. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.8.ppm

Synthesis of methoxymethyl 2-(diphenylphosphino)-4-methoxyphenyl ether (67)



A solution containing 1.6 M *n*-BuLi (8.1 mL, 13 mmol, 1.05 eq) and *N,N,N',N'*-tetramethylendiamine (1.74 mL, 12.2 mmol, 1.01 eq) in dry hexane was added, under nitrogen, to an ice-cooled solution of methoxymethyl phenyl ether **66** (2.02 g, 12 mmol, 1 eq) in dry hexane (final concentration: 0.5 M). The temperature was allowed to warm up to room temperature. The solution became yellow and a pale precipitate formed after 1.5 h. The solution was cooled in ice and chlorodiphenylphosphine (2.19 mL, 12 mmol, 1 eq) was added slowly. The resultant solution was stirred for 16 h at room temperature and the solvent evaporated *in vacuo*. The residue was diluted with DCM and washed with 1 M aqueous NaH_2PO_4. The organic layer was dried over Na_2SO_4 and concentrated to give a orange oil. The oil was dissolved in MeOH. After cooling for over-night, the white crystals were collected and dried *in vacuo*; yield = 61 %.

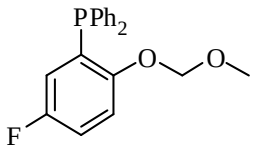
^{1}H-NMR (400 MHz, C_6D_6, 25°C): δ = 7.50-7.40 (m, 4H, Ph), 7.10 (dd, J = 8.9 Hz, J = 3.1 Hz, 1H, H-6), 7.07-7.01 (m, 6H, Ph), 6.71 (dd, J = 8.9, J = 3.1 Hz, 1H, H-5), 6.60 (dd, J = 4.5 Hz, J = 3.1 Hz, 1H, H-3), 4.78 (s, 2H, CH_2O), 3.18 (s, 3H, OCH_3),3.04 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, C_6D_6, 25°C): δ = 156.0, 154.4, 138.3, 135.1, 129.6, 129.5, 129.4, 128.5, 120.3, 116.2, 115.7, 95.7, 56.3, 55.6. ^{31}P-NMR (161 MHz, C_6D_6, 25°C): δ = - 14.1 ppm

General procedure for the synthesis of the 2-(diphenylphosphanyl)-fuorinated-phenyl methoxymethyl ether

A solution of *n*-BuLi in hexane (1.6 M, 1.1 eq) was added dropwise with stirring to a solution of methoxymethyl-fluorinated phenol **68**, **69** and **70** (1 eq) in diethyl ether at -50°C and then allowed to warm to room temperature. After 3 h the solution was cooled again to -50°C, a

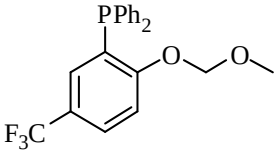
solution of chlorodiphenylphosphane (1.05 eq) in diethyl ether was added dropwise, and the suspension was stirred overnight at room temperature. The precipitate was removed, the solvent was replaced by dichloromethane, and methanol was added. At 0°C white crystals separated and dried *in vacuo*.

2-(Diphenylphosphanyl)-4-fuorophenyl methoxymethyl ether (74)



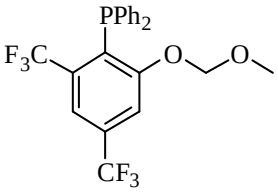
^{1}H-NMR (400 MHz, C_6D_6, 25°C): δ = 7.40-7.32 (m, 4H, Ph), 7.05-7.00 (m,6H, Ph), 6.91-6.84 (m, 1H, H-6), 6.78-6.70 (m, 2H, H-2, H-3), 4.65 (s, 2H, OCH_2) 2.94 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, C_6D_6, 25°C): δ = 158.5, 155.4, 136.8, 134.4, 130.3, 129.1, 128.8, 120.4, 116.5, 115.3, 94.7, 55.7. ^{31}P-NMR (161 MHz, C_6D_6, 25°C): δ = -14.6 ppm. Yield = 47 %

2-(Diphenylphosphanyl)-4-trifuoromethylphenyl methoxymethyl ether (75)



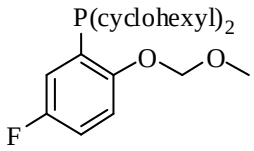
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.54 (m, 1H), 7.36-7.7.27 (m, 10H, Ph), 7.16 (dd, J = 4 Hz, J = 8.8 Hz, 1H, Ar-H), 6.93 (t, J = 3.2 Hz, 1H), 5.11 (s, 1H, CH_2O), 3.15 (s, 3H, OCH_3). ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = -14.4 ppm [oxide ^{31}P = + 26.8 ppm]. Yield = 34 %.

2-(Diphenylphosphanyl)-3,5-ditrifuoromethylphenyl methoxymethyl ether (76)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.52-7.7.28 (m, 12H, Ph), 4.71(s, 1H, CH_2O), 2.96 (s, 3H, OCH_3). Yield = 46 %

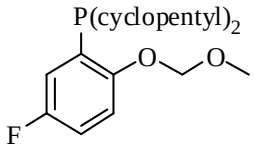
Synthesis of the 2-(dicyclohexylphosphanyl)-4-fluorophenyl methoxymethyl ether (77)



A solution of *n*-BuLi in hexane 1.6 M, (8.27 mL, 13.2 mmol, 1.05 eq) was added dropwise with stirring to a solution of 4-fluorophenyl methoxymethyl ether **68** (1.97 g, 12.6 mmol, 1 eq) in diethyl ether at -50°C and then allowed to warm to room temperature. After 3 h the solution was cooled again to -50°C, a solution of chlorodicyclohexylphosphane (3.02 g, 12.6 mmol, 1 eq) in diethyl ether was added dropwise, and the suspension was stirred overnight at room temperature. The precipitate was removed, then the solvent was evaporated giving a yellow oil. The crude product was purified by flash chromatography using 90:10 hexane/AcOEt as eluent to afford **77** in 61 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.13-7.07 (m, 2H, ArH), 7.02-6.92 (m, 1H, ArH), 5.18 (s, 2H, CH_2O), 3.52 (s, 3H, CH_3), 2.01-1.53 (m, 14H, Cyhex), 1.41-1.13 (m, 8H, Cyhex). ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 8.6 ppm [oxide ^{31}P = + 48.9 ppm].

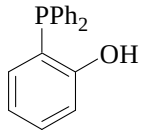
Synthesis of the 2-(dicyclopentylphosphanyl)-4-fluorophenyl methoxymethyl ether (78)



A solution of *n*-BuLi in hexane (2.13 mL, 3.41 mmol, 1.1 eq) was added dropwise with stirring to a solution of 4-fluorophenyl methoxymethyl ether **68** (506.4 mg, 3.24 mmol, 1 eq) in diethyl ether at -50°C and then allowed to warm to room temperature. After 3 h the solution was cooled again to -50°C, a solution of chlorodicyclopentylphosphane (639 μL, 3.24 mmol, 1.05 eq) in diethyl ether was added dropwise, and the suspension was stirred overnight at room temperature. The precipitate was removed and the crude without further purification was used in the deprotection step.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.20-7.02 (m, 2H), 7.01-6.91 (m, 1H), 5.22 (s, 2H, CH_2O), 3.48 (s, 3H, CH_3), 2.26-2.19 (m, 2H), 2.14-2.03 (m, 2H), 2.01-1.32 (m, 14H, Cypent.).

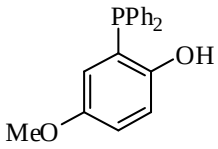
Synthesis of *o*-diphenylphosphinophenol (57)



5 mL of dry methanol were saturated with $HCl_{(g)}$. Methoxymethyl *o*-diphenylphosphinophenyl ether **56** (1 g, 3.1 mmol, 1 eq) was added at room temperature and under argon. The resulting solution was stirred for 1 h then the solvent was evaporated giving a yellow oil. The residue was dissolved in dry MeOH (3 mL), then water was added until the cloud point was reached. The solution was stirred under argon while cooling, and the resulting white solid was filtered; yield = 77 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.43-7.26 (m, 11H, Ph), 7.05-6.86 (m, 3H), 6.42 (bs, 1H, OH). ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 26.6 ppm [oxide ^{31}P = + 40.6 ppm].

Synthesis of 2-(diphenylphosphino)-4-methoxyphenol (59)



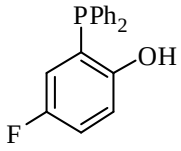
5 mL of dry methanol were saturated with $HCl_{(g)}$. Methoxymethyl *o*-diphenylphosphinophenyl ether **67** (2.48 g, 7 mmol, 1 eq) was added at room temperature and under nitrogen. The resulting solution was stirred for 1 h then the solvent was evaporated giving a orange oil. The residue was diluted with AcOEt (100 mL) and washed with $NaHCO_3$ saturated. The organic layer was dried over Na_2SO_4 and concentrated to give a yellow solid. The crude product was purified by flash chromatography using 80:20 hexane/AcOEt as the eluent to afford **59** in yield = 57 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.72-7.40 (m, 10H, Ph), 7.03-6.86 (m, 1H, H-6), 6.88 (bd, 1H, H-5), 6.50 (bd, 1H, H-3), 5.9 (bs, 1H, OH), 3.61 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, C_6D_6, 25°C): δ = 153.7, 153.5, 133.5, 132.0, 130.0, 128.9, 128.3, 118.8, 118.2, 116.9, 55.7. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 21 ppm

General procedure for the synthesis of the 2-(diphenylphosphanyl)-fuorinated-phenol

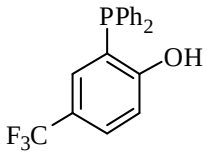
Dry methanol were saturated with $HCl_{(g)}$. Methoxymethyl *o*-diphenylphosphino-fluorinated-phenyl ether (1 eq) was added at room temperature and under nitrogen. The resulting solution was stirred for 1 h then the solvent was evaporated giving a brown oil. The reaction mixture was diluted with AcOEt and extracted with sat. $NaHCO_3$ and water: the organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography, as indicated in each individual case.

2-(Diphenylphosphanyl)-4-fuorophenol (60)



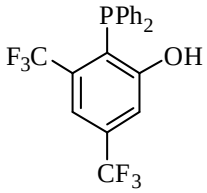
The compound was purified by flash chromatography (hexane/AcOEt 80:20) yield = 90 %. ^{1}H-NMR (400 MHz, C_6D_6, 25°C): δ = 7.30-7.21 (m, 4H,Ph), 7.03-6.93 (m, 6H, Ph), 6.84 (m, 1H, H-3), 6.66 (m, 1H, H-3), 6.51 (m, 1H, H-6) 6.10 (bs, 1H, OH). ^{13}C-NMR (100 MHz, C_6D_6, 25°C): δ = 157.6, 155.7, 135.4, 133.9, 129.3, 129.0, 124.3, 120.1, 118.1, 116.9. ^{31}P-NMR (161 MHz, C_6D_6, 25°C): δ = - 24.8 ppm.

2-(Diphenylphosphanyl)-4-trifuoromethylphenol (61)



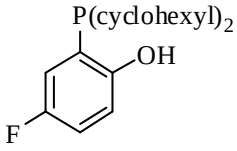
The compound was purified by flash chromatography (hexane/AcOEt 80:20) yield = 85 %. ^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42 (d, J = 8.8 Hz, 1H), 7.27-7.7.15 (m, 10H, Ph 7.12 (s, 1H), 6.86 (dd, J = 4.8 Hz, J = 8.4 Hz, 1H), 6.42 (bs, 1H, OH). ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 26.8 ppm [oxide ^{31}P = + 41.3 ppm].

2-(Diphenylphosphanyl)-3,5-ditrifuoromethylphenol (62)



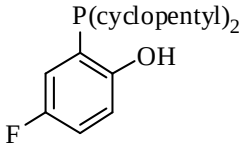
The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 70 %. ^{1}H-NMR (400 MHz, C_6D_6, 25°C): δ = 7.71 (s, 1H), 7.52-7.24 (m, 11H, Ph). ESI-MS: *m/z* 437.28 (M+Na).

Synthesis of the 2-(dicyclohexylphosphanyl)-4-fluorophenol (79)



The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 67 %. ^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.01-6.97 (m, 2H, Ar-H), 6.91-6.84 (m ,1H, Ar-H), 2.01-1.87 (m, 4H, Cyhex), 1.84-1.56 (m, 8H, Cyhex), 1.41-1.02 (m, 10H, Cyhex).

Synthesis of the 2-(dicyclopentylphosphanyl)-4-fluorophenol (80)



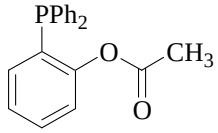
The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 41 %. ^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.02-6.99 (m, 1H), 6.96-6.92 (m, 1H), 6.87-6.82 (m, 1H), 6.76-6.68 (bs, 1H, OH), 2.19-2.04 (m, 2H), 2.02-1.94 (m, 2H), 1.75-1.32 (m, 14H, Cypent).

General procedure for the synthesis of the *o*-diphenylphosphanyl-phenyl acetate

Acetic anhydride (1.2 eq) and *N*,*N*-dimethylaminopyridine (0.1 eq) were added, at room temperature and under nitrogen, to a solution of *o*-diphenylphosphinophenol (1 eq) in dry

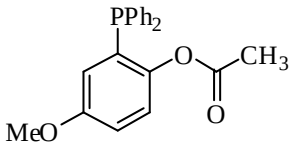
pyridine (0.15 M). The solution was stirred for 2 h and then concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with 10 % aqueous HCl, 5 % aqueous $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated to give the product.

_o_-Diphenylphosphanyl-phenyl acetate (81a)



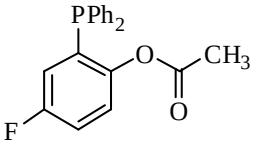
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.40-7.32 (m, 11H, Ph), 7.16 (m, 2H), 6.87 (m, 1H), 2.0 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 169.1, 153.1 (d, *J* = 17 Hz), 149.6, 136.6, 135.8, 135.6, 134.4, 134.0, 133.9, 131.8, 130.1, 129.3, 128.9, 128.7, 128.7, 126.3, 124.1, 122.8, 20.7. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.7 ppm. Quantitative yield.

2-(Diphenylphosphanyl)-4-methoxyphenyl acetate (82a)



1H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.29 (m, 10H, Ph), 7.11 (dd, *J* = 4.4 Hz, *J* = 8.2 Hz, 1H, H-6), 6.91 (dd, *J* = 3.2 Hz, *J* = 8.8 Hz, 1H, H-5), 6.42-6.38 (m, 1H, H-3), 3.16 (s, 3H, OCH3), 1.99 (s, 3H, CH3). Quantitative yield.

2-(Diphenylphosphanyl)-4-fluorophenyl acetate (83a)

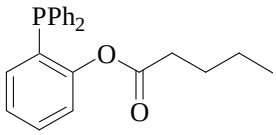


^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45-7.36 (m, 10H, Ph), 7.16-7.09 (m, 1H), 7.07-7.04 (m, 1H), 6.57-6.52 (m, 1H), 1.97 (s, 3H, CH_3). Quantitative yield.

General procedure for the synthesis of the *o*-diphenylphosphanyl-phenyl esters

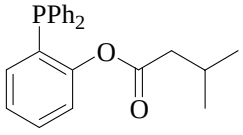
Dry triethylamine (1.1 eq) and the acyl chloride (1.2 eq) were added, at room temperature and under argon, to a solution of the *o*-diphenylphosphinophenol (1 eq) in dry CH_2Cl_2 (0.1 M). The reaction mixture was stirred at room temperature and monitored by TLC (90:10 hexane/AcOEt) until disappearance of the *o*-diphenylphosphinophenol (*ca.* 1 h). The solvent was then evaporated under reduced pressure and the residue was diluted with AcOEt and washed with 5 % aqueous $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated. The crude product obtained was purified by flash chromatography, as indicated in each individual case.

***o*-Diphenylphosphanyl-phenyl pentanoate (81b).**



The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 96 %.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.40-7.31 (m, 11H, Ph), 7.19-7.12 (m, 2H), 6.86-6.82 (m, 1H), 2.28 (t, J = 7.5 Hz, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.29 (m, 2H, CH_2), 0.89 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.8, 153.2 (d, J = 17 Hz), 134.7, 134.5, 134.0, 133.9, 133.8, 132.2, 131.8, 130.1, 129.2, 128.9, 128.8, 128.7, 126.2, 125.9, 125.7, 122.8, 33.9, 26.7, 22.3, 13.9. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.8 ppm [oxide ^{31}P = + 27.2 ppm].

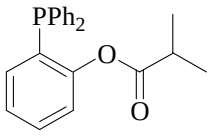
***o*-Diphenylphosphanyl-phenyl 3-methyl-butanoate (81c)**



The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 97 %.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.32 (m, 11H, Ph), 7.20-7.12 (m, 2H), 6.88-6.83 (m, 1H), 2.17 (d, J = 7.0 Hz, 2H, CH_2), 2.05 (m, 1H, CH), 0.94 (d, J = 7.0 Hz, 6H, 2xCH_3).^{13}C-

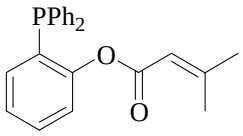
NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.9, 152.9 (d, J = 17 Hz), 135.8 (d, J = 16 Hz), 134.5, 134.4, 134.1, 133.9, 133.8, 133.5, 131.7 (d, J = 9 Hz), 130.2 (d, J = 14 Hz), 130.2, 129.9, 129.4, 129, 128.6, 128.6, 126.0, 125.6, 125.5, 123.9, 123.9, 122.6, 42.9, 25.3, 22.4. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 15.0 ppm [oxide ^{31}P = + 27.1 ppm].

_o_-Diphenylphosphanyl-phenyl _i_-butanoate (81d)



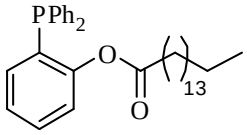
The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 87 %.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.26 (m, 11H, Ph), 7.18-7.12 (m, 2H), 6.83-6.78 (m, 1H), 2.55 (m, J = 7.0 Hz, 1H, CH), 1.10 (d, J = 7.0 Hz, 6H, 2xCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 174.7, 153 (d, J = 17 Hz), 135.7 (d, J = 10 Hz), 134.1, 133.9, 133.7, 131.8 (dd, J = 10 Hz), 130.17 (d, J = 15 Hz), 129.9, 128.9, 128.6, 128.6, 126, 122.5, 34.1, 18.6. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 15.1 [oxide ^{31}P = + 28.9 ppm].

_o_-Diphenylphosphanyl-phenyl 3-methyl-but-2-enoate (81e)



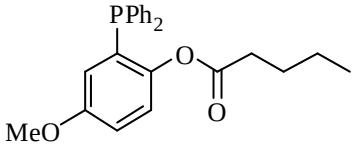
The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 84 %.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.43-7.32 (m, 11H, Ph), 7.22-7.12 (m, 2H), 6.93-6.86 (m, 1H), 5.69 (m, 1H, CH), 2.09 (d, J = 1.2 Hz, 3H, CH_3), 1.90 (d, J = 1.2 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 164.1, 159.6, 152.9 (d, J = 17 Hz), 135.9 (d, J = 10 Hz), 134.1, 133.9, 133.7, 131.7 (d, J = 10 Hz), 130.5 (d, J = 14 Hz), 129.8, 129.0, 128.8, 128.4, 125.8, 122.8, 114.9, 27.5, 20.4. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.8 ppm [oxide ^{31}P = + 26.8 ppm].

_o_-Diphenylphosphanyl-phenyl palmitate (81f)



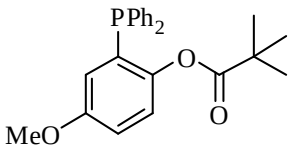
The compound was purified by flash chromatography (hexane/AcOEt 95:5) yield = 85 %. ^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.28 (m, 11H, Ph), 7.18-7.08 (m, 2H), 6.82-6.76 (m, 1H), 2.23 (t, J = 7.2 Hz, J = 8 Hz, 2H, CH_2), 1.48 (t, J = 7.2 Hz, J = 6.8 Hz, 2H, CH_2), 1.41-1.17 (m, 24H, 12xCH_2), 0.88 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.8, 134.3, 134.1, 133.9, 129.2, 126.2, 122.8, 35.2, 34.3, 32.2, 29.9, 29.8, 29.6, 29.4, 29.3, 29.1, 24.7, 22.9, 14.3. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.3 ppm [oxide ^{31}P = + 27.3 ppm].

2-(Diphenylphosphanyl)-4-methoxyphenyl pentanoate (82b)



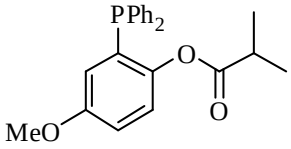
The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 87 % ^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.40-7.31 (m, 10H, Ph), 7.10 (dd, J = 4.4 Hz, J = 8.8 Hz, 1H, H-6), 6.91 (dd, J = 3.2 Hz, J = 8.8 Hz, 1H, H-5), 6.36 (dd, J = 3.2 Hz, J = 4.4 Hz, 1H, H-3), 3.53 (s, 3H, OCH_3), 2.26 (t, J = 8 Hz, 2H, CH_2), 1.51 (m, CH_2), 1.31 (m, CH_2), 0.89 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.9, 157.1, 146.4, 146.3, 135.6, 135.5, 134.1, 131.5, 131.4, 128.5, 128.3, 123.3, 123.2, 122.3, 118.9, 118.8, 114.5, 55.4, 33.7, 26.6, 22.2, 13.7. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.5 ppm [oxide ^{31}P = + 26.9 ppm].

2-(Diphenylphosphanyl)-4-methoxyphenyl _tert_-butanoate (82c)



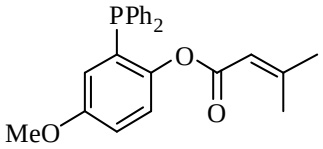
The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 83 %
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.37-7.23 (m, 10H, Ph), 7.04 (dd, *J* = 4.2 Hz, *J* = 8.8 Hz, 1H, H-6), 6.91 (dd, *J* = 3.2 Hz, *J* = 8.8 Hz, 1H, H-5), 6.38 (dd, *J* = 3.2 Hz, *J* = 4.2 Hz, 1H, H-3), 3.61 (s, 3H, OCH_3), 1.12 (s, 9H, 3xCH_3).

2-(Diphenylphosphanyl)-4-methoxyphenyl *i*-butanoate (82d)



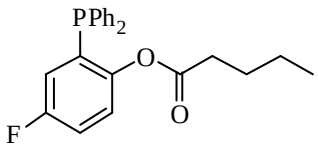
The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 82 %.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.37-7.28 (m, 10H, Ph), 7.06 (dd, *J* = 4.2 Hz, *J* = 8.8 Hz, 1H, H-6), 6.87 (dd, *J* = 3.2 Hz, *J* = 8.8 Hz, 1H, H-5), 6.28 (dd, *J* = 3.2 Hz, *J* = 4.4 Hz, 1H, H-3), 3.61 (s, 3H, OCH_3), 2.53-2.47 (m, 1H, CH), 1.07 (s, 3H, CH_3), 1.05 (s, 3H, CH_3).

2-(Diphenylphosphanyl)-4-methoxyphenyl 3-methyl-but-2-enoate (82e)



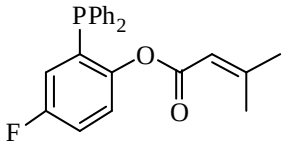
The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 84 %.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.29 (m, 10H, Ph), 7.10 (dd, *J* = 4.4 Hz, *J* = 8.8 Hz, 1H, H-6), 6.91 (dd, *J* = 3.2 Hz, *J* = 8.8 Hz, 1H, H-5), 6.38 (dd, *J* = 3.2 Hz, *J* = 4.4 Hz, 1H, H-3), 5.62 (s, 1H, CH), 3.62 (s, 3H, OCH_3), 2.03 (s, 3H, CH_3), 1.85 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 159.4, 157, 135.7, 135.6, 134.1, 133.9, 129.1, 128.6, 128.5, 128.4, 123.5, 123.3, 123.2, 119, 118.9, 114.9, 114.4, 55.4, 27.5, 20.4. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.1 ppm [oxide ^{31}P = + 26.4 ppm].

2-(Diphenylphosphanyl)-4-fluorophenyl pentanoate (83b).



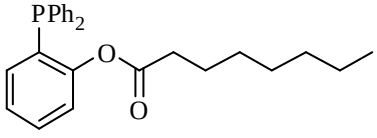
The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 95 %.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.44-7.33 (m, 10H, Ph), 7.18-7.12 (m, 1H), 7.11-7.04 (m, 1H), 6.56-6.51 (m, 1H), 2.30-2.24 (t, J = 7.6 Hz, 2H, CH_2), 1.52 (m, CH_2), 1.32 (m, CH_2), 0.91 (t, J = 7.6 Hz, 3H, CH_3).

2-(Diphenylphosphanyl)-4-fluorophenyl 3-methyl-but-2-enoate (83e).



The compound was purified by flash chromatography (hexane/AcOEt 90:10) yield = 91 %
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.32 (m, 10H, Ph), 7.18-7.12 (m, 2H), 7.11-7.02 (m, 2H), 5.65 (s, 1H, CH), 2.13 (s, 3H, CH_3), 1.91 (s, 3H, CH_3).

Synthesis of *o*-diphenylphosphanyl-phenyl caprylate (81g)

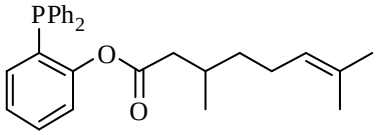


A solution of the *o*-diphenylphosphinophenol **57** (126.2 mg, 0.45 mmol, 1 eq), the caprylic acid (77.9 mg, 0.54 mmol, 1.2 eq) and *N*,*N*-dimethylaminopyridine (5.5 mg, 0.045 mmol, 0.1 eq) in dry CH_2Cl_2 was added, at room temperature and under nitrogen, to a solution of *N*,*N*'-dicyclohexylcarbodiimide (130.1 mg, 0.63 mmol, 1.4 eq) in dry CH_2Cl_2 (total volume: 4.5 mL, 0.1 M). The mixture was stirred at room temperature for 1 h, monitoring by TLC (90:10 hexane/AcOEt). The reaction mixture was filtered and washed with CH_2Cl_2. The crude product

was purified by flash chromatography using 90:10 hexane/AcOEt as the eluent to afford the product in 90 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45-7.26 (m, 11H, Ph), 7.20-7.07 (m, 2H), 6.83-6.78 (m, 1H), 2.25 (t, J = 7.6 Hz, 2H, CH_2), 1.49 (t, J = 7.2 Hz, 2H, CH_2), 1.36-1.19 (m, 8H, 4xCH_2), 0.89 (t, J = 6.8 Hz, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.8, 135.9, 134.7, 134.3, 134.1, 130.1, 129.2, 128.8, 128.7, 126.2, 122.8, 34.2, 33.8, 31.8, 29.2, 24.7, 22.8, 14.3 (CH_3).

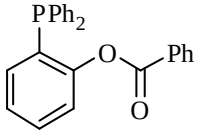
Synthesis of *o*-diphenylphosphanyl-phenyl citronellate (81h)



A solution of the *o*-diphenylphosphinophenol **57** (127.8 mg, 0.46 mmol, 1 eq), the citronellic acid (93.6 mg, 0.55 mmol, 1.2 eq) and *N*,*N*-dimethylaminopyridine (5.5 mg, 0.046 mmol, 0.1 eq) in dry CH_2Cl_2 was added, at room temperature and under nitrogen, to a solution of *N*,*N*'-dicyclohexylcarbodiimide (132.1 mg, 0.64 mmol, 1.4 eq) in dry CH_2Cl_2 (total volume: 4.6 mL, 0.1 M). The mixture was stirred at room temperature for 1 h, monitoring by TLC (90:10 hexane/AcOEt). The reaction mixture was filtered and washed with CH_2Cl_2. The crude product was purified by flash chromatography using 95:5 hexane/AcOEt as the eluent to afford the product in 75 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45-7.27 (m, 11H, Ph), 7.21-7.08 (m, 2H), 6.84-6.78 (m, 1H), 5.07 (t, J = 7.2 Hz, J = 6.8 Hz, 1H, CH), 2.25 (dd, J = 5.6 Hz, $J_{Ha\text{-}Hb}$ = 15.3 Hz, 1H, H_a, CH_2), 2.06 (dd, J = 8.4 Hz, $J_{Ha\text{-}Hb}$ = 15.3 Hz, 1H, H_b, CH_2), 1.99-1.79 (m, 3H, $CHCH_2$), 1.68 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.27-1.12 (m, 2H, CH_2), 0.89 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.2, 153.2, 153.1, 136, 135.9, 134.3, 134.1, 133.9, 131.9, 131.8, 131.7, 130.1, 129.2, 128.8, 128.7, 126.2, 124.5, 122.7, 41.5, 36.9, 29.9, 25.6, 19.8, 17.9. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.9 ppm [oxide ^{31}P = + 27.3 ppm].

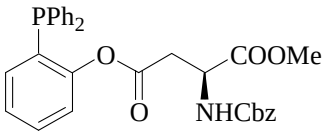
Synthesis of *o*-diphenylphosphanyl-phenyl benzoate (57i)



Dry pyridine (27 μL, 0.34 mmol, 1.2 eq), benzoic anhydride (76.6 mg, 0.34 mmol, 1.2 eq) and *N*,*N*-dimethylaminopyridine (3.4 mg, 0.028 mmol, 0.1 eq) were added, at room temperature and under nitrogen, to a solution of *o*-diphenylphosphinophenol (78.6 mg, 0.28 mmol, 1 eq) in dry CH_2Cl_2 (1.87 mL, 0.15 M). The solution was stirred for 3 h and then concentrated *in vacuo*. The residue was dissolved in AcOEt and washed with 5 % aqueous $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated. The product was purified by flash chromatography using 95:5 hexane/AcOEt as the eluent. Quantitative yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.81-7.78 (m, 2H), 7.46 (m, 1H), 7.37 (td, *J* = 7.6, 1.5 Hz, 1H), 7.32-7.23 (m, 13H, Ph), 7.12 (dd, *J* = 7.6 Hz, 1H), 6.82 (ddd, *J* = 9.8 Hz, *J* = 4.2 Hz, *J* = 2.6 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 164.3, 152.9 (d, *J* = 17 Hz), 135.5 (d, *J* = 10 Hz), 134.6, 134.2, 133.58, 133.4, 131.7 (d, *J* = 11 Hz), 130.7 (d, *J* = 15 Hz), 130.2, 129.9, 129.2, 129.0, 128.6, 128.54, 128.3, 128.1, 126.2, 122.6. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = -14.3 ppm [oxide ^{31}P = + 27.3 ppm].

Synthesis of 1-Methyl 5-[2-(Diphenylphosphanyl)-phenyl] *N*-(Benzyloxycarbonyl)-L-aspartate (81j)

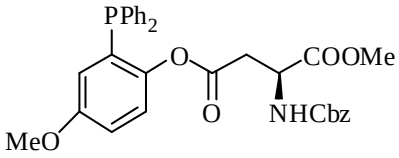


A solution of the *o*-diphenylphosphinophenol **57** (103.7 mg, 0.373 mmol, 1 eq), the commercially available *N*-carbobenzyloxy-L- asparagine acid 1-methyl ester (125.8 mg, 0.447 mmol, 1.2 eq) and *N*,*N*-dimethylaminopyridine (4.6 mg, 0.037 mmol, 0.1 eq) in dry CH_2Cl_2 (3.7 mL, 0.1 M) were added, at room temperature and under nitrogen, to a suspension of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (100 mg, 0.522 mmol, 1.4 eq) and dry *N*,*N*-diisopropylethylamine (89.3 μL, 0.522 mmol, 1.4 eq) in dry CH_2Cl_2. The mixture was stirred at *RT* for 2 h, monitoring by TLC (60:40 hexane/AcOEt). The reaction mixture was diluted with CH_2Cl_2 and extracted with 5 % aqueous HCl and water: the organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography using 60:40 hexane/AcOEt as the eluant to afford **81l** in 85 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.39-7.24 (m, 11H, Ph), 7.17-7.08 (m, 2H), 6.86 (m, 1H), 5.77 (d, *J* = 8.9 Hz, 1H, NH), 5.17 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 5.12 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 4.61 (m, 1H, CH), 3.70 (s, 3H, OCH_3), 3.04 (dd, *J* = 5.1 Hz, 17.4 Hz, 1H, CH_2CO),

2.75 (dd, J = 4.4 Hz, 17.4 Hz, 1H, CH_2CO). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.1, 169.3, 156.2, 152.6 (d, J = 17 Hz), 136.4, 135.6, 135.5, 135.4, 135.3, 134.3, 134.2, 133.9, 133.8, 130.5, 130.3, 130.2, 129.4, 129.3, 128.9, 128.8, 128.7, 128.4, 128.3, 126.7, 122.6, 122.5, 67.3, 53.0, 50.4, 36.6. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 15.6 ppm [oxide ^{31}P = + 28.2 ppm].

1-Methyl 5-[2-(Diphenylphosphanyl)-4-methoxyphenyl] *N*-(Benzyloxy-carbonyl)-L-aspartate (82j)

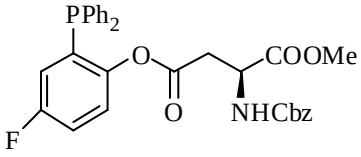


See procedure for the synthesis of Synthesis of 1-Methyl 5-[2-(Diphenylphosphanyl)-phenyl] *N*-(Benzyloxycarbonyl)-L-aspartate (**81j**)

The compound was purified by flash chromatography (hexane/AcOEt 65:35) yield = 75 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.24 (m, 15H, Ph), 7.06 (dd, J = 4 Hz, J = 8.4 Hz, 1H, H-6), 6.86 (dd, J = 3.2 Hz, J = 8.4 Hz, 1H, H-5), 6.37 (t, J = 3.6 Hz, 1H, H-3), 5.75 (d, $J_{NH\text{-}CH}$ = 8.2 Hz, 1H, NH), 5.19 (d, J = 6 Hz, 2H, CH_2-O), 4.60 (q, $J_{CH\text{-}CH2}$ = 4.4 Hz, 1H, CH), 3.72 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 3.01 (dd, J = 4.4 Hz, J = 17.2 Hz, 1H, H_a, CH_2), 2.72 (dd, 1H, J = 4.4 Hz, J = 17.2 Hz, H_b, CH_2).

1-Methyl 5-[2-(Diphenylphosphanyl)-4-fluorophenyl] *N*-(Benzyloxy-carbonyl)-L-aspartate (83j)



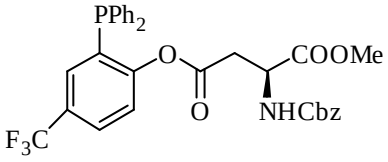
See procedure for the synthesis of Synthesis of 1-Methyl 5-[2-(Diphenylphosphanyl)-phenyl] *N*-(Benzyloxycarbonyl)-L-aspartate (**81j**)

The compound was purified by flash chromatography (hexane/AcOEt 60:40) yield = 85 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.48-7.27 (m, 15H, Ph), 7.12-7.02 (m, 2H, H-2, H-3), 6.55 (m, 1H, H-1), 5.70 (d, $J_{NH\text{-}CH}$ = 8.6 Hz, 1H, NH), 5.18 (d, J = 6 Hz, 2H, CH_2O), 4.61 (q, $J_{CH\text{-}}$

$_{CH2}$ = 4.4 Hz, 1H, CH), 3.72 (s, 3H, OCH_3), 3.01 (dd, *J* = 4.6 Hz, *J* = 17.2 Hz, 1H, H_a, CH_2), 2.75 (dd, *J* = 4.6 Hz, *J* = 17.2 Hz, 1H, H_b, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.9, 169.2, 161.8, 159.4 ($CO_{(Cbz)}$), 134.2, 134.1, 133.9, 133.8, 129.7, 129.6, 129.0, 128.9, 128.7, 128.6, 128.4, 128.3, 128.1 ($5xC_{Ar}$), 123.9, 123.8, 120.2, 120, 116.9, 116.6, 67.2 (CH_2O), 52.9 (OCH_3), 50.3 (CH), 36.5 (CH_2). ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 15.3 ppm [oxide ^{31}P = + 26.7 ppm]. ESI-MS: *m/z* 582.2 (M+Na).

1-Methyl 5-[2-(Diphenylphosphanyl)-4-trifluoromethylphenyl] *N*-(Benzyloxy-carbonyl)-L-aspartate (84j)

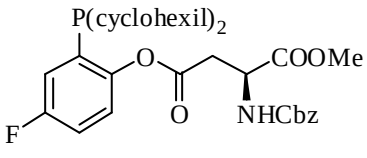


See procedure for the synthesis of Synthesis of 1-Methyl 5-[2-(Diphenylphosphanyl)-phenyl] *N*-(Benzyloxycarbonyl)-L-aspartate (**81j**)

The compound was purified by flash chromatography (hexane/AcOEt 70:30) yield = 90 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.48 (d, *J* = 8.8 Hz, 1H), 7.37-7.21 (m, 16H, Ph), 7.07 (m, 1H), 5.64 (d, $J_{NH\text{-}CH}$ = 8.4 Hz, 1H, NH), 5.14 (d, *J* = 5.6 Hz, 2H, CH_2O), 4.59 (q, $J_{CH\text{-}CH2}$ = 4.4 Hz, 1H, CH), 3.68 (s, 3H, OCH_3), 2.94 (dd, *J* = 4.4 Hz, *J* = 17.2 Hz, 1H, H_a, CH_2), 2.72 (dd, *J* = 4.4 Hz, *J* = 17.2 Hz, 1H, H_b, CH_2).

1-Methyl 5-[2-(Dicyclohexylphosphanyl)-4-fluorophenyl] *N*-(Benzyloxy-carbonyl)-L-aspartate (86j)

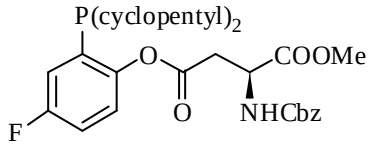


See procedure for the synthesis of Synthesis of 1-Methyl 5-[2-(Diphenylphosphanyl)-phenyl] *N*-(Benzyloxycarbonyl)-L-aspartate (**81j**)

The compound was purified by flash chromatography (hexane/AcOEt 80:20) yield = 80 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.31 (m, 5H, Ph), 7.18-7.12 (m, 1H, H-3), 7.11-7.01 (m, 2H, H-1, H-2), 6.57 (dd, J_{NH-CH} = 8.4 Hz, J = 3.2 Hz, 1H, NH), 5.18 (s, 2H, CH_2O), 4.79 (m, 1H, CH), 3.79 (s, 3H, OCH_3), 3.39 (dd, J = 4.8 Hz, J = 16.8 Hz, 1H, H_a, CH_2), 3.14 (dd, J = 4.4 Hz, J = 16.8 Hz, 1H, H_b, CH_2), 1.93-1.48 (m, 14H, Cyhex), 1.47-0.97 (m, 8H, Cyhex).

1-Methyl 5-[2-(Dicyclopentylphosphanyl)-4-fluorophenyl] *N*-(Benzyloxy-carbonyl)-L-aspartate (87j)

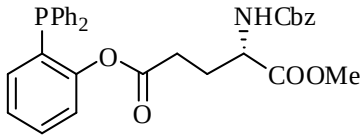


See procedure for the synthesis of Synthesis of 1-Methyl 5-[2-(Diphenylphosphanyl)-phenyl] *N*-(Benzyloxycarbonyl)-L-aspartate (**81j**)

The compound was purified by flash chromatography (hexane/AcOEt 70:30) yield = 80 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.32-7.27 (m, 5H, Ph), 7.18-7.15 (m, 1H), 7.04-6.94 (m, 2H), 6.55-6.50 (m, 1H, NH), 5.13 (s, 2H, CH_2O), 4.78-4.73 (m, 1H, CH), 3.75 (s, 3H, OCH_3), 3.36 (dd, J = 4.4 Hz, J = 16.8 Hz, 1H, H_a, CH_2), 3.01 (dd, J = 4.4 Hz, J = 16.8 Hz, 1H, H_b, CH_2), 2.07-1.98 (m, 2H), 1.86-1.76 (m, 2H), 1.70-1.34 (m, 14H).

Synthesis of 1-Methyl 5-[2-(Diphenylphosphanyl)-phenyl] *N*-(Benzyloxycarbonyl)-L-glutamate (81k)

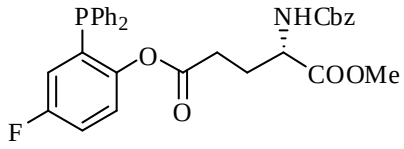


A solution of the *o*-diphenylphosphinophenol **57** (200 mg, 0.715 mmol, 1 eq), the commercially available *N*-carbobenzyloxy-L-glutamic acid 1-methyl ester (255 mg, 0.86 mmol, 1.2 eq) and *N*,*N*-dimethylaminopyridine (8.8 mg, 0.072 mmol, 0.1 eq) in dry CH_2Cl_2 (0.1 M) were added, at room temperature and under nitrogen, to a suspension of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (193.3 mg, 1 mmol, 1.4 eq) and dry *N*,*N*-diisopropylethylamine (172.6 μL, 1 mmol, 1.4 eq) in dry CH_2Cl_2. The mixture was stirred at RT for 2 h, monitoring by TLC (50:50 hexane/AcOEt). The reaction mixture was diluted with CH_2Cl_2 and extracted with

10 % aqueous HCl and water: the organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography using 70:30 hexane/AcOEt as the eluant to afford **81k** in 75 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.29 (m, 11H, Ph), 7.17-7.08 (m, 2H), 6.86 (m, 1H), 5.40 (d, J = 7.2 Hz, 1H, NH), 5.12 (s, J = 12.3 Hz, 1H, CH_2Ph), 4.35 (q, J = 4.8 Hz, 1H, CH), 2.35 (m, 2H, CH_2CO), 2.07 (m, 1H, H_a, CH_2CH), 1.86 (m, 1H, H_b, CH_2CH).

1-Methyl 5-[2-(Diphenylphosphanyl)-4-fluorophenyl] *N*-(Benzyloxy-carbonyl)-L-glutamate (83k)

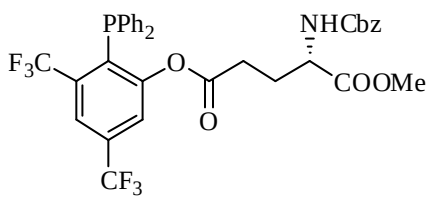


See procedure for the synthesis of 1-Methyl 5-[2-(Diphenylphosphanyl)-phenyl] *N*-(Benzyloxycarbonyl)-L-glutamate (**81k**)

The compound was purified by flash chromatography (hexane/AcOEt 60:40) yield = 85 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.40-7.27 (m, 15 H, Ph), 7.09 (m, 1H, H-2), 7.00 (m, 1H, H-3), 6.47 (m, 1H, H-1), 5.34 (d, J_{NH-CH} = 7.9 Hz, 1H, NH), 5.11 (d, J = 3.8 Hz, 2H, CH_2-O), 4.35 (m, 1H, CH), 3.74 (s, 3H, O-CH_3), 2.28 (m, 2H, CH_2-CO), 2.01 (m, 1H, H_a, CH_2-CH), 1.80 (m, 1H, H_b, CH_2-CH). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.8, 161.9, 159.4 ($CO_{(Cbz)}$), 134.4, 134.4, 134.2, 134.2, 129.8, 129.2, 129.1, 128.9, 128.6, 128.5 (5xC_{Ar}), 124.3, 124.2, 120.2, 119.9, 116.9, 116.7, 67.5 (CH_2-O), 53.5 (CH), 52.8 (O-CH_3), 30.3 (CH_2-CO), 27.6 (CH_2). ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.2 ppm [oxide ^{31}P = + 26.8 ppm]. ESI-MS: *m/z* 596.3 (M+Na).

1-Methyl 5-[2-(Diphenylphosphanyl)-3,5-ditrifluoromethylphenyl] *N*-(Benzyloxy-carbonyl)-L-glutamate (85k)

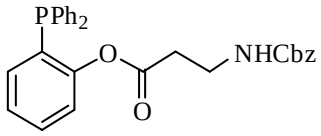


See procedure for the synthesis of 1-Methyl 5-[2-(Diphenylphosphanyl)-phenyl] *N*-(Benzyloxycarbonyl)-L-glutamate (**81k**)

The compound was purified by flash chromatography (hexane/AcOEt 70:30) yield = 95 %.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.94 (s, 1H), 7.61 (s, 1H), 7.38-7.31 (m, 15H, Ph), 5.20 (d, J = 7.6 Hz, 1H, NH), 4.28-4.23 (m, 1H, CH), 3.73 (s, 3H, OCH_3), 1.82-1.58 (m, 4H, 2xCH_2).

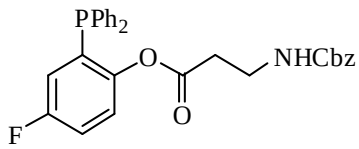
Synthesis of *N*-(Benzyloxycarbonyl)-β-alanine 2-(Diphenylphosphanyl)-phenyl Ester (81l)



A solution of the *o*-diphenylphosphinophenol **57** (90 mg, 0.32 mmoli, 1 eq.), the protected amino acid *N*-carbobenzyloxy-L-β-alanine (87 mg, 0.39 mmoli, 1.2 eq) and *N*,*N*-dimethylaminopyridine (3.9 mg, 0.032 mmol, 0.1 eq) in dry CH_2Cl_2 (0.1 M) was added, at room temperature and under nitrogen, to a solution of *N*,*N'*-dicyclohexylcarbodiimide (94 mg, 0.45 mmol, 1.4 eq) in dry CH_2Cl_2. The mixture was stirred at room temperature for 1.5 h, monitoring by TLC (80:20 hexane/AcOEt). The reaction mixture was filtered and washed with CH_2Cl_2. The crude product was purified by flash chromatography using 70:30 hexane/AcOEt as the eluent to afford **81l** in 82 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.39-7.24 (m, 11H, Ph), 7.17-7.08 (m, 2H), 6.86 (m, 1H), 5.38 (s, J = 8.9 Hz, 1H, NH), 5.13 (s, J = 12.3 Hz, 1H, CH_2Ph), 3.4 (q, J = 6 Hz, 2H, $NHCH_2$), 2.55 (t, J = 6 Hz, 2H, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.1, 169.3, 156.2, 152.6 (d, J = 17 Hz), 136.4, 135.6, 135.5, 135.4, 135.3, 134.3, 134.2, 133.9, 133.9, 130.5, 130.2, 130.1, 130.0, 129.2, 128.7, 128.6, 128.5, 128.4, 128.0, 126.7, 122.5, 122.4, 66.7, 36.6, 34.6.

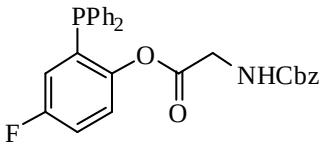
***N*-(Benzyloxy-carbonyl)-β-alanine 2-(Diphenylphosphanyl)-4-fluorophenyl Ester (83l)**



A solution of the 2-diphenylphosphino-*p*-fluorophenol **60** (283.3 mg, 0.96 mmol, 1 eq), the protected amino acid *N*-carbobenzyloxy-L-β-alanine (257.1 mg, 1.15 mmol, 1.2 eq) and *N*,*N*-dimethylaminopyridine (11.7 mg, 0.096 mmol, 0.1 eq) in dry CH_2Cl_2 (9.6 ml, 0.1 M) was added, at room temperature and under argon, to a solution of *N*,*N*'-dicyclohexylcarbodiimide (277.3 mg, 1.34 mmol, 1.4 eq) in dry CH_2Cl_2. The mixture was stirred at room temperature for 2 h, monitoring by TLC (70:30 hexane/AcOEt). The reaction mixture was filtered and washed with CH_2Cl_2. The crude product was purified by flash chromatography using 70:30 hexane/AcOEt as the eluent to afford **83l** in 92 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.27 (m, 15H, Ph), 7.15-7.05 (m, 2H, H-2, H-3), 6.52 (m, 1H, H-1), 5.27 (m, 1H, NH), 5.12 (s, 2H, CH_2O), 3.48 (q, J_{CH2-NH} = 12 Hz, 2H, CH_2NH), 2.50 (t, $J_{CH2-CH2}$ = 6 Hz, 2H, CH_2CO). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.8, 161.9, 159.5 ($CO_{(Cbz)}$), 156.5, 134.8, 134.7, 134.4, 134.2, 132.1, 132, 129.8, 129.3, 129.2, 128.8, 128.5, 128.4 (5xC_{Ar}), 124.3, 124.2, 120.4, 120.2, 117.1, 116.8, 67 (CH_2O), 36.6 (CH_2), 34.8 (CH_2CO). ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 15.2 ppm [oxide ^{31}P = + 27.9 ppm]. ESI-MS: *m/z* 524.2 (M+Na).

_N_-(Benzyloxy-carbonyl)glycine 2-(Diphenylphosphanyl)-4-fluorophenyl Ester (83m)

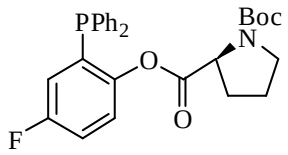


A solution of the 2-diphenylphosphino-*p*-fluorophenol **60** (158,3 mg, 0.53 mmol, 1 eq), the protected amino acid *N*-carbobenzyloxy-L-β-glycine (134 mg, 0.64 mmol, 1.2 eq) and *N*,*N*-dimethylaminopyridine (6.5 mg, 0.053 mmol, 0.1 eq) in dry CH_2Cl_2 (5.3 ml, 0.1 M) was added, at room temperature and under argon, to a solution of *N*,*N*'-dicyclohexylcarbodiimide (152.7 mg, 0.74 mmol, 1.4 eq) in dry CH_2Cl_2. The mixture was stirred at room temperature for 2 h, monitoring by TLC (70:30 hexane/AcOEt). The reaction mixture was filtered and washed with CH_2Cl_2. The crude product was purified by flash chromatography using 70:30 hexane/AcOEt as the eluent to afford **83m** in 90 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.48-7.30 (m, 15H, Ph), 7.15 (m, 1H, H-2), 7.11 (m, 1H, H-3), 6.53 (m, 1H, H-1), 5.12 (s, 2H, CH_2O), 4.99 (m, 1H, NH), 3.85 (d, J_{NH-CH2} = 5.2 Hz, 2H, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 168.4, 159.6 ($CO_{(Cbz)}$), 156.4, 162.1, 136.4 (C_{ipso}), 134.8, 134.7, 134.4, 134.2, 129.9, 129.2, 129.1, 128.9, 128.6, 128.4 (5xC_{Ar}), 124.2,

124.1, 120.4, 120.3, 120.1, 120.1, 117, 116.8, 67.5 (CH_2O), 42.8 (CH_2). ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 14.0 ppm [oxide ^{31}P = + 27.5 ppm]. ESI-MS: *m/z* 510.4 (M+Na).

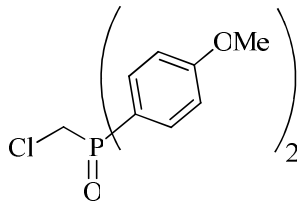
_N_-(_tert_-Butoxycarbonyl)-L-proline 2-(Diphenylphosphanyl)-4-fluorophenyl Ester (83n)



A solution of the 2-diphenylphosphino-*p*-fluorophenol **60** (119.6 mg, 0.40 mmol, 1 eq), the protected amino acid *N*-Boc-L-β-proline (103.3mg, 0.48 mmol, 1.2 eq) and *N*,*N*-dimethylaminopyridine (4.9 mg, 0.04 mmol, 0.1 eq) in dry CH_2Cl_2 (4 ml, 0.1 M) was added, at room temperature and under argon, to a solution of *N*,*N*'-dicyclohexylcarbodiimide (115,5 mg, 0.56 mmol, 1.4 eq) in dry CH_2Cl_2. The mixture was stirred at room temperature for 2 h, monitoring by TLC (80:20 hexane/AcOEt). The reaction mixture was filtered and washed with CH_2Cl_2. The crude product was purified by flash chromatography using 80:20 hexane/AcOEt as the eluent to afford **83n** in 94 % yield.

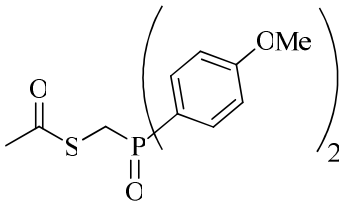
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.30-7.25 (m, 5H, Ph), 7.24-7.09 (m, 5H, Ph), 7.06-6.92 (m, 2H, H-2, H-3), 6.36 (m, 1H, H-1), 4.24 (m, 1H, CH), 3.26 (t, $J_{Hc\text{-}Hd}$ = 6.8 Hz, H_c, 1H, CH_2), 3.18 (t, 1H, H_d, CH_2), 2.03-1.95 (m, 1H, H_a), 1.78-1.62 (m, 3H, H_b, CH_2), 1.45-1.30 (d, 9H, Boc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.2, 161.9, 159.4, 154.8, 153.9, 148.9, 135.1, 134.4, 134.2, 133.9, 132.1, 129.7, 129, 124.3, 123.5, 119.9, 117.2, 116.9, 80.4, 80.1, 59.4, 46.8, 46.6, 30.6, 29.6, 28.8 (Boc), 24.9, 23.8. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = - 15.5, - 15.8 ppm [oxide ^{31}P = + 27.7, + 28.0 ppm].

Synthesis of the compound (89)



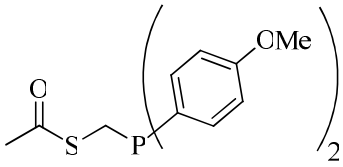
Chloromethylphosphonic dichloride (5g, 30 mmol, 1eq) was dissolved in anyhdrous THF (30 mL). A solution of 4-methoxyphenylmagnesium bromide (0.5M) in THF (120 mL, 240 mmol, 2 eq.) was added dropwise over 1 h. The resulting mixture was stirred at reflux for 24 h. The reaction was then quenched by the addition of water (5 mL), and the solventwas removed under reduced pressure. The residue was dissolved in CH_2Cl_2, and the resulting solution was washed once with water (12 mL). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 97:3). Phosphine oxide was isolated as a white solid in 63 % yield.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.77-7.72 (m, 4H, Ph), 7.04-7.00 (m, 4H, Ph), 4.00 (d, *J* = 7 Hz, 2H), 3.88 (s, 6H, 2xCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 162.9, 133.5, 133.4, 121.5, 120.4, 114.4, 114.2, 55.4, 38.1. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = + 27.4 ppm. ESI-MS: *m/z* 310.05 (M+Na).

Synthesis of the compound (90)



Phosphine oxide **89** (2.9 g, 9.1 mmol, 1 eq) was dissolved in DMF (25 mL). Potassium thioacetate (1.25 g, 10.9 mmol) was then added, and the reaction mixture was stirred under nitrogen for 18 h. the solvent was then removed under reduced pressure. The resulting oil was purified by flash chromatography (CH_2Cl_2/MeOH 97:3). Phosphine oxide **90** was isolated as a clear, colorless oil in 87 % yield.
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.70-7.65 (m,4H, Ph), 6.99-6.97 (m,4H, Ph), 3.85 (s, 6H, 2xOCH_3), 3.70 (d, *J* =8.2 Hz, 2H), 2.28 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 162.9, 133.2, 123.4, 123.3, 114.4, 55.6, 30.4, 28.0. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = + 29.2 ppm. ESI-MS: *m/z* 373.06 (M+Na).

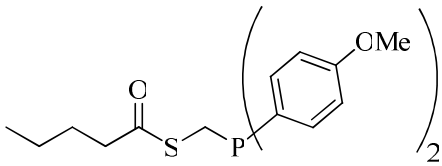
Synthesis of the compound (91)



Phosphine oxide **90** (1.06 g, 2.95 mmol, 1 eq) was dissolved in ahnydrous chloroform (10 mL). Trichlorosilane (8 mL, 3.87 mmol, 26.5 eq) was added, and the resulting solution was stirred under nitrogen for 72 h. The solvent was then removed under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 97:3). Phosphine **91** was isolated as a white solid in 92 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.38-7.36 (m, 4H, Ph), 6.93-6.89 (m, 4H, Ph), 3.85 (s, 6H, 2xOCH_3), 3.47 (bs, 2H), 2.32 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 194.4, 160.5, 134.2, 134.0, 127.9, 127.8, 114.2, 55.2, 30.3, 26.4. ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = -19.7 ppm. ESI-MS: *m/z* 357.06 (M+Na).

Synthesis of the compound (92)



Phosphine **91** (200 mg, 0.56 mmol, 1 eq) was dissolved in ahnydrous DMF (1mL). A solution of MeONa (34 mg, 0.62 mmol, 1.1 eq) was added dropwise at 0°C, under nitrogen. The resulting mixture was stirred at 0°C for 2 h and then valeroyl chloride (67.4 mg, 0.56 mmol, 1eq) was added. The solution was stirred for 1 h at 0°C, dissolved in CH_2Cl_2 and washed with 5 % aqueous HCl, 5 % satured $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated to give the crude product. The resulting oil was purified by flash chromatography (cyclohexane/AcOEt 80:20 to cyclohexane/AcOEt/MeOH 80:20:2). Phosphine **92** was isolated as a clear, colorless oil in 57 % yield.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.44 (t, *J* = 8 Hz, 4H, Ph), 6.94 (d, *J* = 8 Hz, 4H, Ph), 3.68 (s, 6H, 2xOCH_3), 3.56 (d, *J* = 2 Hz, 2H, CH_2S), 2.53 (t, *J* = 7.6 Hz, 2H, CH_2), 1.59 (m, 2H,

CH_2), 1.29 (m, 2H, CH_2), 0.89 (t, J = 7.2 Hz, 3H, CH_3). ^{31}P-NMR (161 MHz, $CDCl_3$, 25°C): δ = -17.6 ppm [oxide ^{31}P = + 29.7 ppm].

3.7 Reference

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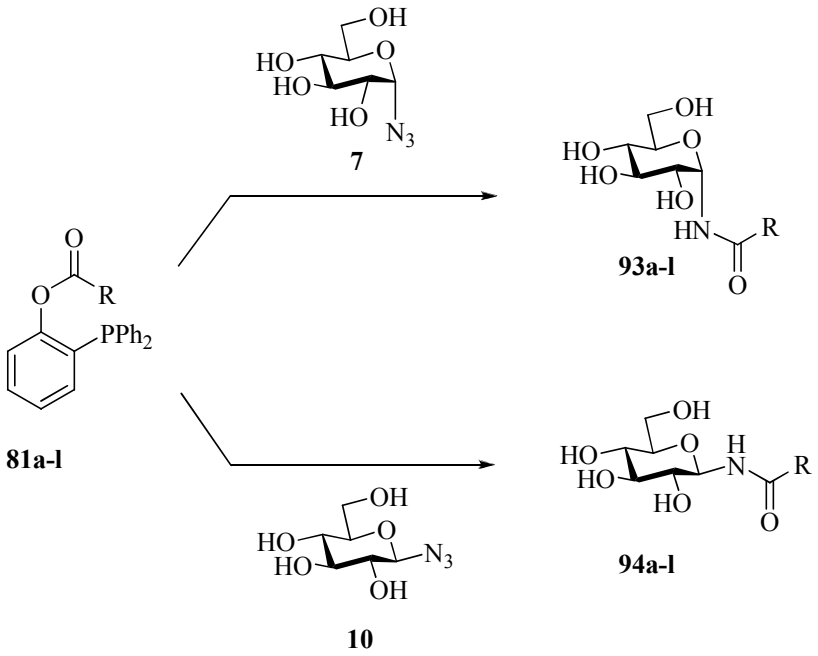
Chapter 4

Staudinger ligation of unprotected azides: a reagent screening

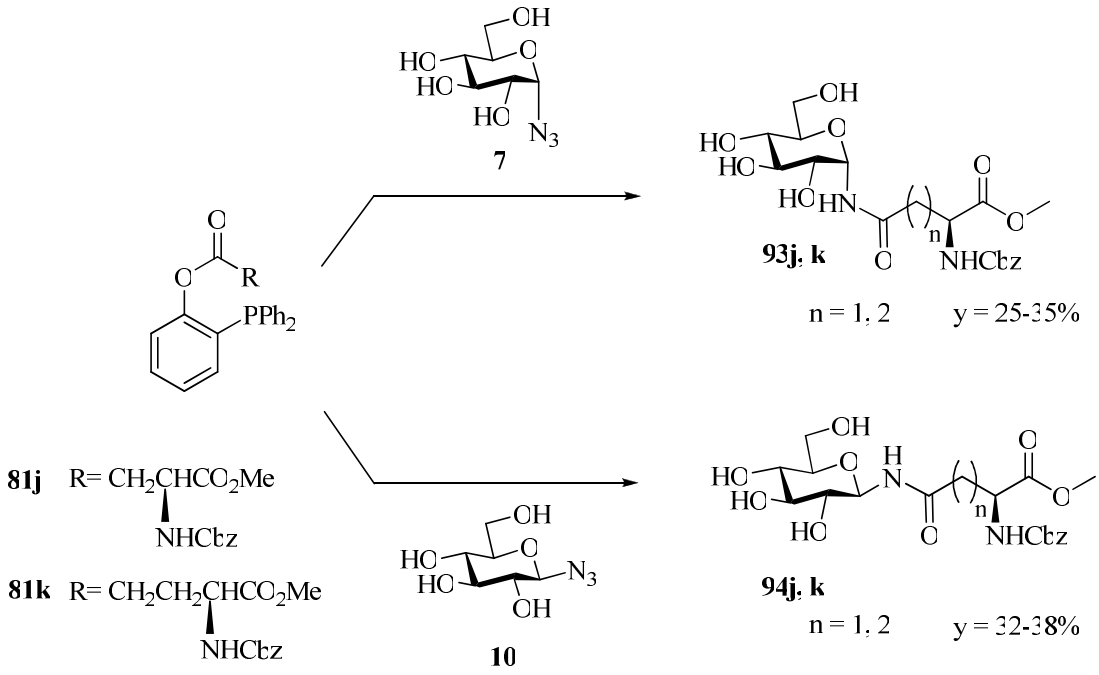
4.1 Background

Our previous studies on the use of Staudinger ligation for the synthesis of glycosyl amides have been summarized in the introduction (Chapter 1). Among them, ligation of unprotected α- and β-glucosyl azides[1] **7** and **10** was particularly remarkable because in both cases it occurred stereoconservatively (Scheme 1) and allowed simple isolation of the resulting glucosyl amides from the phosphane oxide by-product by water extraction. The anomeric ratios of the products were consistently good and the yields moderate-to-good for most of the alkyl chains R, particularly under MW irradiation.[1]



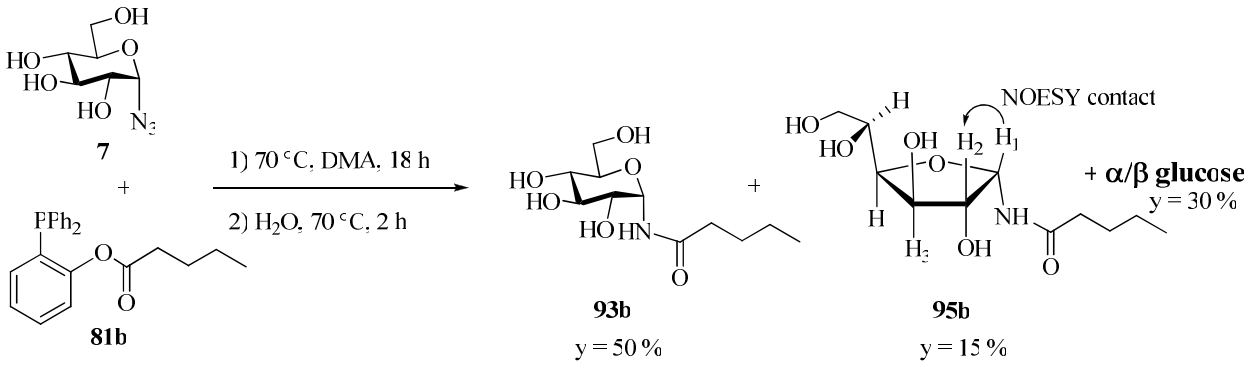
Scheme 1. Stereoconservative Staudinger ligation of **7** and **10** with phosphine **81a-l**

For its novelty and stereoselectivity this method deserved further consideration, but it was far from perfect and left many problems open. As noted above, yields were generally modest and dropped to poor for some α-glycosylamides. Most notably the aspartic and glutamic acid derivativesm **81j** and **81k** (Scheme 2) gave poor yields of the corresponding glucosyl amino acids for both the α and β series. This is particularly disappointing because these amides are important glycoconjugates, mimicking the conserved core of natural *N*-asparagine-linked glycopeptides and glycoproteins.[2, 3]



Scheme 2. Phosphines **81j,k** give poor yields of *N*-glycosyl amino acids

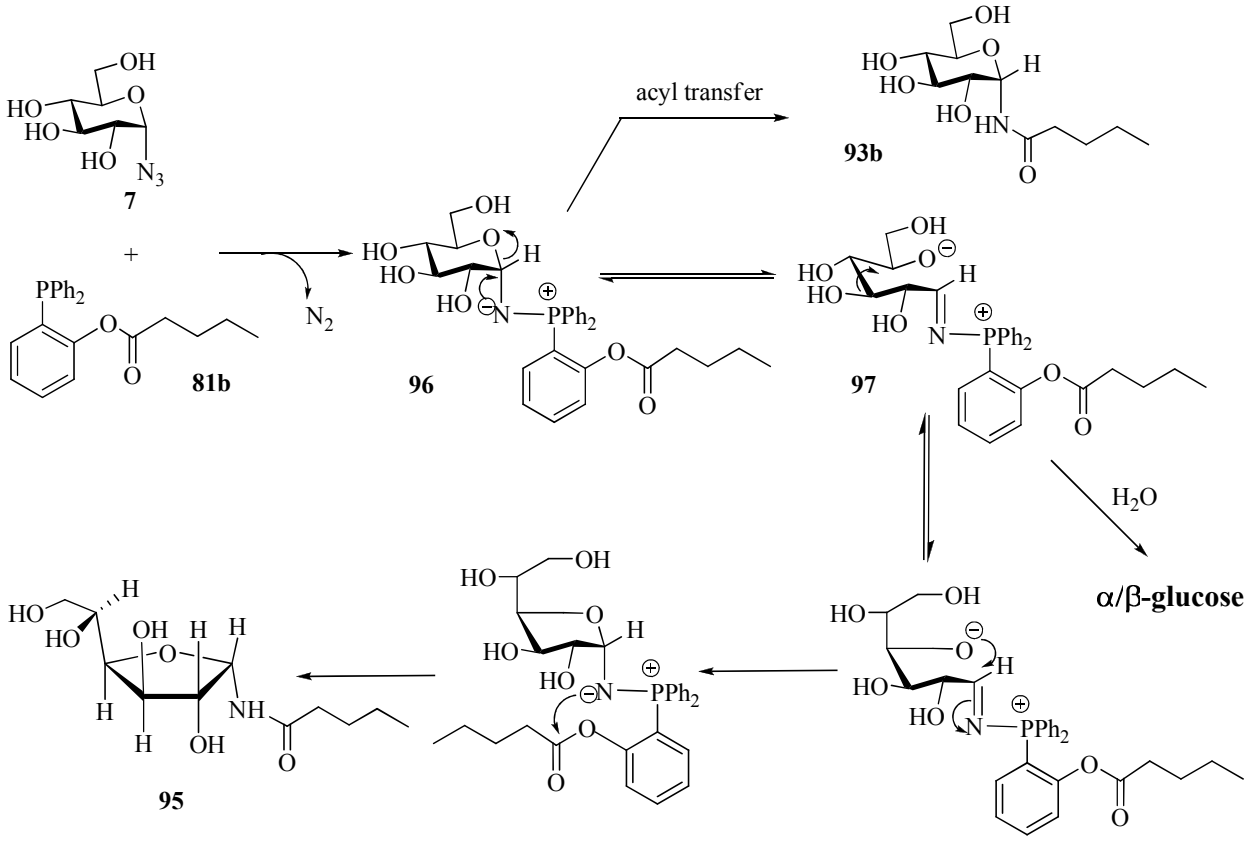
Furthermore, the reaction often yielded mixtures of the desired pyranosylamide and the isomeric furanosylamide. For instance, reaction of **7** with the pentanoyl phosphine **81b** (Scheme 3) in DMA at 70°C yielded a complex mixture of compounds in the water fraction that were separated by reverse phase HPLC and characterized as the expected pentanamide **93b** (50 % yield), the α-*N*-pentanoyl-glucofuranosylamine isomer **95b** (15 % yield) and α and β-glucose (equilibrium mixture of anomers, 30% yield). The configuration of **93b** was assigned on the basis of the anomeric proton signal (5.45 ppm, $J_{1\text{-}2}$ = 5.4 Hz). The structure of **95b** was assigned on the basis of ESI-MS and of the ^{1}H-NMR (D_2O), which is consistent with the spectrum of the known α-D-glucofuranoside (Scheme3).[4]



Scheme 3. Staudinger ligation of **7** with phosphine **81b** in DMA

The anomeric configuration of **95b** was further confirmed to be α by the NOESY spectrum (D_2O), which shows a strong crosspeak for the H_1 and H_2 protons and no crosspeak for the H1 and H3 protons (Scheme 3). The presence of glucose in the reaction crude was identified by ^{1}H-NMR spectroscopy.

The furanoside **95b** must clearly derive from a ring-opening process occurring after the azide reduction step, presumably from the iminophosphorane **96** (Scheme 4) to afford the phosphinimine **97**, which can undergo ring-closure to yield **95b**. Hydrolysis of the same intermediate **97** accounts for the formation of α and β-D-glucose in the reaction mixture. The alternative hypothesis that glucose could be formed by direct hydrolysis of the starting azide was discarded because **7** was recovered unaltered after treatment with water in DMA at 70°C for 24 h.



Scheme 4. Mechanism for the formation of the furanosylamide **95b** and glucose in the ligation of **7** with **81b**

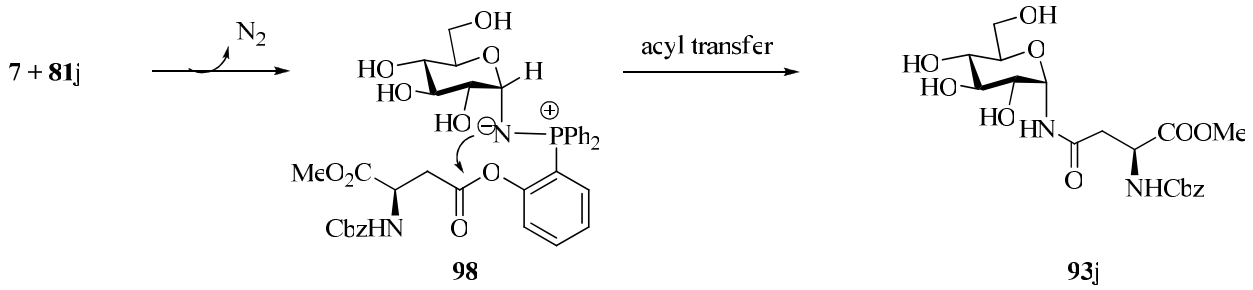
This drawback was partly resolved in our previous work[1, 5] by reducing the temperature of the reaction (high temperatures favor formation of the furanosylamides) while using microwaves to

avoid increasing the reaction time. Adding 2 % DMPU to the solvent (DMA) was also found to be beneficial, since a high polarity of the solvent appears to accelerate the acyl transfer step.[6] Under these conditions, formation of the undesired furanosyl amide could be contained in most cases below 5-10 % and both α and β unprotected glycosyl azides could be transformed stereoselectively in the corresponding amides and easily isolated from reaction mixtures. However, clearly the process called for further optimization.

4.2 Staudinger ligation with a new group of phosphines

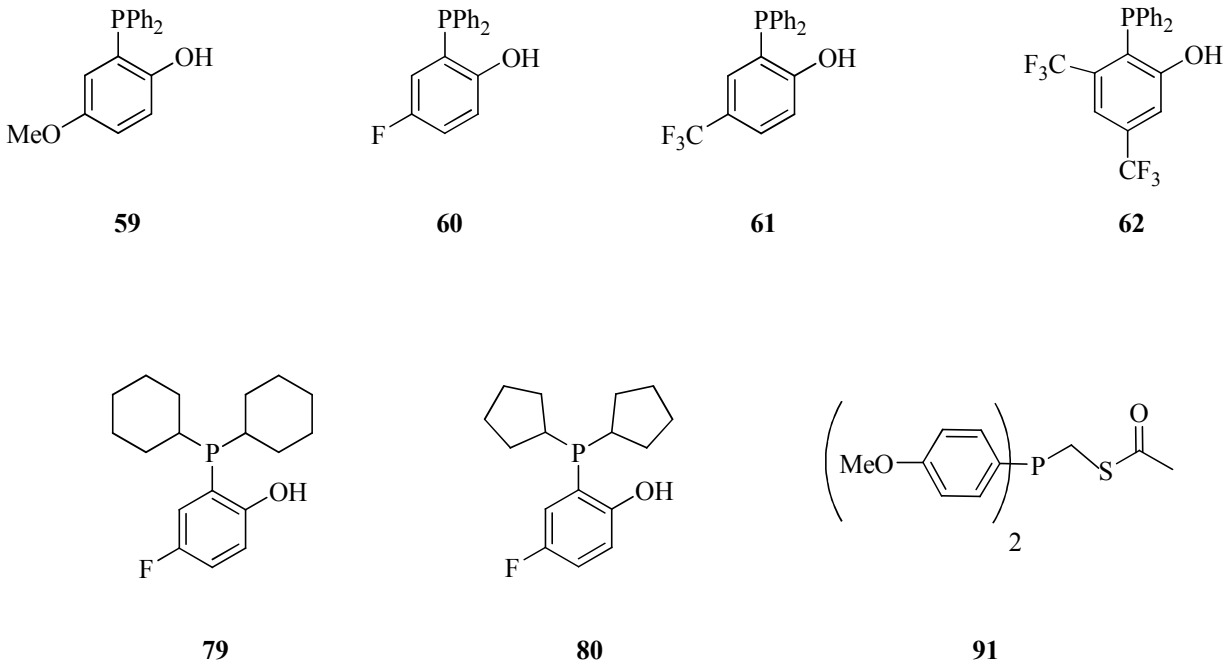
To address the set of problems described above we turned to examining the performance of different phosphines in the reaction using as model substrates the α and β-glucopyranosyl azides **7** and **10**.

The Staudinger ligation of phosphines **81** can be envisaged as taking place in two steps, as described in Scheme 5 for the reaction of **81j** with α-glucosyl azide **7**. In the first step, nucleophilic addition of the phosphine to the γ nitrogen of the azide followed by nitrogen extrusion leads to the iminophosphorane **98**. In the second step, **98** undergoes an intramolecular reaction in which the acyl chain transferred from the phenolic ester to the imminophosphorane nitrogen and an amide bond is generated.[1] Our previous observations indicated that the poor amide yields observed upon reaction of glucosylazides with some phosphines were caused by inefficient acyl chain transfer in the iminophosphorane intermediate **98** (Scheme 5).[1, 7]



Scheme 5. Two-step mechanism for the Staudinger ligation.

Using the syntheses shown in Chapter 3, we prepared the group of new phosphines collected in Figure 1 (**59-62, 79, 80** and **91)** for further studies on the Staudinger ligation process.



59 **60** **61** **62**

79 **80** **91**

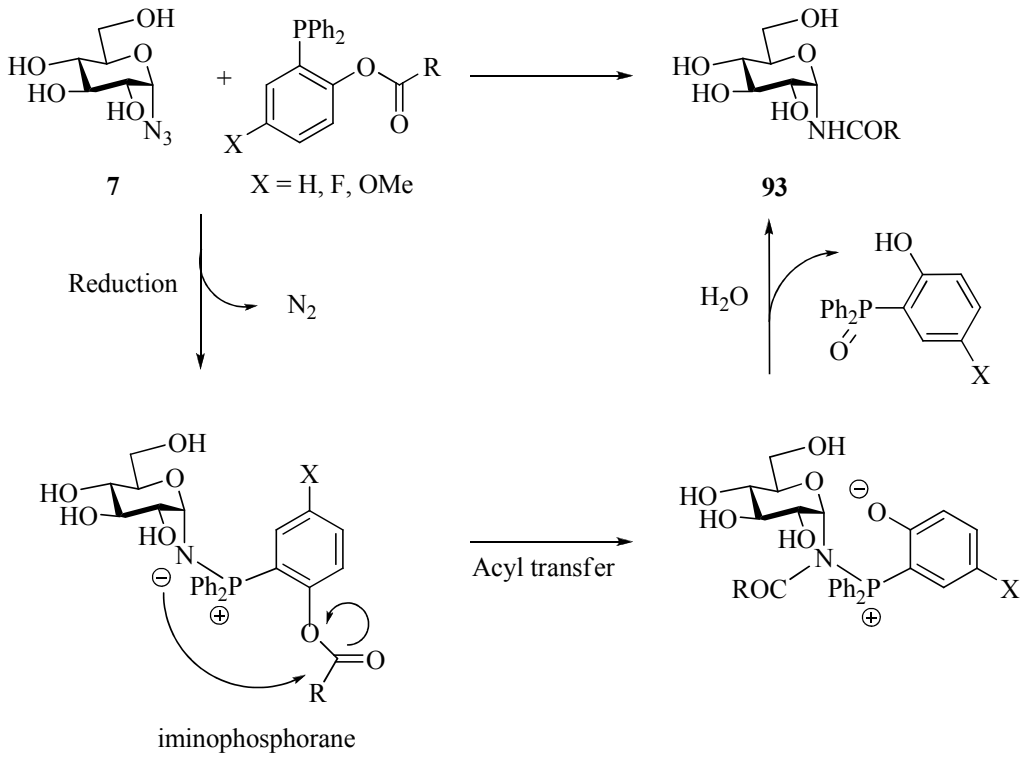
Figure 1. Set of new phosphines

The two phosphines **59** and **60** were studied in more detail and were meant to address two different and opposite features of the process:

1. The azide reduction step (formation of the imminophosphorane, Scheme 6) is facilitated by a strong basicity of the P atom.
2. The acyl transfer step (from phenol oxygen to iminophosphorane *N*, in Scheme 6) would be accelerated by EWG groups on the aromatic ring, which, however, will reduce the basicity of P.

Thus, we expected that increasing the leaving group ability of the phenol, such as with phosphines **60-62** could improve the overall-yield of the reaction by accelerating the transfer step. A possible decrease in reactivity due to the reduced basicity of the P atom induced by the electronpoor phenol could be offset by increasing the electonrichness of the phosphines, for instance with aliphatic substituents, such as cyclohexyl or cyclopentyl rings, as in **79** and **80**. This should result in an increase of the reduction rate, leading to a diminished amount of by-products related to incomplete azide reduction.

More generally, we expected that a change in the structure of the phoshine reagent may be reflected in a different composition of the reaction mixtures and in particular may affect the formation of the furanose isomers in ways that would be difficult to anticipate at this point.



Scheme 6. Mechanism of the traceless Staudinger ligation

As a first step we identified a rapid and diagnostic analytical method to evaluate the course of the reactions by analysis of the crude reaction mixtures. Since the reaction is performed on unprotected sugars, Thin Layer Chromatography cannot be used to monitor its course. Rather, after completion of the reactions under a predefined set of conditions (time, temperature etc), the crude mixtures were diluted with water and extracted with CH_2Cl_2 to eliminate the phosphine by-products. The aqueous layers were analyzed by ^{1}H-NMR (typically in D_2O) and the product ratios established, based on the integration of the anomeric protons (Figure 2). Typical reaction crudes contained both the expected α- or β-pyranosyl amides (**93** or **94**) and variable amounts of the corresponding α-furanosyl amides **95**. As we have previously shown (Scheme 4),[1] the latter are formed by ring-opening/ring-closure of the iminophosphoranes intermediate. Other by-products consist in free glucose (equilibrium anomeric mixtures) formed by hydrolysis of the same intermediates. They were all identified and characterized by isolation and/or comparison with known samples.

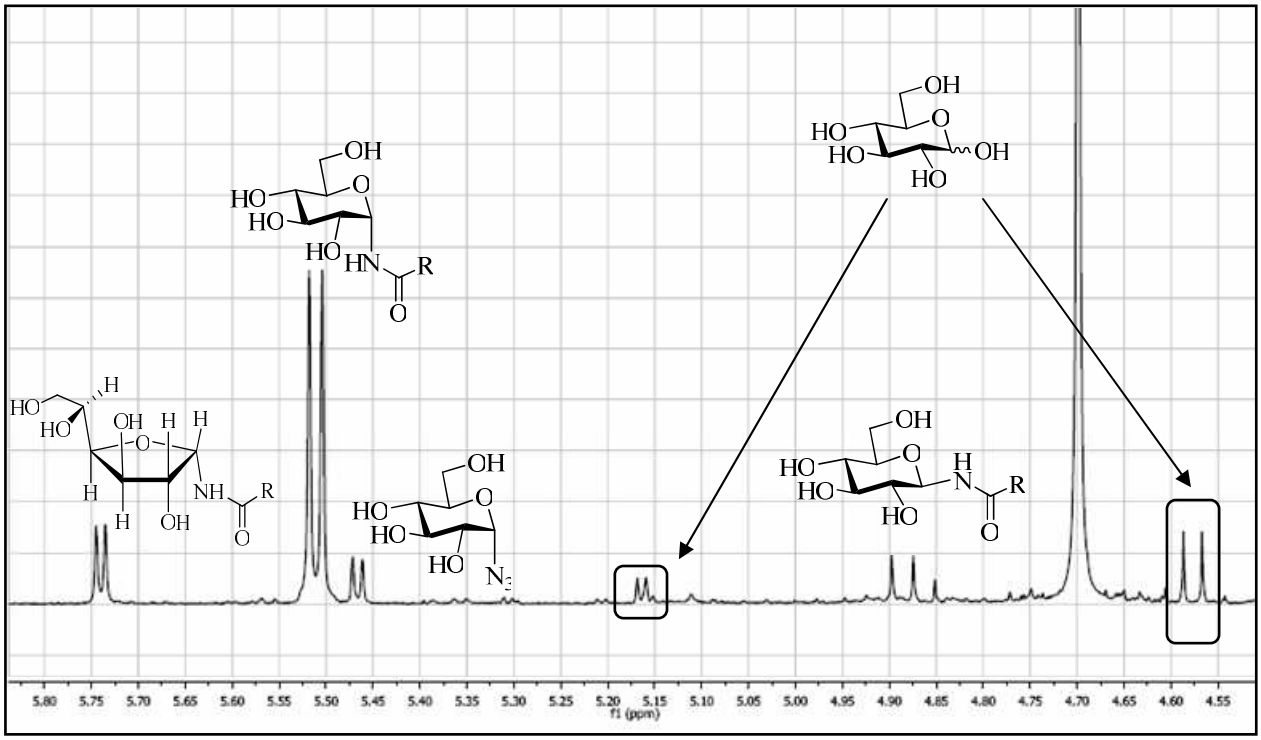


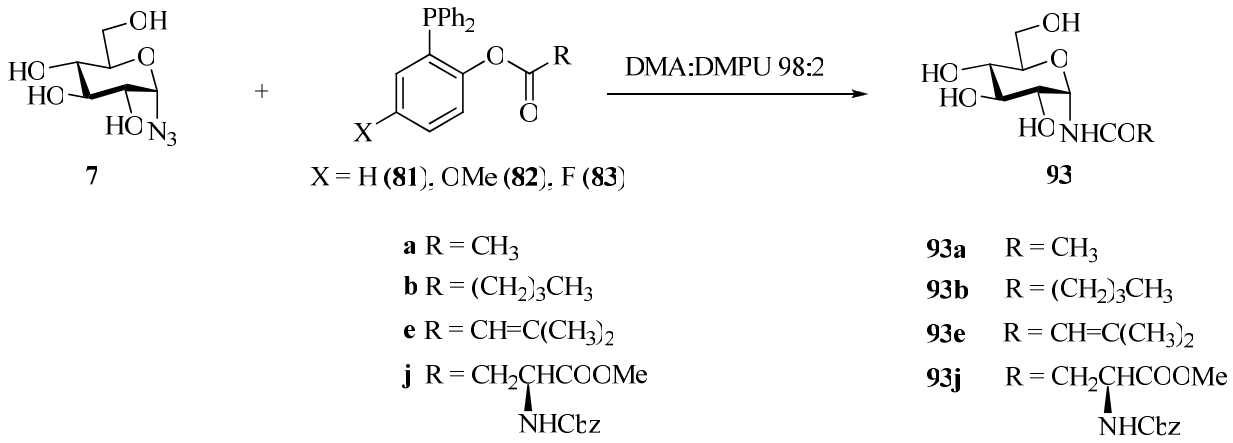
Figure 2. ^{1}H-NMR (D_2O) spectra of the crude water extract

As mentioned above, a first set of experiments was perfomed with phoshines **82** and **83** obtained by acylation of the corresponding phenols **59** and **60**. Phosphine **82** was chosen because fluorophenyl esters are well-known as good activating groups in acylic nucleophilic substitution and increase the acylating ability of the reagent. The methoxy group on the aromatic ring of **83** increases the basicitity of the phosphorous atom, allowing fast reduction of the azide, but reduces the acylating ability.

A set of experiments was designed on the unprotected α-glucosyl azide **7** chosen as the standard substrate, changing reaction temperature, nature of the R group (R = Me, $CH_3CH_2CH_2CH_2$-, $(CH_3)_2C$=CH-, Z-L-aspartic acid 1-methyl ester) and type of process (thermal or microwave assisted) (Scheme 7 and Tables A, B and C in the appendix).

Yields and diasteromeric ratios obtained from the ligation were estimated on the basis of the weight and on the ^{1}H-NMR spectra of the crude water extract, and all reactions were run in the same solvent: 98:2 DMA:DMPU (the results are collected in Tables A and B in the appendix).

For comparison, the data previously obtained[1] in similar conditions for **81** are collected in Table C.



Scheme 7. Screening for the ligation of α-glucosyl azide **7**

Evaluation of results obtained from this screening show that:

The methoxy-substituted phosphines **82** displayed lower levels of α selectivity, proved by the presence of both α- and β-glucosyl amides in the crude water extract, and also in general showed a higher tendency to give the formation of α-glucofuranosyl amides and of other hydrolysis by-products.

Microwave assisted process with compounds **82** afforded low yield, low stereoselectivity and the azide **7** was not completely reduced (see Table A in appendix, entries 5, 6 and 11). In the end, the phosphine **82** presented a reduced reactivity and minor α-stereocontrol in comparison with compound **81** for the Staudinger ligation. Compound **82** afforded high conversion to α-glucofuranosyl amides **95**, if the reaction was performed above 70°C (Table A, entries 3, 4 and 6). However, after water extraction we obtained low yield, probably for an inefficient acyl chain transfer during the ligation, that stop the reaction to iminophosphorane intermediate **98** step (Scheme 5).

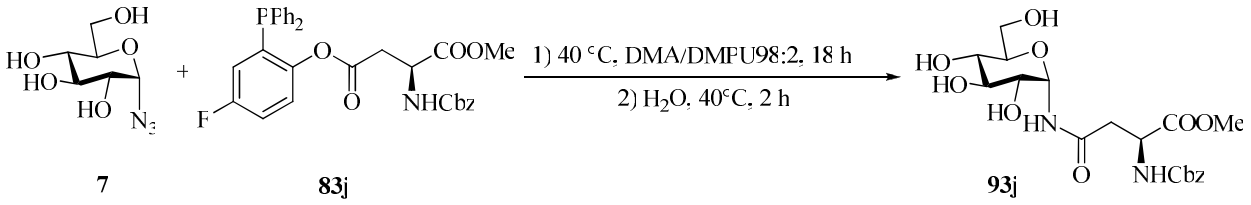
Similarly, the fluorinated compounds **83** showed lower reduction strength and lower acylating ability in the ligation of alkyl chains, but were found to perform dependably in the transfer of Aspartic acid side-chain to **7**. (see Table B in appendix, entries 14 and 15).

Some general observations were extrapolated from this set of experiments for the Staudinger ligation between α-glucosyl azide **7** and functionalized phosphines **82** and **83**:

1. The results showed a strong and somewhat unpredictable dependence of the process on the nature of the R group and the type of phosphine.

2. If the ligation for the thermal process was performed above 40 °C, the quantity of α-glucofuranosyl amide increased in each case observed (Table A and B in appendix, experiment perform at 70°C and 100°C).
3. The methoxy phosphines **82** gave large amount of pyranose-furanose conversion.
4. The fluorinated phosphines **83** gave the best results with amino acids.

The best conditions identified for the synthesis of **93j**, consisted in treating the unprotected α-glucosyl azide with 2 equivalents of phosphines **83j** in DMA/DMPU (98:2) at 40°C during 18 h (Scheme 8).



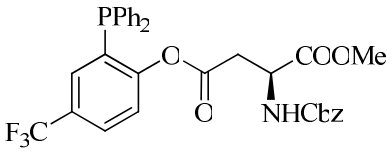
7 **83j** **93j**

Scheme 8. Staudinger ligation of the unprotected α-glucosyl azide **7** with phosphine **83j**

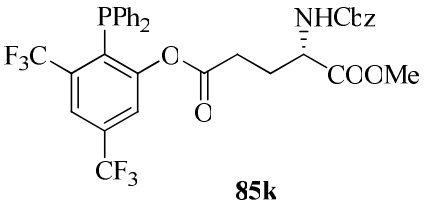
Under these conditions the α-glucopyranosylamide was isolated in good yield and excellent anomeric ratio (55 % isolated yield and 98:2 α/β ratio). The presence of the F atom in the aromatic-ring appears to play a key role for the reactivity of the phosphine **83j** in the acyl transfer of aspartic acid.

As a matter of fact, the reaction occurred with over 55% yield, compared to 25% afforded by the non-fluorinated reagent **81j**. However, the ligation required more equivalents of phosphines (1.2 eq. to 2 eq.) and the reaction time became much longer (4 h to 18 h). This proves the low reactivity of fluorinated phosphine **83j** respect to compound **81j**. Microwave assisted process also in this case afforded low yield and high presence of by-products (Table B in appendix, entries 6, 7, 9 and 13).

To further explore the ability of electron poor phosphines in the Staudinger ligation of aspartic acid residue, azide **7** was treated with **84j** and **85k** (Figure 3) in the same conditions optimized for the precedent ligation (40°C, 18 h and DMA/DMPU 98:2 as mixture solvent).



84j



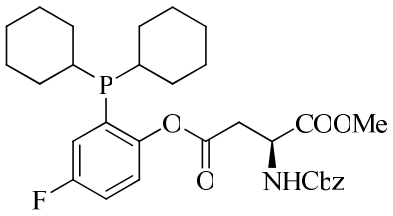
85k

Figure 3. Phosphines **84j** and **85k**

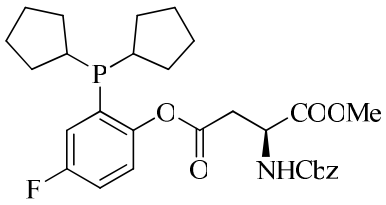
Unfortunately phoshines **84j** and **85k** were found to reduce the azide, but not to transfer the acyl chain and afforded only hydrolysis by-products, such as α and β-glucose and other not identified compounds, whit complet absence of α-glucosyl amide as a product.

This suggested that the azide reduction step could not be induced in the reaction for the low basicity of the phosphorus atom, also increased temperature and time of the reaction.

For this purpose compound **86j** (Figure 4) was synthesized, replacing two phenyl rings with cyclohexyl rings to increase the reduction strength of the phosphines. These became able to reduce the azide **7** (major reaction product was β-glucosylamine as show by anomeric signal at 4.18 ppm $J_{1\text{-}2}$ = 8.8 Hz to prove that the reduction step happened), but didn't have acylating ability at all, probably for the major steric hindrance of the alkyl substituent.



86j



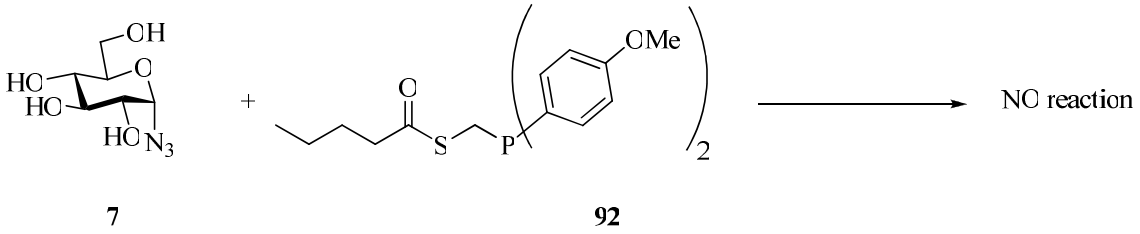
87j

Figure 4. Phosphines **86j** and **87j**

Compound **87j** was synthesized to decrease the steric hindrance of the cyclohexyl ring and tested with different reaction conditions, but also in this case the ligation afforded only hydrolysis by-prodouct with total absence of α-glucosyl amide **93j**.

A final set of experiments were performed treating the unprotected α-glucosyl azides **7** with the functionalized (diphenylphosphino)methanethiol **92** (Scheme 9). [8] This phosphine was largely used in the orthogonal assembly of a protein,[9] site-specific immobilization of peptides and proteins to a surface,[10, 11] and synthesis of glycopeptides. [12]

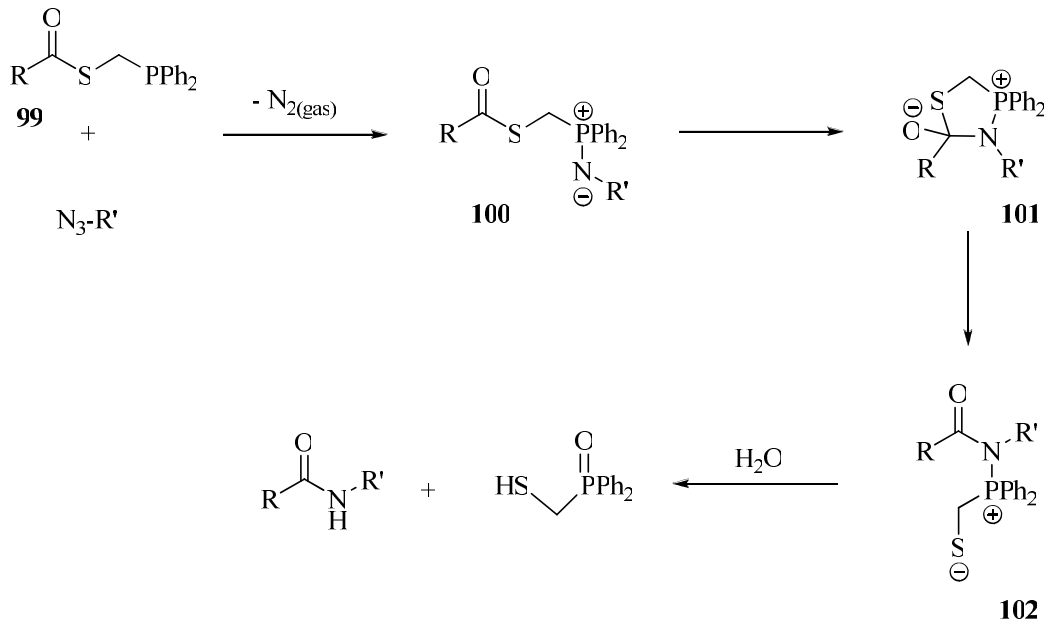
The same procedure employed for the Staudinger ligation between phosphine and protected or unprotected glucosyl azides (70 °C/18 h and 40°C/4h, in DMA:DMPU 98:2 as a solvet mixture)[1, 7] were used, but we observed that **92**, is not able to react with the compound **7**.



7 **92**

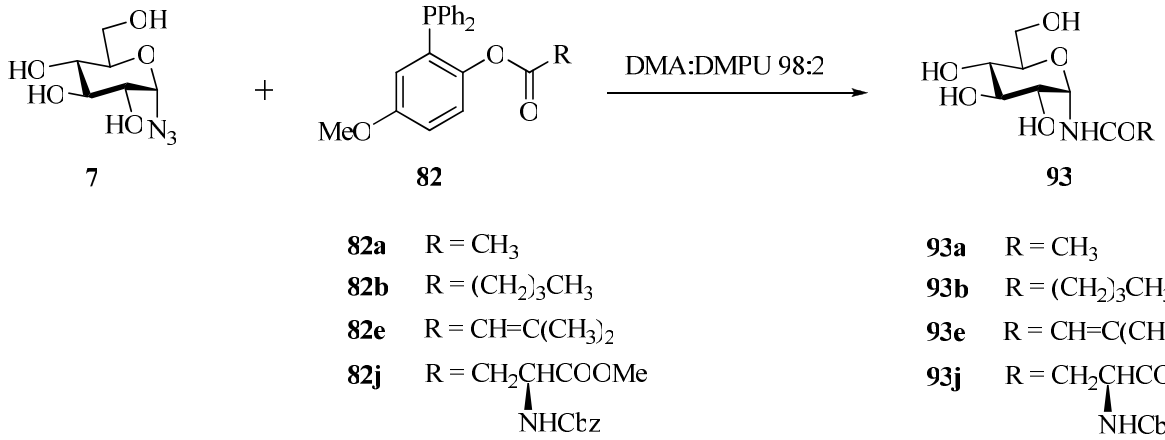
Scheme 9. Staudinger ligation of **7** with the functionalized (diphenylphosphino)methanethiol **92**

The mechanism proposed by Raines and co-workers (Scheme 10)[8] for the ligation mediated by (diphenylphosphino)methanethiol **99**, show that the imminophosphorane **100** can form a tetrahedral intermediate **101**, which collapses to give an amidophosphonium salt **102** that is hydrolyzed in aqueous solution, furnish the final amide. Probably in our case the steric hindrance of the tetrahedral intermediate **101**, that occour during the ligation, avoid the complete reduction of α-glucosyl azide **7** and the acyl transfer of the acyl chain.



Scheme 10. Mechanism for the Traceless Staudinger ligation mediated by (diphenylphosphino)methanthiol [7]

4.3 Appendix



7 **82** **93**

82a R = CH_3
82b R = $(CH_2)_3CH_3$
82e R = $CH{=}C(CH_3)_2$
82j R = $CH_2CHCOOMe$ (NHCbz)

93a R = CH_3
93b R = $(CH_2)_3CH_3$
93e R = $CH{=}C(CH_3)_2$
93j R = $CH_2CHCOOMe$ (NHCbz)

Table A. Screening for the synthesis of α-glucosylamides **93**

Entry	Reagent	Process	Glucose	Azide	β-pyranosyl amide 94	α-pyranosyl amide 93	α-furanosyl amide 95
1	**82a**	70 °C, 18 h	6	/	10	48	36
2	**82a**	100 °C, 18 h	/	/	13	37	50
3	**82b**	70 °C, 18 h	/	/	16	24	60
4	**82b**	100 °C, 18 h	/	/	17	18	65
5	**82b**	M.W.120°C,20’	/	24	15.5	6.5	54
6	**82b**	M.W.120°C,40’	5	8	17	8	59
7	**82e**	100 °C, 18 h	18	/	51	/	31
8	**82j**	40 °C, 18 h	?	43	?	23	27
9	**82j**	70 °C, 18 h	45	/	?	17	38
10	**82j**	70 °C, 4 h	12	26	?	17	26
11	**82j**	M.W.120°C, 1h	12.5	38	?	20	29

Ratio estimated on the basis of the ^{1}H-NMR (D_2O)spectra of the crude water extract
(?) ^{1}H-NMR integration not possible

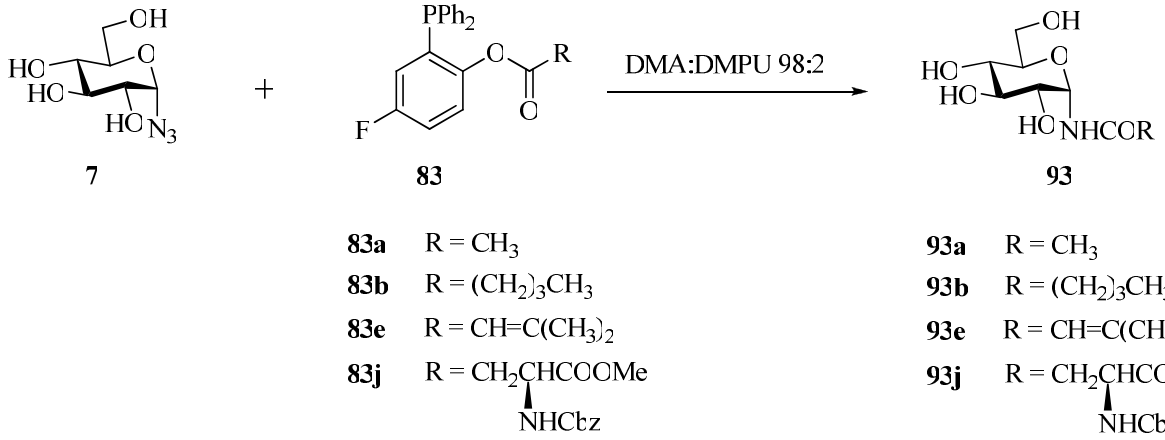
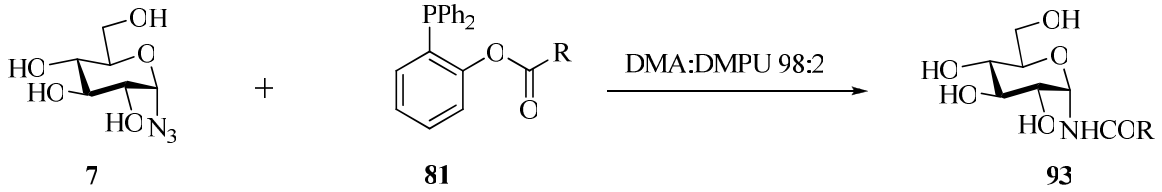


Table B. Screening for the synthesis of α-glucosylamides **93**

Entry	Phosphin.	Process	Glucose	Azide	β-pyranosyl amide 94	α-pyranosyl amide 93	α-furanosyl amide 95
1	**83a**	70 °C, 4 h	16	5	9.5	54	15
2	**83a**	70 °C, 18 h	12	7	8	60	13
3	**83b**	40 °C, 4 h	15	31	7.5	29.5	16
4	**83b**	70 °C, 4 h	15.5	/	10.5	55	19
5	**83b**	70 °C, 18 h	20.5	/	24	8	47
6	**83b**	M.W.50°C,50’	17	8	17	24.5	33
7	**83b**	M.W.120°C,20’	12	7	17	14	50
8	**83e**	70 °C, 4 h	22	/	31	6.5	40
9	**83e**	M.W.120°C,20’	21	/	33.5	32	13.5
10	**83j**	40 °C, 18 h	5	25	?	44	15
11	**83j**	70 °C, 18 h	27	/	?	45	24
12	**83j**	70 °C, 4 h	9	19.5	?	30	19
13	**83j**	M.W.120°C,40’	6	28	7	39	19
14	**83j**	45 °C, 18 h	20	/	/	61	22
15	**83j**	40 °C, 18 h	15	/	/	65(55[a])	20

Ratio estimated on the basis of the ^{1}H-NMR (D_2O)spectra of the crude water extract
(?) ^{1}H-NMR integration not possible. a) Isolated yield



81a R = CH_3
81b R = $(CH_2)_3CH_3$
81e R = $CH{=}C(CH_3)_2$
81j R = $CH_2CHCOOMe$ (NHCbz)

93a R = CH_3
93b R = $(CH_2)_3CH_3$
93e R = $CH{=}C(CH_3)_2$
93j R = $CH_2CHCOOMe$ (NHCbz)

Table C. Screening for the synthesis of α-glucosylamides **93**

Entry	Phosphin.	Process	Glucose	Azide	β-pyranosyl amide 94	α-pyranosyl amide 93	α-furanosyl amide 95
1	**81a**	70 °C, 18 h	20	/	/	77	3
2	**81b**	40 °C, 4 h	20	/	/	75(65[a])	5
3	**81b**	70 °C, 18 h	20	/	/	65(50[a])	15
4	**81b**	M.W.50°C,50’	20	/	/	75(65[a])	2
5	**81b**	M.W.120°C,10’	20	/	/	75(65[a])	2
6	**81e**	40 °C, 4 h	20	/	/	40	40
7	**81e**	M.W.50°C,50’	/	/	/	10[a]	52[a]
8	**81j**	40 °C, 4 h	35	/	/	25[a]	15
9	**81j**	M.W.50°C,50’	35	/	/	25[a]	10
10	**81j**	M.W.120°C,10’	/	/	/	25[a]	10

Ratio estimated on the basis of the ^{1}H-NMR (D_2O)spectra of the crude water extract
(?) ^{1}H-NMR integration not possible. a) Isolated yield

4.4 References

[1] Nisic, F.; Bernardi, A. *Carbohydr. Res*. **2008**, 343, 1636-1643.

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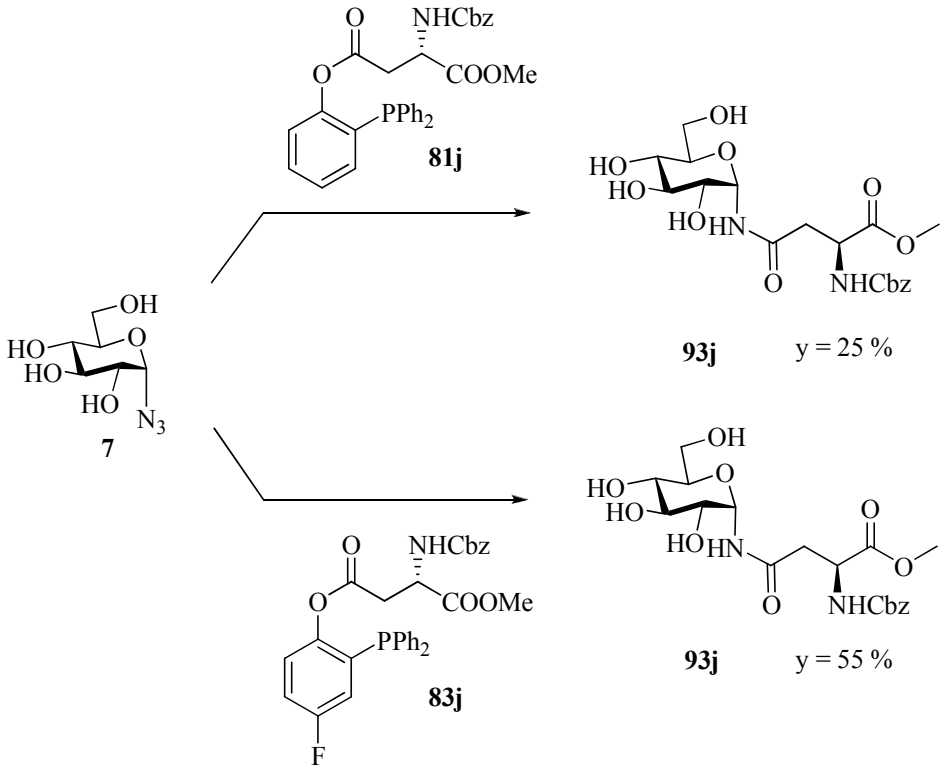
[12] For examples, see: (a) He, Y.; Hinklin, R. J.; Chang, J. Y.; Kiessling, L. L. *Org. Lett.* **2004**, *6*, 4479-4482. (b) Bianchi, A.; Bernardi, A. *Tetrahedron Lett.* **2004**, *45*, 2231-2234. (c) Bianchi, A.; Russo, A.; Bernardi, A. *Tetrahedron Asymmetry* **2005**, *16*, 381-386. (d) Liu, L.; Hong, Z.-Y.; Wong, C.-H. *ChemBioChem* **2006**, *2006*, 429-432.

Chapter 5

Stereoselective synthesis of *N*-glycosyl amino acids by traceless Staudinger ligation of unprotected glycosyl azides

In chapter 4 we showed that monofluorophosphine **83j** improve the yields of aspartic acid transfer, which occurred with over 55 % yield, compared to 25 % afforded by the non-fluorinated analogue **81j** (Scheme 1). Hence, improving the leaving group ability of the activated ester appears to result in higher yields for this difficult transfer step and the novel functionalized phosphanis **83j** affords the corresponding *N*-glucosyl aspartic acid **93j** in a stereoconservative and synthetically pratical manner. This preliminary result prompted us to further explore the scope of phosphines derived from monofluorophosphino phenol **60** in the ligation of amino acids and glycosyl azides.

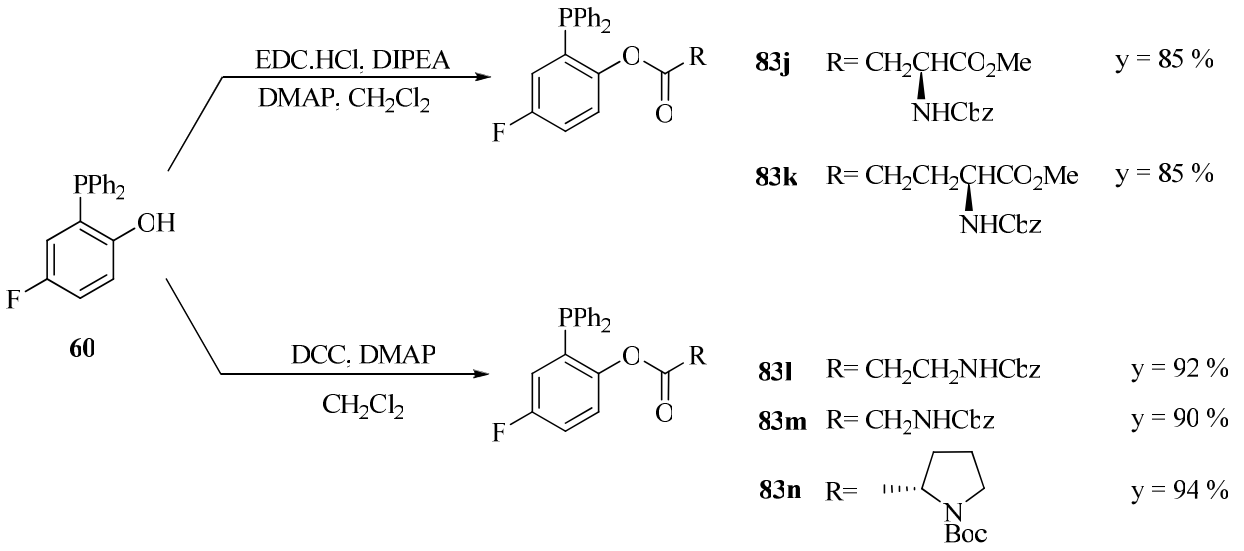


Scheme1. Ligation of α-glucosyl azide **7** with functionalized phosphines **81j** and **83j**

5.1 Synthesis of α- and β-glucosyl amino acids

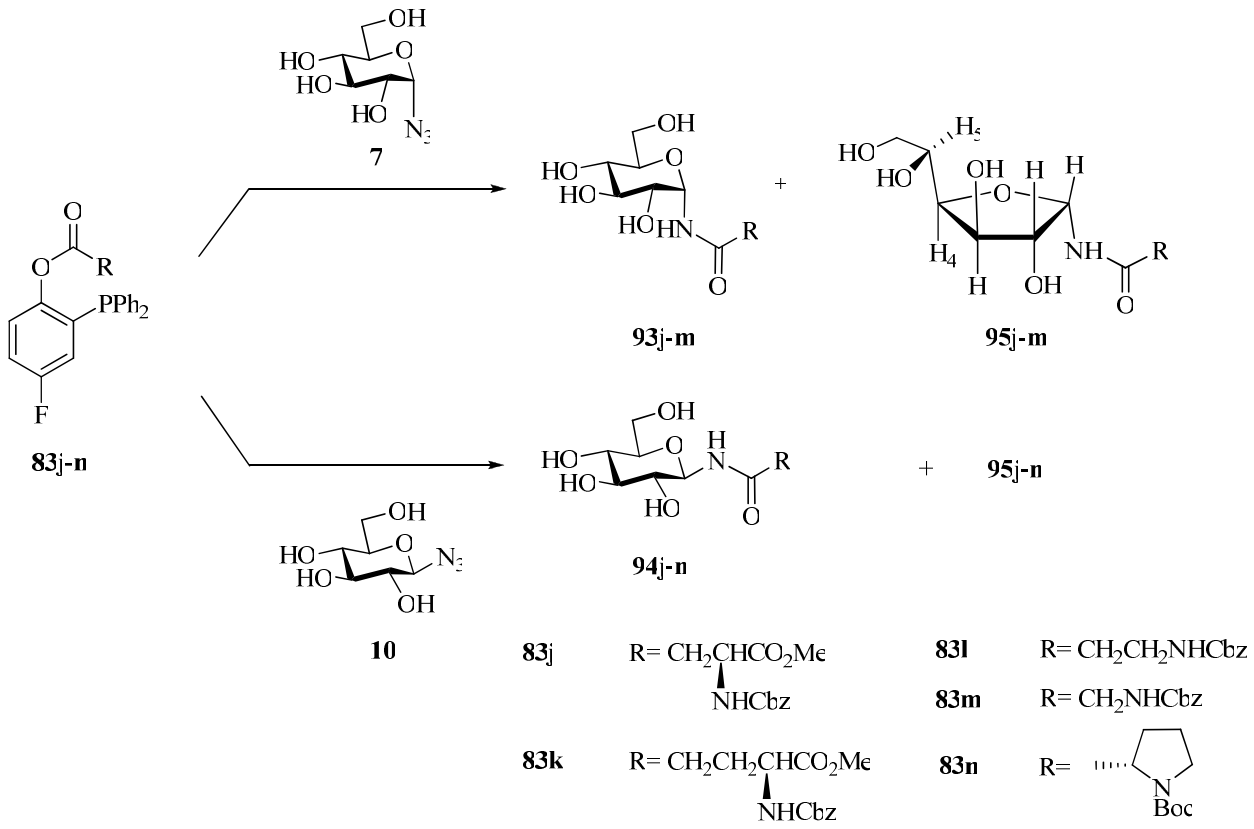
The monofluoro phosphines **83j-n**, could be obtained in high yields from the known phosphine **60**[1] by reaction with the appropriate amino acids and condensing agents as show in Scheme 2, (See chapter 3 experimental ct for the procedures). The functionalized phosphines **83j-n** were synthesized to examine the Staudinger ligation of the aspartic and glutamic acid side-chains (**83j** and **83k**, respectively) as well as the reactions of simple α- and β-amino acids such as β-alanine

(**83l**), glycine (**83m**) and proline (**83n**). Optimal yields were obtained by using either *N*-[3-(dimethylamino) propyl]-*N*-ethylcarbodiimide (EDC) (**83j** and **83k**) or DCC (**83l-n**) as the condensing agent. The ligation reagents could be isolated by simple filtration through a silica gel plug. Analytical samples were purified by flash chromatography. In general, and like the parent non-fluorinated compounds, phosphines **83j-n** were found to be air-stable compounds that could be handled under standard conditions with minimal precautions.



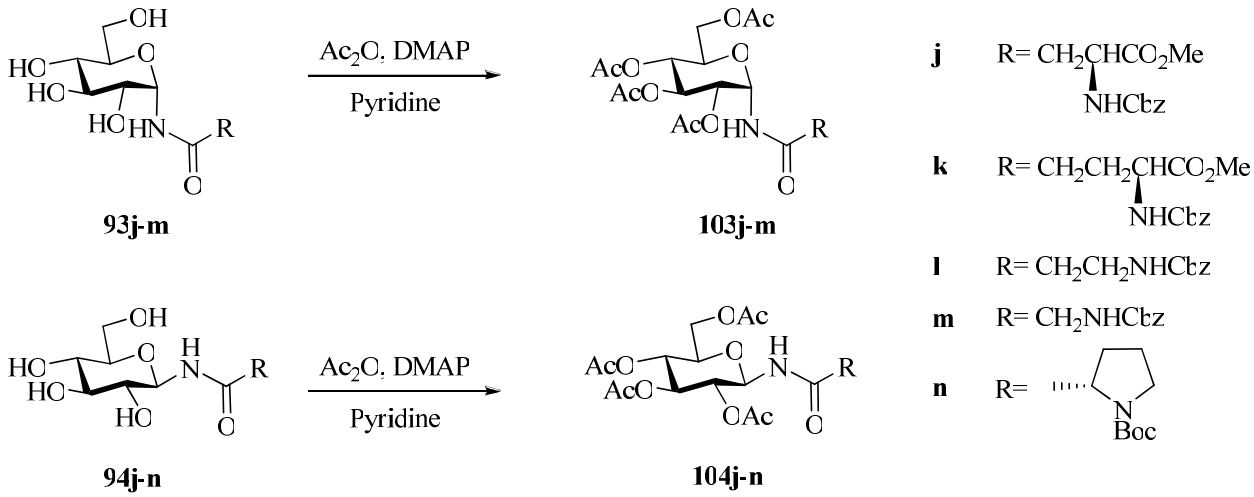
Scheme 2. Synthesis of the fluorinared phosphines **83j-n**

The ligation experiments were performed by treating the unprotected α- and β-glucosyl azides **7** and **10** with 2 equivalents of phosphine **83j-n** in DMA/DMPU (98:2) at 40 °C for 18 h. After completion of the reaction, the crude mixtures were stirred with water for an additional 2 h, then diluted with water and extracted with CH_2Cl_2 to eliminate the phosphine by-products. The aqueous layers were analyzed by ^1H NMR (typically in D_2O) and the product ratios established on the basis of the integration of the anomeric protons. Typical reaction crudes contained both the expected α- or β-pyranosyl amides **93** or **94** and variable amounts of the corresponding furanosyl amides **95** (Scheme 3). The anomeric configuration of **95j-n** was consistent with the previously data reported for the α-glucofuranosyl amides.[2] As we have previously shown (Scheme 4, chapter 4),[2] the latter are formed by ring-opening/ring-closure of the intermediate iminophosphoranes.[3] Free glucose (equilibrium anomeric mixtures) is also formed by hydrolysis of the same intermediates.



Scheme 3. Synthesis of α- and β- glucosyl aminoacids **93j-m** and **94 j-n**.

The glucosyl amides were purified by flash chromatography on silica gel ($CHCl_3$/MeOH/H_2O 80:20:2) and fully characterized as their acetylated counterparts **103j-m** and **104j-n** (Scheme 4).



Scheme 4. Acetylated compounds **103j-m** and **104j-n**

Ligation of the α-azide was generally accompanied by ring contraction and sizeable quantities of the glucofuranosyl amides **95** were formed (Table 1, entries 1-4). Strict control of anhydrous

conditions and temperature were found to minimize the formation of ring-contraction by-products. In our previous studies with phosphines **81j**,[2] the use of MW irradiation proved beneficial for reducing the amount of furanose formation, but this was not the case with phosphines **83j** as MW irradiation (50 °C, 50 min) gave lower yields of the desired pyranosyl amides and more by-products.

The reactions with β-glucosyl azide **10** (Table 1, entries 5-9) gave good-to-excellent yields of the corresponding pyranosyl amides **94j-n**, which were essentially pure after water extraction. The ^{1}H NMR spectra of the reaction crudes showed no trace of the anomeric α-pyranosyl epimer and very minor quantities (3 %) of a by-product to which the furanosyl amide structure **95** was tentatively assigned; α-, β- and γ-amino acids were transferred with equal efficiency. The aspartic acid amide **94j** (Table 1, entry 5) was the most difficult to obtain, but could still be isolated in 77 % yield, as opposed to the yield of 32 % obtained under the same conditions with phosphane **81j**.[2]

Table 1. Synthesis of α- and β-glucosylaminoacids **93** and **94** by ligation of α- and β-glucosylazides **7** and **10** with **83j-n**

Entry	Phosphine	Azide	Product	Isolated yields[a] (%)	α/β ratio[b]	Pyranose/Furanose ratio[c]
1	**83j**	**7**	**93j**	56	98:2	85:15
2	**83k**	**7**	**93k**	59	98:2	87:13
3	**83l**	**7**	**93l**	60	98:2	90:10
4	**83m**	**7**	**93m**	36 (12)[d]	98:2	67:33
5	**83j**	**10**	**94j**	77	≤ 1:99	98:2
6	**83k**	**10**	**94k**	87	≤ 1:99	98:2
7	**83l**	**10**	**94l**	85	≤ 1:99	98:2
8	**83m**	**10**	**94m**	75	≤ 1:99	97:3
9	**83n**	**10**	**94n**	70	≤ 1:99	98:2

a) Yields based on glucopyranosylamide b) Ratio of the anomeric proton signals of the α- and β-pyranosylamides **93** and **94** in the crude ^{1}H-NMR spectrum c) Ratio of the anomeric proton signals in the crude ^{1}H-NMR spectrum. d) Isolated yield of furanosyl amide **95m**.

A significant difference in the reactivity of α- and β-glucopyranosyl azides towards the traceless Staudinger ligation was noted, in particular reaction of α-glucosyl azide **7** (Table 1, entries 1-4) with **83j-m** afforded the corresponding α-amide **93j-m** in lower yields compared to the β-anomers **94j-n** formed from **10** (Table 1, entries 5-9).

In 2010 Field and co-worker[4] reported a comparison of X-ray crystallographic data for both the α- and β-peracetylated glucopyranosyl azides **1** and **2** highlighted the impact of anomeric effect on the dipolar character of the anomeric azides, which likely accounts for the observed reactivity difference. This comparison revealed that the acetylated glucopyranose rings of **1** and **2** are very similar, except for the anomeric azido groups having different configurations (axial vs equatorial). In addition, the interatomic distances within the C(1)-N(A)-N(B)-N(C) fragment (Table 2 and Figure1) are in keeping with the expected influence of the anomeric effect. The C(1)-N(A) distance in **1** is shorter than the corresponding distance in **2**; the terminal N(B)-N(C) bond is shorter than the inner N(A)-N(B) bond in β-isomer **1**, whereas in α-isomer **2** these bonds are more similar value.

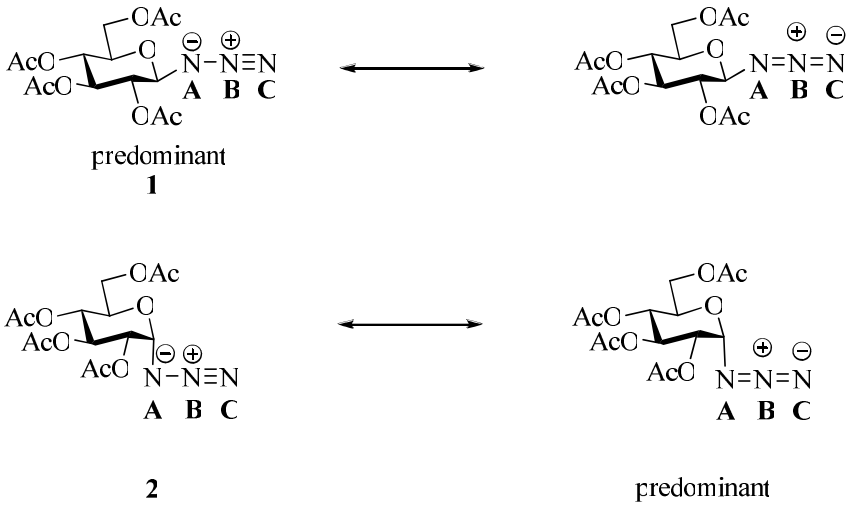


Figure 1. Resonance structures for α- and β-glucopyranosyl azides

Table 2. Interatomic distance (Å) for CN_3 fragment in tetra-*O*-acetyl-glucosyl azides **1** and **2**

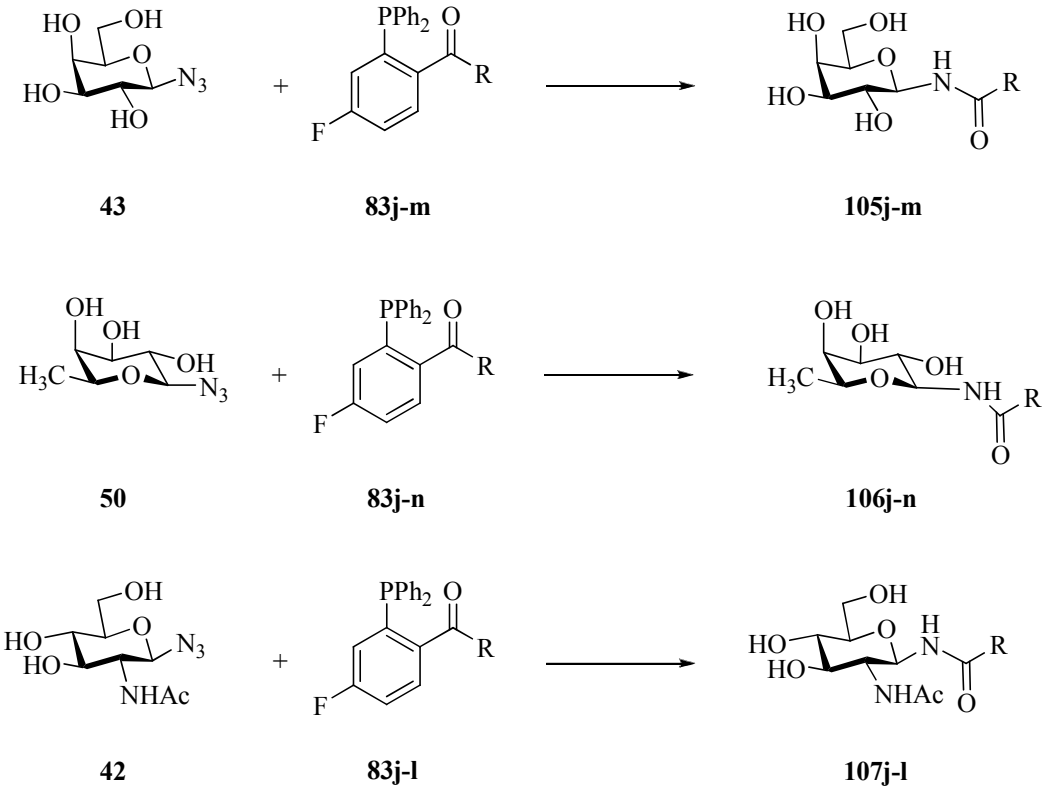
	β-azide 1	**α-azide 2**
C(1)-N(A)	1.460	1.510
N(A)-N(B)	1.243	1.165
N(B)-N(C)	1.119	1.195

This particular difference between the azido group in **1** and **2** indicates a significant difference in electron distribution within this group for two isomeric compounds, which manifest itself as a difference in dipolar charge character and hence the different reaction rates.

The higher reactivity of β-azides **1** can be interpreted as a result of a partial negative charge on atom N(A).

5.2 Synthesis of other β-glycosyl amino acids

To probe the scope of the fluorinated phosphines **83**, other β-unprotected glycosyl azides were tested in the Staudinger ligation (Scheme 5, Table 3). The reactions of phosphines **83j-n** with β-galactosyl azide **43**, β-fucosyl azide **50** and β-glucosyl-*N*-acetyl azide **42** were performed and afforded the corresponding glycosyl amides **105**, **106** and **107** in good yields.



Scheme 5. Synthesis of β- amides **105-107** with **83j-n**

^{1}H NMR analysis of the crude reaction mixtures showed no trace of the anomeric α-pyranosyl epimer. Variable quantities (5-13 %) of the isomeric furanosyl amides were formed, depending both on the nature of the azide and the amino acid transferred (see Table 3). In all cases, this by-product could be separated from the pyranose isomer by flash chromatography. With the β-galactosyl azide **43**, β- and γ-amino acids were transferred with an average yield of 70 % (Table 3, entries 1-4). Similar results were obtained with β-fucosyl azide **50** (Table 3, entries 5-9). β-Glucosyl-*N*-acetyl azide **105** was found to be less reactive and the corresponding amides were formed in an average yield of 55 % (Table 3, entries 10-12).[5] Comparison with the yields obtained with the corresponding β-glucosyl azide **10** (Table 1, entries 5-7), which on average are higher by 30 %, suggests a deactivating role of the acetamido group in **42**.

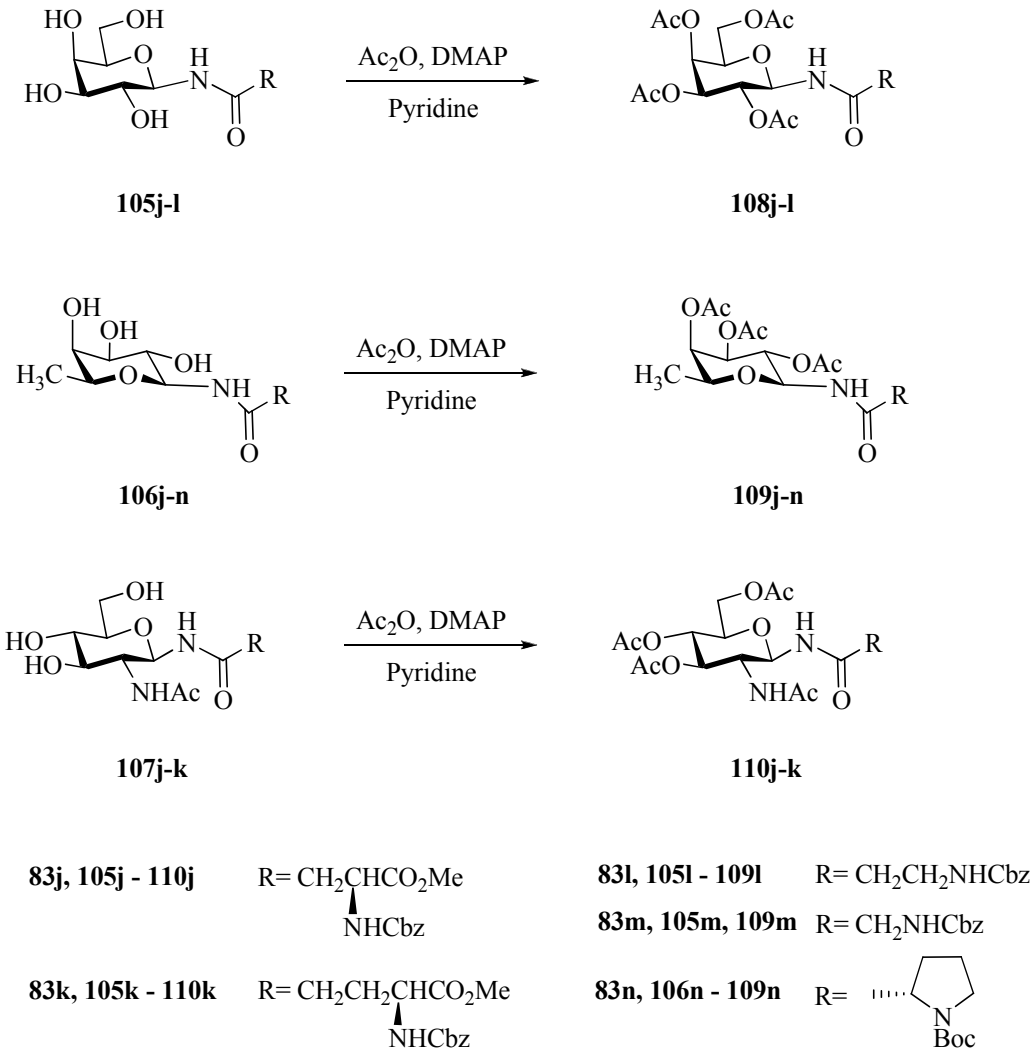
Table 3. Synthesis of β-amides **105-107** with **83j-n**.

Entry	Phosphine	Azide	Product	Isolated yields[a] (%)	Pyranose/Furanose ratio[b]
1	**83j**	**43**	**105j**	74	-
2	**8k**	**43**	**105k**	70	5 %
3	**83l**	**43**	**105l**	71	10 %
4	**83m**	**43**	**105m**	73	5 %
5	**83j**	**50**	**106j**	60[c]	9 %
6	**83k**	**50**	**106k**	68 [c]	9 %
7	**83l**	**50**	**106l**	70 [c]	13 %
8	**83m**	**50**	**106m**	75 [c]	6 %
9	**83n**	**50**	**106n**	87 [c]	-
10	**83j**	**42**	**107j**	56	-
11	**83k**	**42**	**107k**	54	-
12	**83l**	**42**	**107l**	50	-

a) Yields based on β-pyranosylamide . b) Ratio of the anomeric proton signals estimated from ^{1}H-NMR spectra of the crude reaction mixtures. c. ca. 10 % of hydrolysis product (fucose) also observed

The propensity of galactopyranose and fucopyranose (Table 3, entries 2-8) to gives the formation of furanose configuration, in non aqueous media was already known in literature.[6]

The β-galactosyl, β-fucosyl and β-Glucosyl-*N*-acetyl amides were purified by flash chromatography on silica gel ($CHCl_3$/MeOH/H_2O 80:20:2 or $CHCl_3$/MeOH 80:20) and fully characterized as their acetylated counterparts **108j-l**, **109j-n** and **110j-k** (Scheme 6).



Scheme 6. Acetylated compounds **108j-l**, **109j-n** and **110j-k**

5.3 Conclusions

The results reported shown that unprotected α- and β-glycosyl azides can be stereoselectively ligated to α-, β- and γ-amino acids by traceless Staudinger ligation using fluorinated phosphines **83**. All the *N*-glycosyl amino acid products could be isolated from the crude reaction mixtures by simple water extraction and were further purified by flash chromatography on silica gel.

The starting phosphine **60** is not commercially available, but it can be easily prepared on the gram scale by following the reported procedure (Chapter 3).[1] It is stable to air at room temperature for weeks and can be handled with no special precautions.

Acylation followed by silica gel filtration affords the ligation agents **83j-n**, which can be used without further purification. The stereoselective synthesis of the required azide precursors has been reported in the Chapter 2. The ligation method described herein works reliably well for unprotected β-azides of the *gluco*, *galacto* and *fuco* series. Lower yields (ca. 55 %) were obtained with β-glucosyl-2-*N*-acetyl and α-glycosyl azides. Only one method for the synthesis of β-D-2-deoxy-2-*N*-acetylglucopyranosyl-asparagine from unprotected *N*-acetylglucosamine has been described.[7]

There are only a handful of methods that can be used to synthesize α-*N*-glycosyl amides,[2, 8, 9, 10] among them, the traceless Staudinger ligation of α-glycosyl azides with functionalized phosphines was found to perform poorly with amino acids. The fluorophenylphosphines **83j-n** described allow the synthesis of α-*N*-glucosyl amino acids in reasonable yields and in a synthetically practical manner.

The elaboration of unprotected carbohydrates has received considerable attention in recent years.[11, 12, 13] Unprotected glycosyl amino acids can be used directly for the linear synthesis of glycopeptides (direct insertion of the glycosyl amino acid into a growing peptide chain).[10, 14] Methods of glycopeptide synthesis that do not involve protected sugars do not require further manipulation of the sugar, which can lead to racemization of the amino acid.[15]

Beyond affording *N*-glycosylated Asn and Gln building blocks, the functionalized phosphines described in this chapter could also be used to achieve traceless ligation of glycosyl azides to glycine, proline and to the non-proteinogenic amino acid β-Ala.

The resulting compound are not meant to be incorporated into *N*-glycopeptides sequences, but can be used for the bioconjugation of carbohydrates to aglycons using chemically stable bonds and for the design of carbohydrate mimics which may resist in vivo to hydrolytic enzymes.[16, 17]

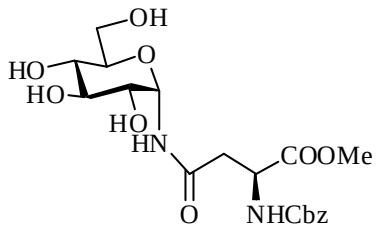
5.4 Experimental Section

Solvents were dried by standard procedures: dichloromethane, methanol, *N*,*N*-diisopropylethylamine and triethylamine were dried over calcium hydride; *N*,*N*-dimethylacetamide (DMA), 1,3-dimethyltetrahydro-2(1*H*)pyrimidinone (DMPU), chloroform and pyridine were dried over activated molecular sieves. Reactions requiring anhydrous conditions were performed under nitrogen. ^{1}H, ^{13}C and ^{31}P-NMR spectra were recorded at 400 MHz on a Bruker AVANCE-400 instrument. Chemical shifts (δ) for ^{1}H and ^{13}C spectra are expressed in ppm relative to internal Me_4Si as standard. Signals were abbreviated as s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained with a Bruker ion-trap Esquire 3000 apparatus (ESI ionization) or an Autospec Fission Instrument (FAB ionization) and FT-ICR Mass Spectrometer APEX II & Xmass software (Bruker Daltonics) - 4.7 Magnet. Thin layer chromatography (TLC) was carried out with pre-coated Merck F_{254} silica gel plates. Flash chromatography (FC) was carried out with Macherey-Nagel silica gel 60 (230-400 mesh).

General procedure for stereoselective ligation of 3 in DMA:DMPU mixtures.

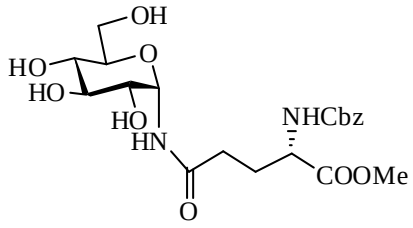
The phosphine (2 eq) was added, at room temperature, to a 0.1 M solution of azide (1 eq) in 98:2 *N*,*N*-dimethylacetamide and DMPU. The solution was stirred for 16 h at 40 °C, then water was added and the mixture was stirred for an additional 2H at the same temperature. The solvent was evaporated under reduced pressure, and the residue was diluted with water and extracted with CH_2Cl_2. The water layer was evaporated under reduced pressure and the crude was purified as indicated below for each compound.

N^{α}-(Benzyloxycarbonyl)-N^{γ}-(α-D-glucopyranosyl)-L-asparagine *O*-Methyl Ester (93j)



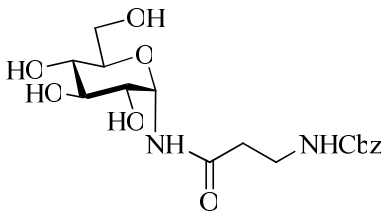
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.35) or automated reverse phase chromatography (H_2O:CH_3CN gradient);
^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.42-7.28 (m, 5H, Ph), 5.48 (d, $J_{1,2}$ = 5.6 Hz, 1H, H-1), 5.06 (bs, 2H, CH_2O), 4.55 (t, J = 6.4 Hz, 1H, CH), 3.72 (dd, $J_{2,3}$ = 5.6 Hz, 1H, H-2), 3.54-3.76 (m, 4H, H-3, H-4, H-5, H-6), 3.68 (s, 3H, OCH_3), 3.34 (m, 1H, H-6'), 2.88 (d, J = 6.4 Hz, 2H, CH_2). ^{13}C-NMR (100 MHz, D_2O):δ 179.4, 128.8, 128.4, 127.6, 76.6 (C-1), 72.9 (C-3), 72.6 (C-5), 69.2 (C-2), 69.1 (C-4), 67.3 (CH_2O), 60.5 (C-6), 53.14 (OCH_3), 50.8 (CH). MS (ESI): *m/z* = 465.2 $[M + Na]^+$. $[\alpha]^D_{25}$ = + 57.2 (c 0.2, MeOH). Yield = 55 %.

N^{α}-(Benzyloxycarbonyl)-N^{δ}-(α-D-glucopyranosyl)-L-glutamine *O*-Methyl Ester (93k)



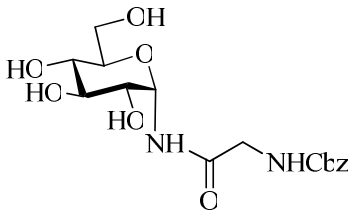
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.33); ^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.42-7.28 (m, 5H, Ph), 5.52 (d, $J_{1,2}$ = 5.5 Hz, 1H, H-1), 5.10 (s, 2H, CH_2O), 4.20 (q, J = 5 Hz, $J_{CH,CH2}$ = 9 Hz, 1H, CH), 3.72 (m, 4H, H-2, OCH_3), 3.54-3.76 (m, 6H, H-3, H-4, H-5, H-6, H-6'), 2.47 (t, J = 7 Hz, 2H, $COCH_2$), 2.18 (m, 1H, H_a, CH_2), 1.95 (m, 1H, H_b, CH_2). MS (ESI): *m/z* = 457.3 $[M + H]^+$. $[\alpha]^D_{25}$ = + 50.4 (c 0.3, MeOH). Yield = 59 %.

_N_-(_N_-Benzyloxycarbonyl-β-alanyl)-α-D-glucopyranosylamine (93l)



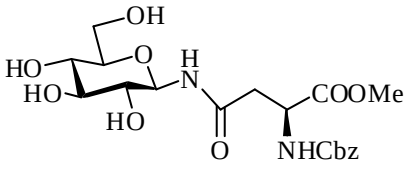
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.38); ^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.41-7.33 (m, 5H, Ph), 5.52 (d, 1H, $J_{1,2}$ = 5.4 Hz, H-1) 5.05 (s, 2H, CH_2O), 3.78-3.58 (m, 4H, H-2, H-6, H-6'), 3.50-3.27 (m, 4H, CH_2), 2.53-2.48 (m, 2H, CH_2). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 175.9, 158.2 ($CO_{(Cbz)}$), 136.4 (C_{ipso}), 128.8, 128.5, 127.7 (5xC_{Ar}), 76.6 (C-1), 73.0, 72.6, 69.3, 69.2, 66.9 (CH_2O), 60.4 (C-6), 38.1 (CH_2), 36.1 (CH_2). $[\alpha]^D_{25}$ = + 46.4 (c 1, MeOH). Yield = 60 %.

_N_-(_N_-Benzyloxycarbonylglycyl)-α-D-glucopyranosylamine (93m)



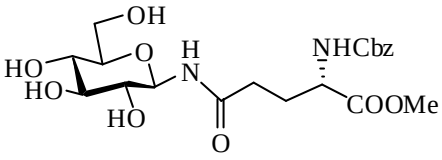
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.23); ^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.43-7.29 (m, 5H, Ph), 5.53 (d, $J_{1,2}$ = 5.6 Hz, 1H, H-1), 5.09 (s, 2H, CH_2O), 3.89 (bs, 2H, CH_2), 3.87-3.34 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6'). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 170.3, 128.8, 128.4, 127.8 (5xC_{Ar}), 79.4 (C-1), 76.7, 72.7, 72.4, 69.4, 67.3 (CH_2O), 60.6 (C-6), 43.4 (CH_2). $[\alpha]^D_{25}$ = + 16.3 (c 0.5, MeOH). Yield = 36 %.

N^{α}-(Benzyloxycarbonyl)-N^{γ}-(β-D-glucopyranosyl)-L-asparagine *O*-Methyl Ester (94j)



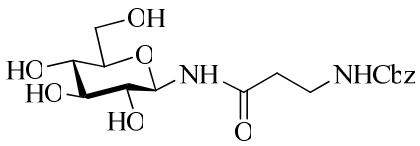
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.43); ^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.45-7.35 (m, 5H, Ph), 5.10 (s, 2H, CH_2O), 4.96 (d, $J_{1,2}$ = 9.2 Hz, 1H, H-1), 4.65 (t, J = 6.4 Hz, 1H, CH-N), 3.88 (dd, $J_{6,6'}$ = 12.4 Hz, $J_{5,6}$ = 2.4 Hz, 1H, H-6), 3.80-3.69 (m, 4H, H-6', OCH_3), 3.60-3.51 (m, 2H, H-4, H-5), 3.46-3.37 (m, 2H, H-3, H-2), 2.95-2.89 (m, 2H, CH_2). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 173.4, 173.1, 157.7 ($CO_{(Cbz)}$), 136.2 (C_{ipso}), 128.8, 128.4, 127.6 (5xC_{Ar}), 79.2 (C-1), 77.5 (C-5), 76.4 (C-4), 71.7 (C-2), 69.2 (C-3), 67.2 (CH_2O), 53.1 (OCH_3), 50.6 (CH), 37.1 (CH_2). MS (FAB): *m/z* = 465 $[M + Na]^+$. $[\alpha]^D_{25}$ = - 0.6 (c 1, MeOH). Yield = 77 %.

N^{α}-(Benzyloxycarbonyl)-N^{δ}-(β-D-glucopyranosyl)-L-glutamine *O*-Methyl Ester (94k)



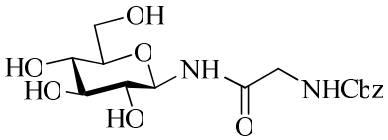
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.47); ^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.49-7.38 (m, 5H, Ph), 5.14 (s, 2H, CH_2O), 4.95 (d, $J_{1,2}$ = 9.2 Hz, 1H, H-1), 4.25 (m, 1H, CH-N), 3.86 (dd, $J_{5,6}$ = 2 Hz, $J_{6,6'}$ = 12.4 Hz, 1H, H-6), 3.72 (s, 3H, OCH_3), 3.67 (dd, $J_{5,6'}$ = 5.2 Hz, 1H, H-6'), 3.54-3.47 (m, 2H, H-4, H-5), 3.44-3.35 (m, 2H, H-3, H-2), 2.44 (t, J = 7.6 Hz, 2H, CH_2CO), 2.27-2.18 (m, 1H, H_a, CH_2CH), 2.04-1.95 (m, 1H, H_b, CH_2CH). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 176.2, 174.2, 158 ($CO_{(Cbz)}$), 136.3 (C_{ipso}), 128.8, 128.4, 127.6 (5xC_{Ar}), 79.2 (C-1), 77.5 (C-5), 76.4 (C-4), 71.8 (C-2), 69.2 (C-3), 67.2 (CH_2O), 60.5 (C-6), 53.5 (CH), 52.9 (OCH_3), 31.7 (CH_2CO), 26.2 (CH_2). MS (FAB): *m/z* = 479 $[M + Na]^+$. $[\alpha]^D_{25}$ = - 10 (c 1, MeOH). Yield = 87 %.

_N_-(_N_-Benzyloxycarbonyl-β-alanyl)-β-D-glucopyranosylamine (94l)



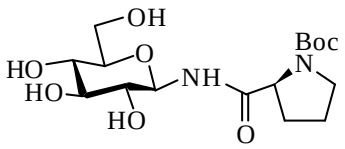
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.34); ^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.50-7.38 (m, 5H, Ph), 5.11 (s, 2H, CH_2O), 4.97 (d $J_{1,2}$ = 8.8 Hz, 1H, H-1), 3.88 (d, $J_{6,6'}$ = 12.4 Hz, 1H, H-6), 3.72 (dd, $J_{6,6'}$ = 12.4 Hz, 1H, H-6'), 3.60-3.30 (m, 6H, H-5, H-4, H-2, H-3, CH_2NHCbz), 2.54 (t, 2H, CH_2). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 175.3, 158.2 ($CO_{(Cbz)}$), 136.4 (C_{ipso}), 128.8, 128.3, 127.6 (5xC_{Ar}), 79.2 (C-1), 77.5 (C-5), 76.4 (C-4), 71.7 (C-2), 69.2 (C-3), 66.9 (CH_2O), 60.5 (C-6), 36.7 (CH_2), 35.8 (CH_2). MS (FAB): *m/z* = 407 $[M + Na]^+$. $[\alpha]^D_{25}$ = + 3.5 (c 0.7, MeOH). Yield = 85 %.

_N_-(_N_-Benzyloxycarbonylglycyl)-β-D-glucopyranosylamine (94m)



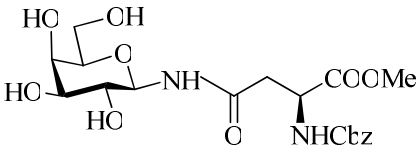
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.32); ^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.45-7.30 (m, 5H, Ph), 5.09 (s, 2H, CH_2O), 4.94 (d, $J_{1,2}$ = 9.2 Hz, 1H, H-1), 3.88-3.77 (m, 3H, CH_2, H-6), 3.64 (dd, $J_{5,6'}$ = 5.2 Hz, $J_{6,6'}$ = 12.4 Hz, 1H, H-6'), 3.53-3.44 (m, 2H, H-5, H-3), 3.39-3.28 (m, 2H, H-2, H-4). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 173.4, 158.6 ($CO_{(Cbz)}$), 136.2 (C_{ipso}), 128.8, 128.2, 127.7 (5xC_{Ar}), 79.3 (C-1), 77.6, 76.4 , 71.7, 68.9, 66.4 (CH_2O), 60.5 (C-6), 43.7 (CH_2). $[\alpha]^D_{25}$ = + 4.1 (c 1, MeOH). Yield = 75 %.

_N_-(_N_-_tert_-Butoxycarbonyl-L-prolyl)-β-D-glucopyranosylamine (94n)



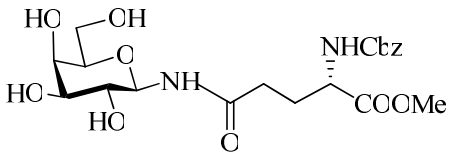
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.36); ^{1}H-NMR (400 MHz, MeOD, 25°C): δ = 4.95 (d, $J_{1,2}$ = 8.8 Hz, 1H, H-1), 4.26 (dd, J = 3.6 Hz, J = 8.4 Hz, 1H, CH), 3.87 (bdd, $J_{6,6'}$ = 11.6 Hz, 1H, H-6), 3.75-3.68 (m, 1H, H-6'), 3.63-3.52 (m, 1H, CH_2CH, H_a), 3.51-3.42 (m, 2H, H-3, CH_2CH, H_b), 3.41-3.30 (m, 3H, H-2, H-4, H-5), 2.37-2.18 (m,1H, H_c), 2.08-1.88 (m, 3H, H_d, CH_2), 1.50 (m, 9 H, Boc). ^{13}C-NMR (100 MHz, MeOD, 25°C): δ = 81.4 (C-1), 79.8, 79.1, 74.2, 71.6, 62.8 (C-6), 61.8 (CH), 48 (CH_2), 32 (CH_2), 28.8 (Boc), 25.1 (CH_2). FT-ICR MS (ESI): calcd. for $C_{16}H_{28}N_2O_8$ Na $[M+Na]^+$ 399.17379; found 399.17314. $[\alpha]^D_{25}$ = - 39.2 (c 0.6, MeOH). Yield = 70 %.

_N_$^{\alpha}$-(Benzyloxycarbonyl)-_N_$^{\gamma}$-(β-D-galactopyranosyl)-L-asparagine _O_-Methyl Ester (105j)



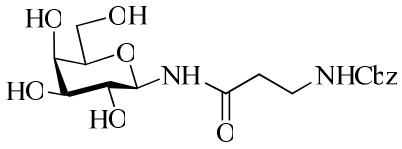
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.18); ^{1}H-NMR (400 MHz, MeOD, 25°C): δ = 7.42-7.28 (m, 5H, Ph), 5.11 (s, 2H, CH_2O), 4.58 (m, 1H, CH), 3.89 (d, J = 2.4 Hz, 1H, H-4), 3.77-3.64 (m, 5H, H-6, H-6', OCH_3), 3.63-3.47 (m, 3H, H-2, H-3, H-5), 2.89-2.78 (m, 2H, CH_2). ^{13}C-NMR (100 MHz, MeOD, 25°C): δ = 129.6, 129.1, 129 (5xC_{Ar}), 81.6 (C-1), 78.4, 75.9, 71.4, 70.6 (C-4), 67.9 (C-6), 62.7 (CH_2O), 53.1 (OCH_3), 52.1 (CH), 38.2 (CH_2). Yield = 74 %.

N^{α}-(Benzyloxycarbonyl)-N^{δ}-(β-D-galactopyranosyl)-L-glutamine *O*-Methyl Ester (105k)



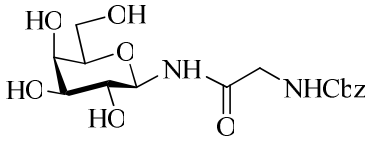
^{1}H-NMR (400 MHz, DMSO, 25°C): δ = 7.47-7.7.26 (m, 5H, Ph), 5.04 (s, 2H, CH_2O), 4.63 (d, $J_{1,2}$ = 5.2 Hz, 1H, H-1), 4.10-4.01 (m, 1H, CH), 3.70-3.67 (m, 1H, H-5), 3.64 (s, 3H, OCH_3), 3.52-3.31 (m, 5H, H-2, H-3, H-4, H-6, H-6'), 2.25-2.20 (t, J = 7.6 Hz, 2H, CH_2), 2.03-1.95 (m, 1H, H_a, CH_2CH), 1.78-1.73 (m, 1H, H_b, CH_2CH). ^{13}C-NMR (100 MHz, DMSO, 25°C): δ = 172.6, 171.6, 156.1 ($CO_{(Cbz)}$), 136.8 (C_{ipso}), 128.3, 127.7, 127.1 (5xC_{Ar}), 79.9 (C-1), 76.7, 74.1, 69.7, 68.2, 65.5 (CH_2O), 60.4 (C-6), 53.5 (CH), 51.8 (OCH_3), 31.6 (CH_2CO), 26.3 (CH_2). Yield = 70 %.

***N*-(*N*-Benzyloxycarbonyl-β-alanyl)-β-D-galactopyranosylamine (105l)**



^{1}H-NMR (400 MHz, DMSO, 25°C): δ = 7.46-7.29 (m, 5H, Ph), 5.04 (s, 2H, CH_2O), 4.70 (d, $J_{1,2}$ = 8.8 Hz, 1H, H-1), 3.71 (d, J = 2.4 Hz, 1H, H-4), 3.54-3.29 (m, 5H, H-2, H-3, H-5, H-6, H-6'), 3.27-3.23 (m, 2H, CH_2), 2.41-2.28 (m, 2H, CH_2). ^{13}C-NMR (100 MHz, DMSO, 25°C): δ = 170.6, 155.9 ($CO_{(Cbz)}$), 137.2 (C_{ipso}), 128.4, 127.8, 127.7 (5xC_{Ar}), 79.8 (C-1), 76.6, 73.9, 69.5, 68.1 (C-4), 62.2 (CH_2O), 60.4 (C-6), 36.6 (CH_2), 35.5 (CH_2). Yield = 71 %.

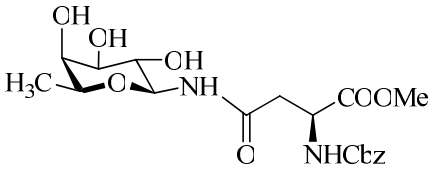
***N*-(*N*-Benzyloxycarbonylglycyl)-β-D-galactopyranosylamine (105m)**



The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.35);

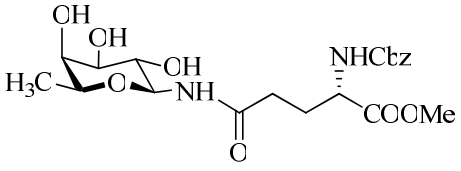
^{1}H-NMR (400 MHz, MeOD, 25°C): δ = 7.42-7.28 (m, 5H, Ph), 5.14 (s, 2H, CH_2O), 4.92 (d, $J_{1,2}$ = 8.8 Hz, 1H, H-1), 3.95-3.83 (m, 3H, H-5, CH_2), 3.78-3.70 (m, 2H), 3.60-3.58 (m, 2H, H-2), 3.55-3.52 (dd, $J_{5,6'}$ = 3.2 Hz, $J_{6,6'}$ = 9.6 Hz, 1H, H-6'). ^{13}C-NMR (100 MHz, MeOD, 25°C): δ = 172.9, 159.1 ($CO_{(Cbz)}$), 138.1 (C_{ipso}), 129.5, 129.0, 128.9 (5xC_{Ar}), 81.6 (C-1), 78.3, 75.7, 71.4, 70.4, 67.9 (CH_2O), 62.6 (C-6), 46.1 (CH_2). Yield = 73 %.

N^{α}-(Benzyloxycarbonyl)-N^{γ}-(β-D-fucopyranosyl)-L-asparagine *O*-Methyl Ester (106j)



The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20, R_f = 0.45);
^{1}H-NMR (400 MHz, MeOD, 25°C): δ = 7.41-7.31 (m, 5H, Ph), 5.12 (s, 2H, CH_2O), 4.60 (m, 1H, H_a, CH-N), 3.68 (s, 3H, OCH_3), 3.67-3.56 (m, 3H, H-2, H-3, H-5), 3.52 (d, J = 6.4 Hz, 1H, H-4), 2.84 (m, 2H, CH_2CH), 1.25 (d, $J_{5,6}$ = 8 Hz, 3H, H-6). ^{13}C-NMR (100 MHz, MeOD, 25°C) : δ = 173.5, 172.8, 158.4 ($CO_{(Cbz)}$), 138.1 (C_{ipso}), 129.5, 128.8 (5xC_{Ar}), 81.3 (C-1), 75.9, 73.7, 73.1, 71.1, 67.1 (CH_2O), 53.0 (OCH_3), 52.0 (CH), 38.5 (CH_2), 16.9 (C-6). FT-ICR MS (ESI): calcd. for $C_{19}H_{26}N_2O_9Na$ $[M+Na]^+$ 449.15305; found 449.15235. $[\alpha]^D_{25}$ = -0.04 (c 0.6, MeOH). Yield = 60 %

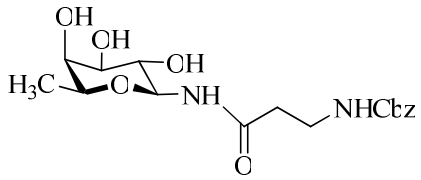
N^{α}-(Benzyloxycarbonyl)-N^{δ}-(β-D-fucopyranosyl)-L-glutamine *O*-Methyl Ester (106k)



The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20, Rf = 0.68);
^{1}H-NMR (400 MHz, D_2O, 25°C) : δ = 7.52-7.43 (m, 5H, Ph), 5.18 (s, 2H, CH_2O), 4.92 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.30 (m, 1H, CH), 3.89-3.67 (m, 6 H, H-3, H-4, H-5, OCH_3), 3.59 (t, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 2.47 (m, 2H, CH_2CO), 2.24 (m, 1H, H_a, CH_2), 2.06 (m, 1H, H_b, CH_2), 1.25 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6). ^{13}C-NMR (100 MHz, D_2O, 25°C) : δ = 175.8, 174.0, 157.8 ($CO_{(Cbz)}$), 136.0 (C_{ipso}), 128.6, 128.2, 127.4 (5xC_{Ar}), 79.3 (C-1), 73.3 (C-2), 72.4, 71.2, 68.9, 67.0 (CH_2O),

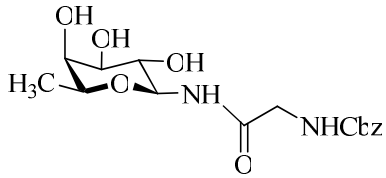
53.4 (CH), 52.7 (OCH_3), 31.5 (CH_2CO), 26.0 (CH_2CH), 15.3 (C-6). FT-ICR MS (ESI): calcd. for $C_{20}H_{28}N_2O_9$ Na $[M+Na]^+$ 463.16870; found 463.16891. $[\alpha]^D_{25}$ = -23.4 (c 0.2, MeOH). Yield = 68 %.

_N_-(_N_-Benzyloxycarbonyl-β-alanyl)-β-D-fucopyranosylamine (106l)



The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.35); ^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.49-7.39 (m, 5H, Ph), 5.11 (s, 2H, CH_2O), 4.91 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 3.85 (q, 1H, $J_{5,6}$ = 6.4 Hz, H-5), 3.79 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-4), 3.71 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-3), 3.59 (t, 1H, H-2), 3.43 (t, 2H, CH_2) 2.53 (m, 2H, CH_2CO), 1.24 (d, 3H, H-6). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 175.1, 158.2 ($CO_{(Cbz)}$), 136.4 (C_{ipso}), 128.7, 128.3, 127.7 (5xC_{Ar}), 79.4 (C-1), 73.5 (C-3), 72.5 (C-5), 71.4 (C-4), 69.1 (C-2), 66.9 (CH_2O), 36.7 (CH_2), 35.7 (CH_2CO), 15.6 (C-6). FT-ICR MS (ESI): calcd. for $C_{17}H_{24}N_2O_7Na$ $[M+Na]^+$ 391.14757; found 391.14714. $[\alpha]^D_{25}$ = - 14.1 (c 1, MeOH). Yield = 70 %.

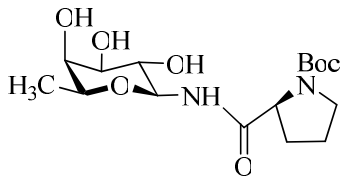
_N_-(_N_-Benzyloxycarbonylglycyl)-β-D-fucopyranosylamine (106m)



The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.34); ^{1}H-NMR (400 MHz, D_2O, 25°C): δ = 7.52-7.37 (m, 5H, Ph), 5.19 (s, 2H, CH_2O), 4.96 (d, 1H, $J_{1,2}$ = 9 Hz, H-1), 3.95 (d, 2H, J = 3.4 Hz, CH_2), 3.89 (q, 1H, $J_{5,6}$ = 6.4 Hz, H-5), 3.82 (dd, 1H, $J_{3,4}$ = 3.4 Hz, H-4), 3.74 (dd, 1H, $J_{2\text{-}3}$ = 9.4Hz, H-3), 3.64 (t, 1H, H-2), 1.26 (d, 3H, H-6). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 173.2, 158.6 ($CO_{(Cbz)}$), 136.2 (C_{ipso}), 128.8, 128.5, 127.8

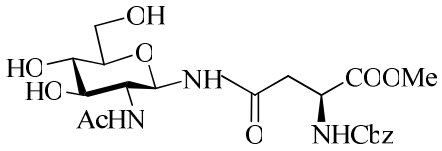
(5xC_{Ar}), 79.6 (C-1), 73.5 (C-3), 72.7 (C-5), 71.4 (C-4), 69.1 (C-2), 67.4 (CH_2O), 43.5 (CH_2), 15.5 (C-6). FT-ICR MS (ESI): calcd. for $C_{16}H_{22}N_2O_7Na$ $[M+Na]^+$ 377.13192; found 377.13165. $[\alpha]^D_{25}$ = - 12.1 (c 1, MeOH). Yield = 75 %.

***N*-(*N*-*tert*-Butoxycarbonyl-L-prolyl)-β-D-fucopyranosylamine (106n)**



The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.48); ^{1}H-NMR (400 MHz, MeOD, 25°C): δ = 4.21-4.13 (m, 1H, CH), 3.70 (q, $J_{5,6}$ = 6.4 Hz, 1H, H-5), 3.64 (s, 1H, H-4), 3.56-3.47 (m, 3H, H-2, H-3, H_a), 3.43-3.37 (m, 1H, H_b), 2.31-2.17 (m, 1H, H_c, CH_2), 2.15-1.78 (m, 3H, CH_2, H_d), 1.52-1.39 (bs, 9 H, Boc), 1.21 (d, 3H, H-6). ^{13}C-NMR (100 MHz, MeOD, 25°C): δ = 176.8, 156.3 (CO_{Boc}), 81.5 (C-1), 76.3, 73.7 (C-5), 73,4 (C-4), 71.3, 61.9 (CH), 48.0 (CH_2), 32.6 (CH_2), 28.8 (Boc), 24.8 (CH_2), 17.1 (H-6). FT-ICR MS (ESI): calcd. for $C_{16}H_{28}N_2O_7Na$ $[M+Na]^+$ 383.17887; found 383.17848. $[\alpha]^D_{25}$ = - 49.7 (c 1, MeOH). Yield = 87 %.

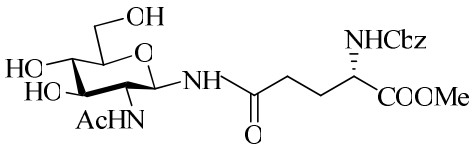
***N*$^{\alpha}$-(Benzyloxycarbonyl)-*N*$^{\gamma}$-(2-acetamido-2-deoxy- β-D-glucopyranosyl)-L-asparagine *O*-Methyl Ester (107j)**



The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20, Rf = 0.20); ^{1}H-NMR (400 MHz, MeOD, 25°C) : δ = 7.39-7.31 (m, 5H, Ph), 5.11 (s, 2H, CH_2O), 4.96 (d, $J_{1,2}$ = 9.7 Hz, 1H, H-1), 4.60 (t, $J_{CH\text{-}CH2}$ = 6.8 Hz, 1H, CH-N), 3.85 (bd, $J_{6,6'}$ = 11.8 Hz, 1H, H-6), 3.77-3.70 (m, 4H, H-2, OCH_3), 3.66 (dd, $J_{5,6'}$ = 3.8 Hz, 1H, H-6'), 3.47 (m, 1H, H-3), 3.40-3.32 (m, 2H, H-4, H-5), 2.73 (t, 2H, CH_2), 1.94 (s, 3H, NHAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ

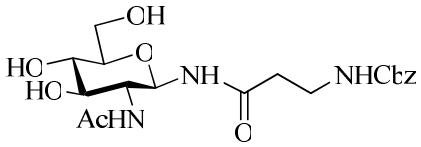
= 174.6, 173.5, 172.6, 129.6, 129.2, 129.0 (5xC_{Ar}), 80.4 (C-1), 79.9, 76.5 (C-3), 72.0, 67.9 (CH_2O), 62.8 (C-6), 56.2 (C-2), 53.1 (OCH_3), 52.2 (CH), 38.7 (CH_2), 23.0 (CH_3). MS (FAB): *m/z* = 484 $[M+1]^+$. $[\alpha]^D_{25}$ = + 19.2 (c 1, MeOH). Yield = 56 %.

N^{α}-(Benzyloxycarbonyl)-N^{γ}-(2-acetamido-2-deoxy- β-D-glucopyranosyl)-L-glutamine-*O*-Methyl Ester (107k)



The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20, Rf = 0.42); ^{1}H-NMR (400 MHz, MeOD, 25°C): δ = 7.45 (m, 5H, Ph), 5.15 (s, 2H, CH_2O), 5.05 (d, $J_{1,2}$ = 9.7 Hz, 1H, H-1), 4.20 (m, 1H, CH), 3.47-3.81 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6'), 3.75 (s, 3H, OCH_3), 2.38 (t, *J* = 7.4 Hz, 2H, CH_2CO), 2.15-2.05 (m, 1H, H_a, CH_2), 2.03-1.92 (m, 4H, H_b, NHAc). ^{13}C-NMR (100 MHz, MeOD, 25°C) : δ = 175.7, 174.8, 152.6 ($CO_{(Cbz)}$), 136.4 (C_{ipso}), 128.9, 128.5, 127.7 (5xC_{Ar}), 78.4 (C-1), 77.6, 74.2, 69.6, 67.3 (CH_2O), 60.6 (C-6), 54.4 (CH), 53.6 (C-2), 53.0 (OCH_3), 31.8 (CH_2CO), 26.3 (CH_2), 22.0 (OCH_3). FT-ICR MS (ESI): calcd. for $C_{22}H_{31}N_3O_{10}Na$ $[M+Na]^+$ 520.19017; found 520.19006. $[\alpha]^D_{25}$ = + 3.5 (c 0.6, MeOH). Yield = 54 %.

***N*-(*N*-Benzyloxycarbonyl-β-alanyl)-2-acetamido-2-deoxy-β-D-glucopyranosylamine (107l)**



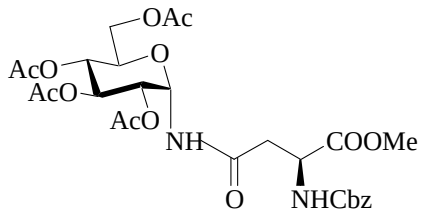
The compound was purified by flash chromatography ($CHCl_3$:MeOH:H_2O 80:20:2, R_f = 0.24); ^{1}H-NMR (400 MHz, MeOD, 25°C): δ = 7.31-7.24 (m, 5H, Ph), 5.01 (s, 2H, CH_2O), 4.91 (d, $J_{1,2}$ = 9.6 Hz, 1H, H-1) 3.78 (d, $J_{6,6'}$ = 11.6 Hz, 1H, H-6), 3.69 (t, $J_{2,3}$ = 10 Hz, 1H, H-2), 3.60 (dd, $J_{5,6'}$ = 2.4 Hz, 1H, H-6'), 3.39 (m, 1H, H-3), 3.31-3.24 (m, 4H, H-4, H-5, CH_2), 2.34 (t, *J* = 6.7 Hz, 2H, CH_2), 1.88 (s, 3H, NHAc). ^{13}C-NMR (100 MHz, D_2O, 25°C): δ = 173.0, 172.8, 157.2 ($CO_{(Cbz)}$), 136.9 (C_{ipso}), 128.1, 127.6, 127.4 (5xC_{Ar}), 78.9 (C-1), 78.1, 74.9 (C-3), 71.1, 66.1

(CH_2O), 61.3 (C-6), 54.8 (C-2), 37.9 (CH_2), 35.8 (CH_2), 21.4 (CH_3). MS (FAB): *m/z* = 448 [M + Na]$^+$. $[\alpha]^D_{25}$ = + 18.6 (c 1, MeOH). Yield = 60 %.

General procedure for acetylation of unprotected glycosyl amides.

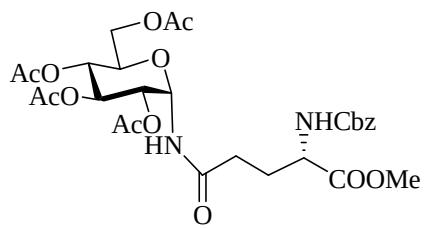
Acetic anhydride (10 eq) and a catalytic amount of *N*,*N*-dimethylaminopyridine were added, at room temperature, to a solution of substrate (1 eq) in pyridine dried on molecular sieves (0.1 M). The solution was stirred for 24H and then was concentrated in vacuo. The residue was dissolved in AcOEt and washed with aqueous 5 % HCl, aqueous 5 % $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated to give the product in quantitative yield.

***N*$^{\alpha}$-(benzyloxycarbonyl)-*N*$^{\gamma}$-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-L-asparagine-*O*-Methyl Ester (103j)**



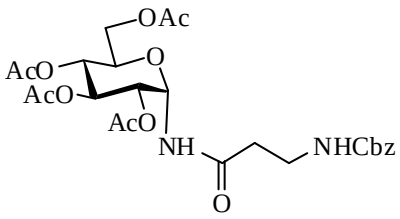
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.41-7.32 (m, 5H, Cbz), 6.78 (s,1H, NH), 5.95 (m, 1H, NH-Cbz), 5.85 (t, $J_{1,2}$ = 6.4Hz, $J_{N\text{-}H}$ = 6.4 Hz, 1H, H-1), 5.48 (t, $J_{3,4}$ = 9.6 Hz, $J_{2,3}$ = 9.6 Hz, 1H, H-3), 5.17 (m, 1H, H-2), 5.17 (m, 2H, CH_2O), 5.08 (m, 1H, H-4) 4.65 (m, 1H, CH), 4.30 (dd, $J_{5,6}$ = 2.4 Hz, $J_{6,6'}$ = 12.4Hz, 1H, H-6), 4.08 (m, 1H, H-6'), 3.92 (m, 1H, H-5), 3.75 (s, 3H, OCH_3), 3.03 (m, 1H, H_a, CH_2), 2.85 (m, 1H, H_b, CH_2).

***N*$^{\alpha}$-(benzyloxycarbonyl)-*N*$^{\delta}$-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-L-glutamine-*O*-Methyl Ester (103k)**



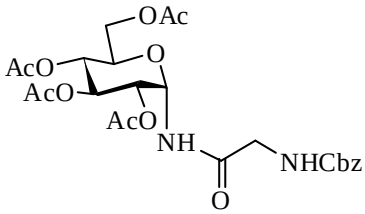
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.50-7.48 (m, 1H, NH), 7.42-7.32 (m, 5H, Cbz), 5.90 (t, $J_{1,2}$ = 6 Hz, $J_{N\text{-}H}$ = 6 Hz, 1H, H-1), 5.62 (d, $J_{1,NH\text{-}Cbz}$ = 8.6 Hz, 1H, NHCbz,), 5.51 (m,1H, H_3), 5.20 (m, 2H, CH_2O), 5.18 (m, 1H, H-2), 5.09 (m, 1H, H-4), 4.34 (m, 1H, CH), 4.27 (m, 1H, H-6), 4.06 (m, 1H, H-6'), 4.04 (m, 1H, H-5), 3.75 (s, 3H, OCH_3), 2.35 (m, 2H, CH_2CO), 2.30 (m, 1H, H_a, CH_2), 2.05 (m, 12H, 4xOAc), 1.91 (m, 1H, H_b, CH_2).
HETCOR-NMR (400 MHz, $CDCl_3$, 25°C): δ = 128.8, 128.5, 127.7, 73 (C-1), 72 (C-3), 71 (C-4), 70 (C-5), 69 (C-2), 68 (CH), 64 (C-6), 54 (OCH_3), 37-35 (2xCH_2), 29 (4xOAc).

_N_-(_N_-Benzyloxycarbonyl-β-alanyl)-2,3,4,6-tetra-_O_-acetyl-α-D-glucopyranosyl amine (103l)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45-7.26 (m, 5H, Ph), 6.95 (m, 1H, NH), 5.87 (m, 1H, H-1), 5.48-5.30 (m, 2H, NHCbz, H-3), 5.23-5-03 (m, 4H, CH_2O, H-2, H-4), 4.26 (dd, $J_{5,6}$ = 3.6 Hz, $J_{6,6'}$ = 12 Hz, 1H, H-6), 4.10-4.01 (bd, 1H, H-6'), 3.97-3.82 (m, 1H, H-5), 3.51 (m, 2H, CH_2), 2.55 (m, 2H, CH_2CO), 2.14-1.96 (m, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 176.4, 172.4, 170.8, 169.5, 169.2, 156.9 ($CO_{(Cbz)}$), 136.4 (C_{ipso}), 128.7, 128.3, 128.2 (5xC_{Ar}), 74.4 (C-1), 70.3 (C-3), 68.6 (C-2), 68.5 (C-4), 68.3 (C-5), 67.1 (CH_2O), 61.9 (C-6), 37 (CH_2), 36.9 ($COCH_2$), 20.7, 20.6, 19.7 (4xOAc). MS (FAB): *m/z* = 575 $[M + Na]^+$. $[\alpha]^D_{25}$ = + 44.0 (c 0.5, $CHCl_3$). R_f = 0.25 (hexane:AcOEt 40:60).

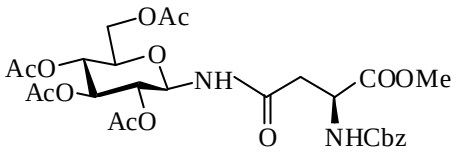
_N_-(_N_-Benzyloxycarbonylglycyl)-2,3,4,6-tetra-_O_-acetyl-α-D-glucopyranosyl amine (103m)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.32-7.20 (m, 5H, Ph), 6.78 (m, 1H, NH), 5.77 (bd, 1H, H-1),5.48-5.27 (m, 2H, H-3, NHCbz), 5.17-4.98 (m, 4H, H-2, H-4, CH_2O), 4.75 (bdd, 1H, H-6),

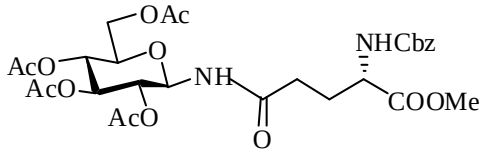
4.27 (bdd, 1H, H-6'), 4.05-3.88 (m, 3H, H-5, CH_2), 2.05-1.87 (m, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.8, 170.3, 169.7, 169.3, 169.1, 158.2 ($CO_{(Cbz)}$), 136.0 (C_{ipso}), 128.8, 128.5, 128.3 (5xC_{Ar}), 80.1 (C-1), 76.5 (C-3), 75.1, 73.9, 72.7, 68.4, 67.7(CH_2O), 61.9 (C-6), 45.2 (CH_2), 20.8, 20.7, 20.6 (4xOAc). MS (FAB): *m/z* = 561 $[M + Na]^+$. $[\alpha]^D_{25}$ = + 14.7 (c 0.6, $CHCl_3$). R_f = 0.24 (hexane:AcOEt 40:60).

N^{α}-(benzyloxycarbonyl)-N^{γ}-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-asparagine-*O*-Methyl Ester(104j)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45-7.30 (m, 5H, Ph), 6.37 (d, $J_{NH,1}$ = 9.2 Hz, 1H, NH), 5.96 (d, J = 8.8 Hz, 1H, NHCbz), 5.29 (t, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.20 (t, $J_{1,2}$ = 9.6 Hz, 1H, H-1), 5.12 (s, 2H, CH_2O), 5.05 (t, 1H, H-4), 4.90 (t, 1H, H-2), 4.60 (m, 1H, CH), 4.29 (dd, $J_{5,6}$ = 4.4 Hz, $J_{6,6'}$ = 12.6 Hz, 1H, H-6), 4.06 (m, $J_{5,6'}$ = 2 Hz, $J_{6,6'}$ = 12.6 Hz, 1H, H-6'), 3.77 (m, 1H, H-5), 3.73 (s, 3H, OCH_3), 2.89 (dd, $J_{CH,Ha}$ = 4.4 Hz, $J_{Ha,Hb}$ = 16.4 Hz, H_a, 1H, CH_2CO), 2.70 (dd, $J_{CH,Hb}$ = 4 Hz, H_b, 1H, CH_2CO), 2.10-1.98 (m, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.7, 171.6, 170.9, 170.2, 169.9, 156.4 ($CO_{(Cbz)}$), 136.5 (C_{ipso}), 128.9, 128.5, 128.4 (5xC_{Ar}), 78.5 (C-1), 74.1 (C-5), 72.8 (C-3), 70.8 (C-2), 68.4 (C-4), 67.5 (CH_2O), 61.9 (C-6), 53.1 (OCH_3), 50.7 (CH), 38.1 (CH_2), 21.1, 20.9, 20.9 (4xOAc). MS (FAB): *m/z* = 611 $[M + H]^+$. $[\alpha]^D_{25}$ = + 11.4 (c 0.7, $CHCl_3$). R_f = 0.31 (hexane:AcOEt 40:60).

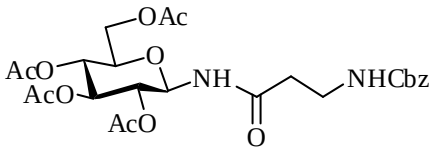
N^{α}-(benzyloxycarbonyl)-N^{δ}-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-glutamine-*O*-Methyl Ester (104k)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.40-7.32 (m, 5H, Ph), 6.58 (d, 1H, $J_{NH,1}$ = 9.2 Hz, NH), 5.55 (d, J = 8 Hz, 1H, NHCbz), 5.29 (t, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.25 (m, 1H, H-1),

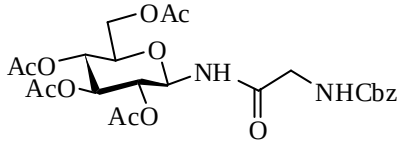
5.10 (s, 2H, CH_2O), 5.07 (t, $J_{3,4}$ = 9.6 Hz, $J_{4,5}$ = 10 Hz, 1H, H-4), 4.92 (t, $J_{1,2}$ = 9.2 Hz, $J_{2,3}$ = 9.6 Hz, 1H, H-2), 4.34 (m, 1H, CH-N), 4.28 (dd, $J_{5,6}$ = 4.4 Hz, $J_{6,6'}$ = 12.4 Hz, 1H, H-6), 4.07 (dd, $J_{5,6'}$ = 2 Hz, $J_{6,6'}$ = 12.4 Hz, 1H, H-6'), 3.80 (m, 1H, H-5), 3.74 (s, 3H, OCH_3), 2.30-2.20 (m, 2H, CH_2CO), 2.08-1.98 (m, 12H, 4xOAc), 1.90-1.80 (m, 2H, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 172.5, 172.5, 171.3, 170.9, 170.2, 169.9, 156.5 ($CO_{(Cbz)}$), 136.4 (C_{ipso}), 128.9, 128.6, 128.5 (5xC_{Ar}), 78.5 (C-1), 73.9 (C-5), 73.2 (C-3), 70.9 (C-2), 68.4 (CH_2O), 61.9 (C-6), 53.5 (CH), 52.9 (OCH_3), 32.4 (CH_2CO), 28.4 (CH_2), 20.9 (4xOAc). MS (FAB): *m/z* = 625 $[M + H]^+$. $[\alpha]^D_{25}$ = + 44.1 (c 1, $CHCl_3$). R_f = 0.27 (hexane:AcOEt 40:60).

_N_-(_N_-Benzyloxycarbonyl-β-alanyl)-2,3,4,6-tetra-_O_-acetyl-β-D-glucopyranosyl amine (104l)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45-7.28 (m, 5H, Ph), 6.40 (d, J_{NH-1} = 9.2 Hz, 1H, NH), 5.30 (bd, 1H, NHCbz), 5.27 (t, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.24 (t, $J_{1,2}$ = 9.4 Hz, J_{NH-1} = 9.2 Hz, 1H, H-1), 5.05 (s, 2H, CH_2O), 5.04 (m, 1H, H-4), 4.90 (t, $J_{1,2}$ = 9.4 Hz, $J_{2,3}$ = 9.6 Hz, 1H, H-2), 4.30 (dd, $J_{5,6}$ = 4.4 Hz, $J_{6,6'}$ = 12.6 Hz, 1H, H-6), 4.10 (dd, $J_{5,6'}$ = 2Hz, $J_{6,6'}$ = 12.6 Hz, 1H, H-6'), 3.81 (m, 1H, H-5), 3.46 (q, 2H, CH_2), 2.41 (m, 2H, CH_2CO), 2.10-1.98 (m, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 172.1, 171.4, 170.9, 170.2, 169.9, 156.4 ($CO_{(Cbz)}$), 136.7 (C_{ipso}), 128.9, 128.5, 128.4 (5xC_{Ar}), 78.4 (C-1), 73.9 (C-5), 72.9 (C-3), 70.9 (C-2), 68.4 (C-4), 67.1 (CH_2O), 61.9 (C-6), 36.9 (CH_2), 36.4 (CH_2-CO), 21.1, 20.9 (4xOAc). MS (FAB): *m/z* = 553 $[M + H]^+$. $[\alpha]^D_{25}$ = + 45.4 (c 1, $CHCl_3$). R_f = 0.24 (hexane:AcOEt 40:60).

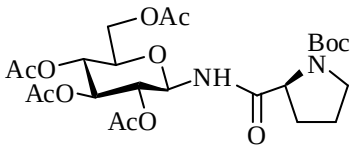
_N_-(_N_-Benzyloxycarbonylglycyl)-2,3,4,6-tetra-_O_-acetyl-β-D-glucopyranosyl amine (104m)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.47-7.30 (m, 5H, Ph), 6.93 (d, 1H, $J_{NH,1}$ = 8.4 Hz, NH), 5.33-5.23 (m, 3H, H-3, H-1, NHCbz), 5.14 (s, 2H, CH_2O), 5.06 (t, $J_{3,4}$ = 9.6 Hz, 1H, H-4), 4.94

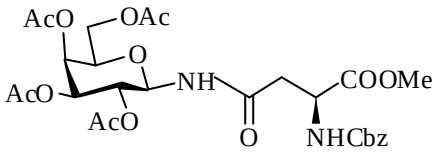
(t, $J_{1,2}$ = 9.6 Hz, $J_{2,3}$ = 9.6 Hz, 1H, H-2), 4.28 (dd, $J_{5,6}$ = 4.4 Hz, $J_{6,6'}$ = 12.6 Hz, 1H, H-6), 4.15-4.05 (m, 1H, H-6'), 3.97-3.75 (m, 3H, H-5, CH_2), 2.15-1.97 (m, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.6, 170.9, 170.2, 169.9, 169.8, 156.8 ($CO_{(Cbz)}$), 136.2 (C_{ipso}), 128.9, 128.7, 128.6 (5xC_{Ar}), 78.6 (C-1), 74 (C-5), 72.9 (C-3), 70.7 (C-2), 68.5 (C-4), 67.9 (CH_2O), 61.9 (C-6), 45 (CH_2), 21, 20.9 (4xOAc). MS (FAB): *m/z* = 539 $[M + H]^+$. $[\alpha]^D_{25}$ = + 6.3 (c 1, $CHCl_3$). R_f = 0.24 (hexane:AcOEt 40:60).

_N_-(_N-tert_-Butoxycarbonyl-L-prolyl)-2,3,4,6-tetra-_O_-acetyl-β-D-glucopyranosyl amine (104n)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.31-5.21 (m, 2H, H-3, H-1), 5.03 (t, 1H, H-4), 4.89 (m, 1H, H-2), 4.25-4.05 (m, 3H, CH, H-6, H-6'), 3.82 (m, 1H, H-5), 3.51-3.25 (m, 2H, CH_2), 2.30-1.75 (m, 16 H, 2CH_2, 4xOAc), 1.43 (bs, 9 H, Boc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.7, 170.6, 170, 169.6, 78.1 (C-1), 73.7 (C-5), 72.8 (C-3), 70.8 (C-2), 68.4 (C-4), 61.9 (C-6), 47.1 (CH_2), 29.8 (CH_2), 28.2 (Boc), 20.8, 20.7, 20.6 (4xOAc). MS (FAB): *m/z* = 545 $[M + H]^+$. $[\alpha]^D_{25}$ = - 20.1 (c 1, $CHCl_3$). R_f = 0.25 (hexane:AcOEt 40:60).

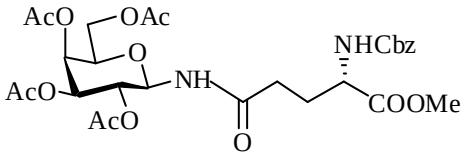
N^{α}-(benzyloxycarbonyl)-N^{γ}-(2,3,4,6-tetra-_O_-acetyl-β-D-galactopyranosyl)-L-asparagine-_O_-Methyl Ester (108j)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.41-7.28 (m, 5H, Ph), 6.39 (d, $J_{NH,1}$ = 8.8 Hz, 1H, NH), 5.97 (d, *J* = 8.8 Hz, 1H, NHCbz), 5.42 (bs, 1H, H-4), 5.18 (t, 1H, H-1), 5.13-5.06 (m, 4H, H-2, H-3, CH_2O), 4.60 (m, 1H, CH), 4.17-3.96 (m, 3H, H-5, H-6, H-6'), 3.73 (s, 3H, OCH_3), 2.88 (dd, 1H, $J_{CH,Ha}$ = 4.4 Hz, $J_{Ha,Hb}$ = 16.4 Hz, H_a, CH_2), 2.70 (dd, 1H, $J_{CH,Hb}$ = 4 Hz, $J_{Ha,Hb}$ = 16.4 Hz, H_b, CH_2) 2.08-1.98 (m, 12H, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.6, 171.4, 170.6, 170.4, 169.9, 169.8, 156.1 ($CO_{(Cbz)}$), 136.2 (C_{ipso}), 128.6, 128. 2, 128.1 (5xC_{Ar}), 78.4 (C-1), 72.5

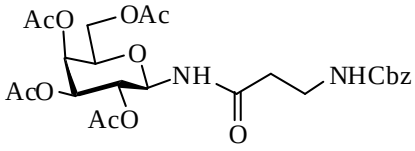
(C-5), 70.7, 68.3, 67.2 (CH_2O), 67.1, 61.1 (C-6), 52.8 (OCH_3), 50.4 (CH), 37.8 (CH_2), 20.7-20.6 (4xOAc). MS (FAB): *m/z* = 611 $[M + H]^+$. $[\alpha]^D_{25}$ = + 17.4 (c 1, $CHCl_3$). R_f = 0.36 (hexane:AcOEt 40:60).

N^{α}-(benzyloxycarbonyl)-N^{δ}-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-glutamine-*O*-Methyl Ester (108k)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.28 (m, 5H, Ph), 6.47 (d, $J_{NH,1}$ = 8.8 Hz, $J_{Ha,Hb}$ = 16.4 Hz, NH), 5.56 (d, J = 7.6 Hz, $J_{Ha,Hb}$ = 16.4 Hz, NHCbz), 5.43 (bs, 1H, H-4), 5.22 (t, 1H, H-1), 5.20-5.03 (m, 4H, H-2, H-3, CH_2O), 4.36 (m, 1H, CH), 4.20-3.97 (m, 3H, H-5, H-6, H-6'), 3.74 (s, 3H, OCH_3), 2.33-1.90 (m, 16 H, 2xCH_2, 4xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 172.4, 171.7, 170.7, 170.3, 170.1, 156 ($CO_{(Cbz)}$), 136.5 (C_{ipso}), 128.9, 128.6, 128.5 (5xC_{Ar}), 78.8 (C-1), 72,6 (C-5), 71.2 , 68.6, 67.5, 67,4 (CH_2O), 61.4 (C-6), 53.6 (CH), 52.9 (OCH_3), 32.4 (CH_2CO), 28.2 (CH_2), 21.1, 20.9, 20.9 (4xOAc). MS (FAB): *m/z* = 625 $[M + H]^+$. $[\alpha]^D_{25}$ = + 15.7 (c 1, $CHCl_3$). R_f = 0.36 (hexane:AcOEt 40:60).

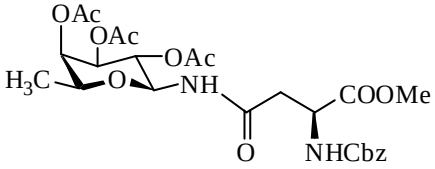
***N*-(*N*-Benzyloxycarbonyl-β-alanyl)-2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl amine (108l)**



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.47-7.28 (m, 5H, Ph), 6.36 (d, $J_{NH,1}$ = 9.2Hz, 1H, NH), 5.43 (d, $J_{3,4}$ = 3.2 Hz, 1H, H-4), 5.35 (m, 1H, NHCbz), 5.21 (t, $J_{NH,1}$ = 9.2Hz, 1H, H-1), 5.18-5.05 (m, 4H, H-2, H-3, CH_2O), 4.15-3.98 (m, 3H, H-5, H-6, H-6'), 3.46 (m, 2H, CH_2), 2.42 (m, 2H, CH_2CO), 2.13 (s, 3H, OAc), 2.04-1.98 (m, 9 H, 3xOAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 172, 171.7, 170.7, 170.3, 170.1, 156.6 ($CO_{(Cbz)}$), 136.7 (C_{ipso}), 128.9, 128.5, 128.4 (5xC_{Ar}), 78.7 (C-1), 72.7 (C-5), 71.1, 68.7, 67.4, 67.1 (CH_2O), 61.4 (C-6), 36.9 (CH_2), 36.4

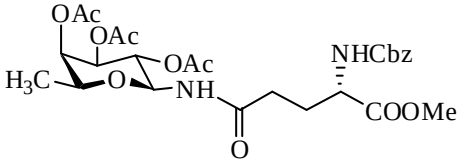
(CH_2CO), 21.0, 20.9, 20.9 (4xOAc). MS (FAB): *m/z* = 553 $[M + H]^+$. $[\alpha]^D_{25}$ = + 13.9 (c 1, $CHCl_3$). R_f = 0.21 (hexane:AcOEt 40:60).

N^{α}-(benzyloxycarbonyl)-N^{δ}-(2,3,4,6-tetra-*O*-acetyl-β-D-fucopyranosyl)-L-asparagine-*O*-Methyl Ester (109j)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45-7.27 (m, 5H, Ph), 6.30 (dd, $J_{NH,1}$ = 8.8 Hz, 1H, NH), 5-78 (d, *J* = 7.2 Hz, 1H, NHCbz), 5.29 (s, 1H, H-4), 5,26 (bs, 1H, H-2), 5.20-5.02 (m, 4H, H-1, H-3, CH_2O), 4.58 (m, 1H, CH), 3.88 (q, $J_{5,6}$ = 6.4 Hz, 1H, H-5), 3.75 (s, 3H, OCH_3), 2.94 (dd, $J_{Ha,Hb}$ = 16.2 Hz, $J_{Ha,CH}$ = 3.6 Hz, 1H, H_a, CH_2), 2.71 (dd, $J_{Ha,Hb}$ = 16.2 Hz, $J_{Hb,CH}$ = 4 Hz, 1H, H_b, CH_2), 2.16 (s, 3H, OAc), 2.02-1.97 (m, 6 H, 2xOAc), 1.17 (d, $J_{5,6}$ = 6.4 Hz, 3H, H-6). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 171.4, 170.7, 170.1, 128.9, 128.6, 128.4 (5xC_{Ar}), 78.6 C-1, 71.5, 71.3 (C-5), 70.6 (C-4), 68.8, 67.5 (CH_2O), 53.1 (OCH_3), 50.8 (CH), 38.5 (CH_2), 20.9, 20.9, 16.4 (C-6). MS (FAB): *m/z* = 553 $[M + H]^+$. $[\alpha]^D_{25}$ = -3.1(c 0.4, $CHCl_3$). R_f = 0.33 (hexane:AcOEt 40:60).

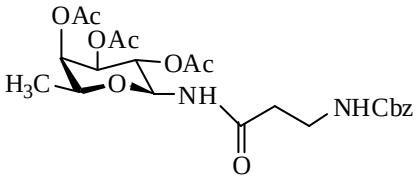
N^{α}-(benzyloxycarbonyl)-N^{δ}-(2,3,4,6-tetra-*O*-acetyl-β-D-fucopyranosyl)-L-glutamine-*O*-Methyl Ester (109k)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.44-7.28 (m, 5H, Ph), 6.35 (d, $J_{NH,1}$ = 8.8 Hz, 1H, NH), 5.52 (d, *J* = 7.2 Hz, 1H, NHCbz), 5.26 (bs, 1H, H-4), 5.20-5.02 (m, 5H, H-1, H-2, H-3, CH_2O), 4.35 (m, 1H, CH), 3.89 (bq, 1H, H-5), 3.74 (s, 3H, OCH_3), 2.38-1.94 (m, 11H, CH_2, 3xOAc), 1.17 (d, $J_{5,6}$ = 6.4 Hz, 3H, H-6). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 172.5, 172.3, 171.9, 170.7, 170.2, 156.3 ($CO_{(Cbz)}$), 136.5 (C_{ipso}), 128.9, 128.6, 128.5 (5xC_{Ar}), 78.7 (C-1), 71.6, 71.2 (C-5), 70.7 (C-4), 68.7, 67.4 (CH_2O), 53.7 (CH), 52.9 (OCH_3), 32.5 (CH_2CO), 27.9 (CH_2), 21.1,

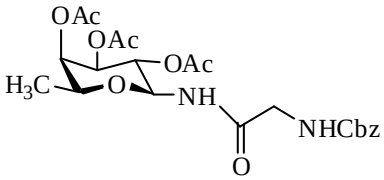
20.9, 20.8 (3xOAc), 16.4 (C-6). MS (FAB): *m/z* = 567 $[M + H]^+$. $[\alpha]^D_{25}$ = - 7.1 (c 0.7, $CHCl_3$). R_f = 0.30 (hexane:AcOEt 40:60).

_N_-(_N_-Benzyloxycarbonyl-β-alanyl)-2,3,4-tri-_O_-acetyl-β-L-fucopyranosyl amine (109l)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.40-7.27 (m,5H, Ph), 6.34 (d, $J_{NH,1}$ = 8.8 Hz, 1H, NH), 5.36 (m, 1H, NHCbz), 5.27 (bd, $J_{3,4}$ = 2.4 Hz, 1H, H-4), 5.18 (t, $J_{NH,1}$ = 8.8 Hz, 1H, H-1), 5.15-5.02 (m, 4H, H-2, H-3, CH_2O), 3.92 (q, $J_{5,6}$ = 6.4 Hz, 1H, H-5), 3.46 (bq, 2H, CH_2), 2.49-2.32 (m, 2H, CH_2CO), 2.17 (s, 3H, OAc), 2.04-1.98 (m, 6 H, 2xOAc), 1.18 (d, $J_{5,6}$ = 6.4 Hz, 3H, H-6). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 172.0, 171.8, 170.7, 170.2, 156.7($CO_{(Cbz)}$), 136.8 (C_{ipso}), 128.8, 128.4, 128.4 (5xC_{Ar}), 78.6 (C-1), 71.5 (C-5), 71.2, 70.6 (C-4), 68.8, 67.1 (CH_2O), 36.9 (CH_2), 36.3 (CH_2CO), 21.0, 20.9, 20.9 (3xOAc), 16.4 (C-6). MS (FAB): *m/z* = 495 $[M + H]^+$. $[\alpha]^D_{25}$ = - 19.6 (c 1, $CHCl_3$). R_f = 0.23 (hexane:AcOEt 40:60).

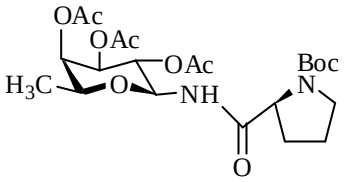
_N_-(_N_-Benzyloxycarbonylglycyl)-2,3,4-tri-_O_-acetyl-β-L-fucopyranosyl amine (109m)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45-7.28 (m, 5H, Ph), 6.90 (d, *J* = 8.8 Hz, 1H, NH), 6.43 (d, *J* = 8.8 Hz, 1H, NH), 5.27-5.11 (m, 6 H, H-1, H-2, H-3, H-4, CH_2O), 3.96-3.88 (m, 2H, H-5, H_a, CH_2), 3.80 (dd, *J* = 6 Hz, $J_{Ha,Hb}$ = 17.2 Hz, 1H, H_b, CH_2), 2.22 (s, 3H, OAc), 2.17 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.18 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 172.9, 170.1, 169.9, 169.6, 156.4 ($CO_{(Cbz)}$), 136.1 (C_{ipso}), 128.8, 128.7, 128.4 (5xC_{Ar}), 78.6 (C-1), 71.2 (C-5), 72.0, 70.5, 68.5, 67.6 (CH_2O), 44.8 (CH_2), 20.9, 20.8, 20.7 (3xOAc), 16.2

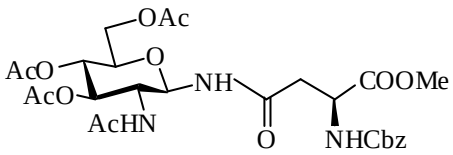
(C-6). MS (ESI): *m/z* = 503.5 $[M + Na]^+$. $[\alpha]^D_{25}$ = - 16.1 (c 0.7, $CHCl_3$). R_f = 0.28 (hexane:AcOEt 40:60).

_N_-(_N_-_tert_-Butoxycarbonyl-L-prolyl)-2,3,4-tri-_O_-acetyl-β-L-fucopyranosyl amine (109n)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.27 (s, 1H, H-4), 5.25-5.05 (m, 3H, H-1, H-2, H-3), 4.30-4.23 (m, 1H, CH), 3.91 (q, $J_{5,6}$ = 6.4 Hz, 1H, H-5), 3.53-3.30 (m, 2H, CH_2), 2.24-1.81 (m, 13H, 2xCH_2, 3xOAc), 1.16 (d, 3H, H-6). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 173.3, 171, 170.6, 170.1, 78.5 (C-1), 71.6, 71.1 (C-5), 70.6 (C-4), 60.9 (CH), 48.0 (CH_2), 29.8 (CH_2), 28.5 (CH_2), 20.8, 20.7 (3xOAc), 16.2 (C-6). MS (ESI): *m/z* = 487.2 $[M + H]^+$. $[\alpha]^D_{25}$ = - 61.5 (c 1, $CHCl_3$). R_f = 0.38 (hexane:AcOEt 40:60).

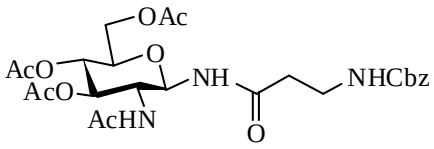
N^{α}-(Benzyloxycarbonyl)-N^{γ}-(2-acetamido-3,4,6-tri-_O_-acetyl-2-deoxy)-β-D-glucopyranosyl-L-asparagine-_O_-Methyl Ester (110j)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42-7.28 (m, 5H, Ph), 7.08 (d, $J_{NH,1}$ = 3.6 Hz, 1H, NH), 5.87 (bd, 2H, NH), 5.05 (m, 1H, H-4), 5.05 (s, 2H, CH_2O), 4.96-4.91 (bt, 2H, H-1, H-3), 4.52 (m, 1H, CH), 4.22 (dd, $J_{5,6}$ = 4.0 Hz, $J_{6,6'}$ = 12.4 Hz, 1H, H-6), 4.06-3.99 (m, 2H, H-2, H-6'), 3.66 (s, 3H, OCH_3), 3.67-3.62 (m, 1H, H-5), 2.65 (bd, 1H, H_a, CH_2), 2.05 (dd, $J_{Ha,Hb}$ = 16.5 Hz, $J_{Hb,CH}$ = 4.3 Hz, 1H, H_b, CH_2), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.90 (s, 3H, NHAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 172.4, 170.1, 169.5, 128.9, 128.4 (5xC_{Ar}), 80.8 (C-1), 74.5 (C-3), 73.1 (C-5), 67.8 (C-4), 67.4 (CH_2O), 61.9 (C-6), 53.9 (C-2), 53.0 (OCH_3),

50.7 (CH), 38.0, 30.0 (CH_2), 23.4 (NHAc), 21.0, 20.9 (3xOAc). MS (FAB): *m/z* = 610 $[M + H]^+$. $[\alpha]^D_{25}$ = + 6.4 (c 0.6, $CHCl_3$). R_f = 0.55 (hexane:AcOEt 40:60).

_N_-(_N_-Benzyloxycarbonyl-β-alanyl)-2-acetamido-3,4,6-tri-_O_-acetyl-2-deoxy-β-D-glucopyranosyl amine (110l)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.41-7.28 (m, 5H, Ph), 7.07 (d, $J_{NH,1}$ = 8.4 Hz, 1H, NH), 6.06 (d, *J* = 7.6 Hz, 1H, NHAc), 5.42 (bs, 1H, NHCbz), 5.09-4.98 (m, 5H, H-1, H-3, H-4, CH_2O), 4.28 (dd, $J_{5,6}$ = 4.2 Hz, $J_{6,6'}$ = 12.6 Hz, 1H, H-6), 4.1-4.03 (m, 2H, H-2, H-6'), 3.74 (m, 1H, H-5), 3.44 (q, 2H, CH_2), 2.51-2.31 (m, 2H, CH_2), 2.09-1.98 (m, 9 H, 3xOAc), 1.91 (s, 3H, NHAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 172.3, 172.1, 170.8, 169.4, 156.4 ($CO_{(Cbz)}$), 136.7 (C_{ipso}), 128.6, 128.3, 128.2 (5xC_{Ar}), 80.4 (C-1), 73.7 (C-5), 73.1, 67.8, 66.8 (CH_2O), 61.8 (C-6), 53.7 (C-2), 36.8 (CH_2), 36.2 (CH_2CO), 23.2 (NHAc), 20.9, 20.8, 20.7 (3xOAc). MS (ESI): *m/z* = 551.2 $[M + H]^+$. $[\alpha]^D_{25}$ = + 2.75 (c 0.8, $CHCl_3$). R_f = 0.23 (hexane:AcOEt 40:60).

5.5 References

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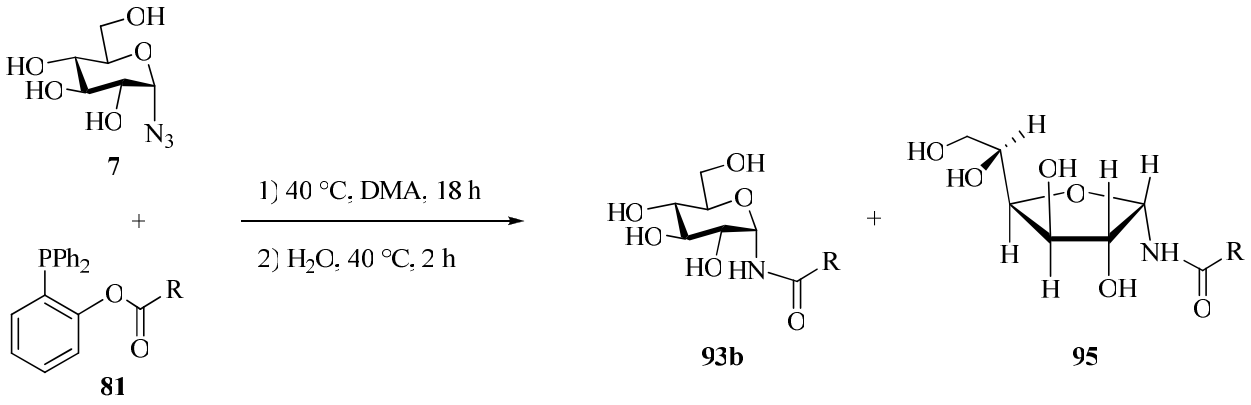
Chapter 6

Stereoselective synthesis of glycofuranosyl amides

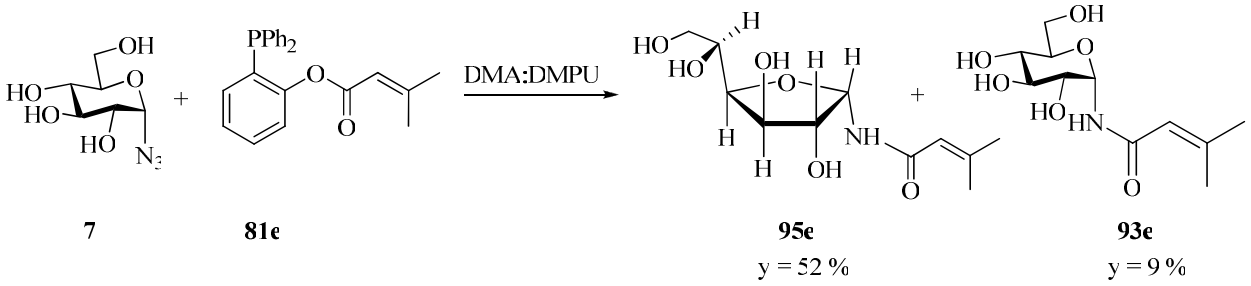
6.1 Glycofuranosyl amides: preliminary studies

The formation of glucofuranosides **95** in Staudinger ligation reactions of glucosyl azide **7** (Chapter 4) drew our attention towards this atypical class of compounds (Scheme 1). In particular the furanose form of D-glucose is known to be unstable[1] and rarely isolated only as bis-acetonides [2] or esters of boronic acid.[3]



Scheme 1. Formation of α-gluco-furanosyl amide in the Staudinger ligation of **7**

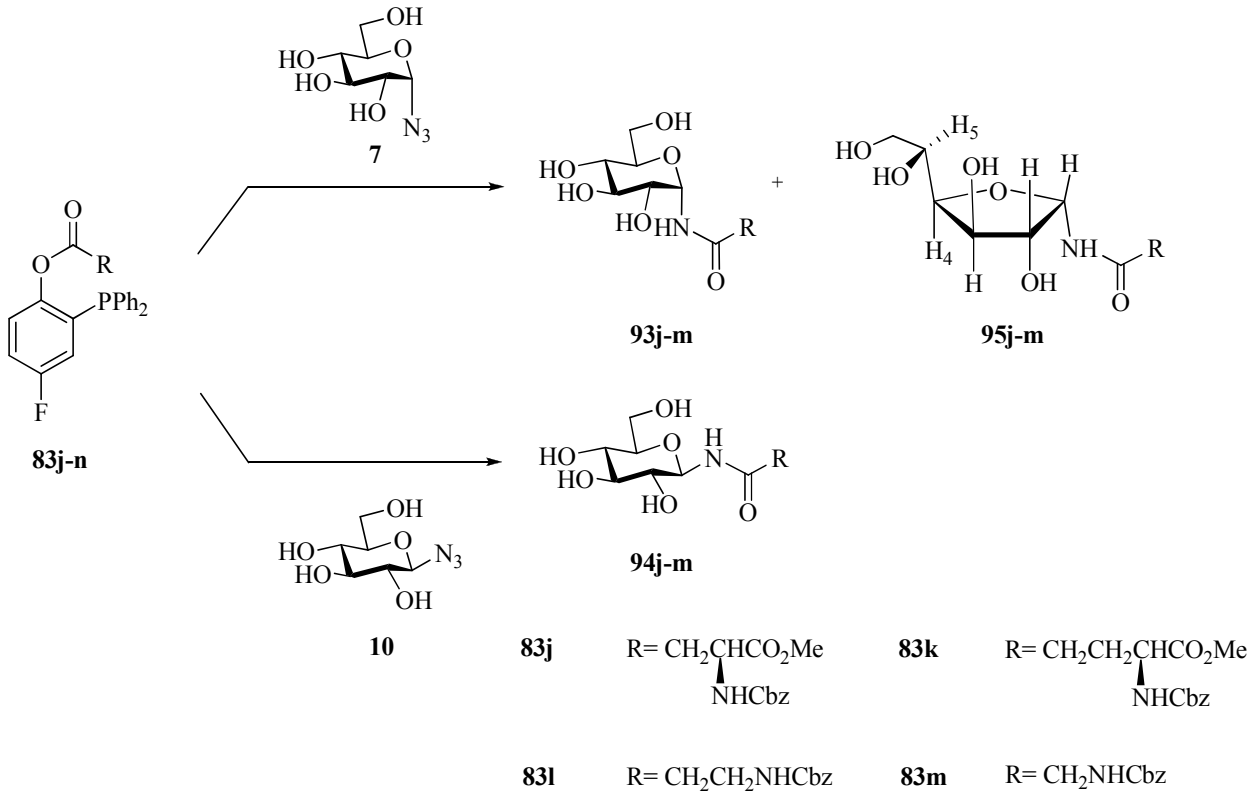
Usually, at 40°C the reaction of **7** with functionalized phosphines **81** afforded the α-glucofuranosyl amides **95** in average yield of 5-15 % depending on the acyl chain. The amount of furanose increases at higher temperatures. During the ligation of particular acyl chains (for instance **81e**), up to 50 % of furanose was obtained (**95e**, Scheme 2 and entries 6 and 7 of Table C, Chapter 4).[4]



Scheme 2. Formation of *N*-3-methyl-2-butenoyl-α-D-glucofuranosylamide **95e**

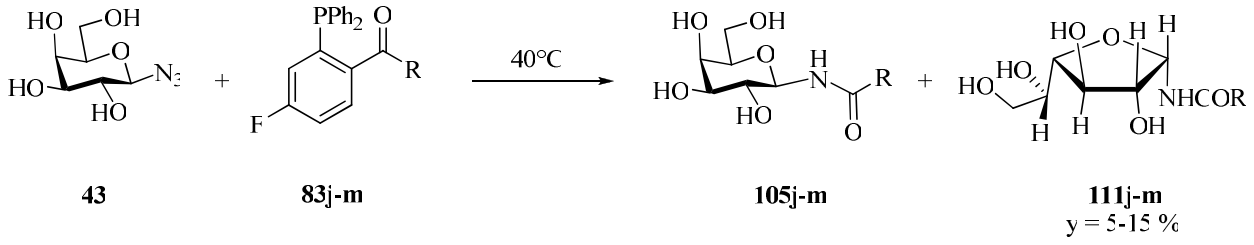
Other interesting observations were obtained during the screening performed on α-glucopyranosyl azides **7** and **10** with fluorinated phosphines **83** for the transfer of amino acids

residues (Chapter 4 and 5). With the α-glucosyl azide **7** variable amounts of furanosylamide were formed, depending on the nature of the acyl chain transferred during the ligation (entries 1-4, Table1, Chapter 5). When the same reaction conditions were extended to unprotected β-glucosyl azides **10,** formations of **95** was not observed and only β-pyranosyl amides **94** were obtained (Scheme 3 and entries 5-9 in Table1, Chapter 5).[5]



Scheme 3. Synthesis of α- and β- glucosyl aminoacids **93j-m** and **94 j-m**

On the other hand, ligation of unprotected β-galactopyranosyl azide **43** with fluorinated phosphines **83j-m** under similar conditions afforded α-furanosyl amides **111** in average yield of 5-15 % (Scheme 4, and entries 1-9 in Table 3, Chapter 5).



Scheme 4. Formation of α-galactofuranosyl amides **111j-m** as by-products in the ligation of **43**

The propensity of glucose and galactose to give furanosides in non-aqueous media was described in the literature.[1] Data show (Table 1) that no furanose is observed for glucose. Galactose forms ca. 30 % of furanose, and the β-furanose configuration (1,2-*trans*) is more stable than the α (1,2-*cis*) one. So, the Staudinger ligation of **7** and **43** appears to promote only the formation of the less stable α furanosyl compounds.

Table 1. Furanose configuration of monosaccharide, in DMSO media[a]

Entry	Monosaccharide	β-pyranose (%)	α-pyranose (%)	β-furanose (%)	α-furanose (%)
1	Glucose	63.1	36.9	-	-
2	Galactose	39	28	24	9

a) From *ref 1*

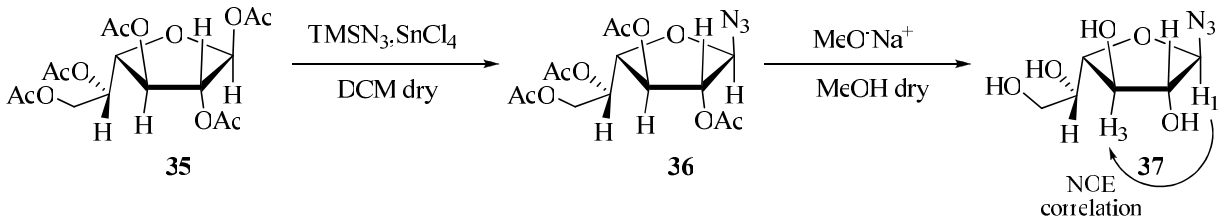
This series of experiments allowed us to draw the following general conclusion:

- The formation of α-furanosyl amides **95** and **111** is favored by a temperature increase and can be increased by running the ligation at temperatures above 40°C.
- The nature of the acyl chains transferred plays a fundamental and still unpredictable role for the furanosyl ring formation.
- In each of the reactions evaluated the glycofuranosyl amide was formed as a unique α-furanose isomer.
- Strikingly, despite the pyranose-furanose isomerization must involve a ring opening step, formation of the β-pyranosyl amide, a more obvious candidate product of an equilibration process, is not observed.

To clarify the mechanism of this intriguing reaction and to analyze the potential activity of furanosyl compounds as sugar mimics, a new set of experiments was performed starting from the β-D-galactofuranosyl azide (β-D-Gal*f* azide, Scheme 5) as a model substrate. The synthesis of penta-*O*-acetyl-galactofuranose (D-AcGal*f*) **35** was described.[6] We reasoned that the reactivity of the corresponding azide **36** could shed light on the formation of the unusual and alternative α-furanosyl by-products obtained in the ligation of unprotected α-pyranosyl azides. Furthermore, D-Gal*f* derivatives have been reported to posses biological activity against *Mycobacterium tubercolosis*,[7, 8] and thus the corresponding amides constitute by themselves an interesting synthetic target.

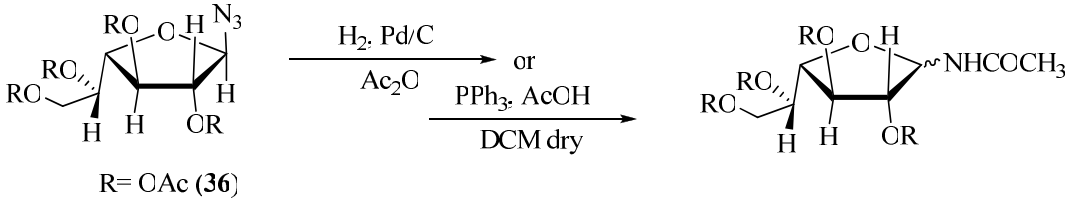
6.2 Synthesis of α- and β-galactofuranosyl amides

The starting galactofuranosyl azide **36** was prepared in 90 % yields from known penta-*O*-acetyl-galactofuranose **35** [5] using $TMSN_3/SnCl_4$ (Scheme 5). Zemplen's deacetylation afforded the unprotected Gal*f* azide **37**, whose anomeric configuration was established by NOE difference experiments, showing a clear correlation between protons H1 and H3.



Scheme 5. Synthesis of Gal*f* azide

Initial studies focused on the functionalization of **36** using standard methodology, such as catalytic hydrogenation followed by *in situ* acetylation or intermolecular Staudinger reaction with Ph_3P/AcOH (Scheme 6). However, both methods led to a mixture of epimers at the anomeric carbon. Similar results were obtained upon treatment of **37** with H_2 and Pd/C.



Scheme 6. Derivatization of **36** with conventional methods leads to mixtures of epimers

The reaction mixtures could be analyzed by ^{1}H-NMR spectrscopy. As shown in Figure 1, the anomeric protons of all four isomeric galactosyl amides are clearly observed in the ^{1}H-NMR of the reaction mixture (Figure 1, peaks 1-4). They are well separated from one another, as well as from the starting azide (doublet at 5.22 ppm, not shown in Figure 1) and from the hydrolysis products (α- and β-galactose, peaks 5 and 6 in Figure 1, respectively) which originate from incomplete acyl transfer in the intermediate iminophosphorane.

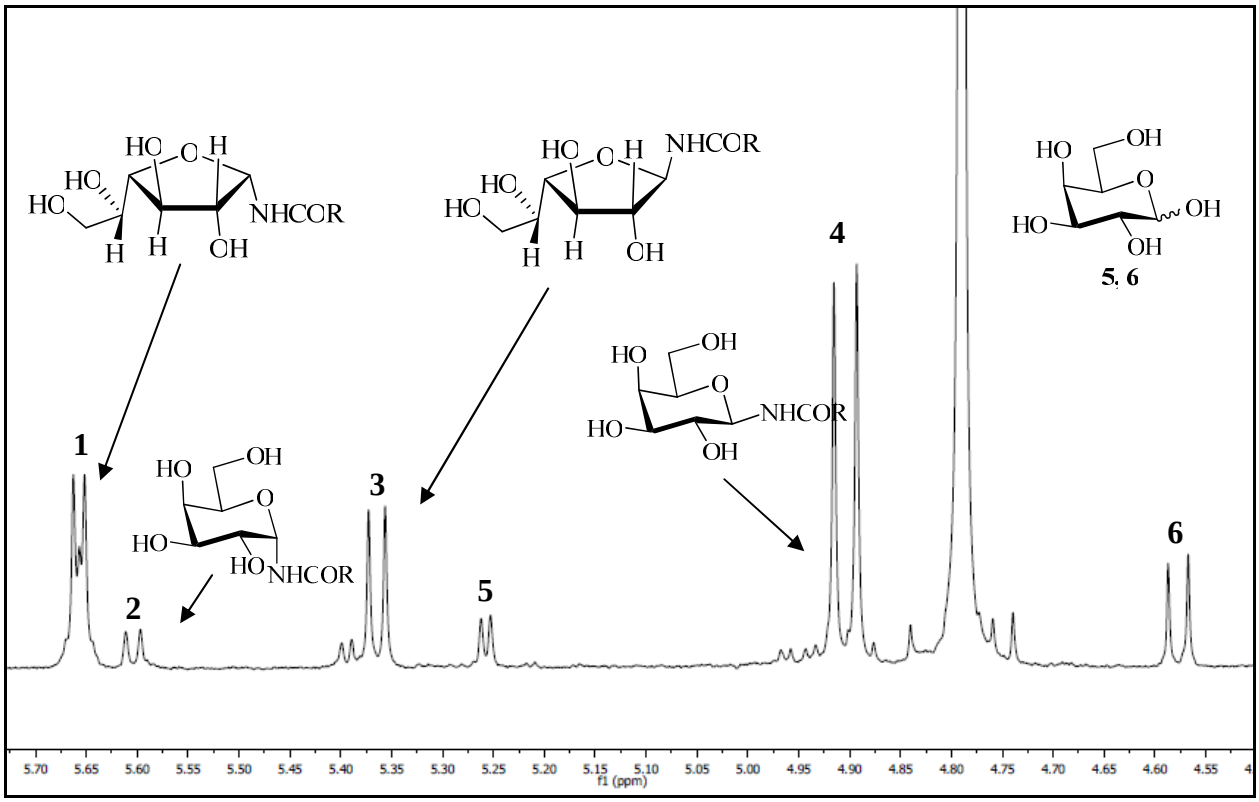
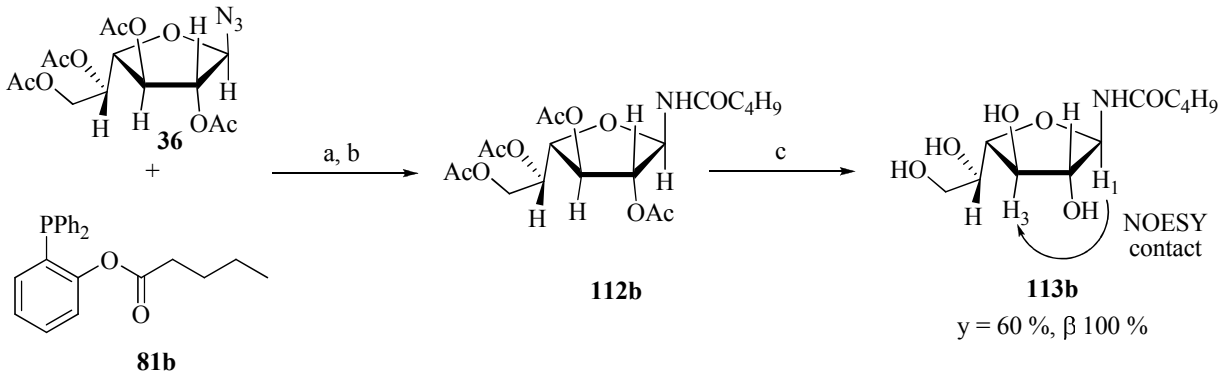


Figure 1. ^{1}H-NMR (D_2O) spectra of the anomeric proton of the four isomeric galactosyl amides (peaks 1-4). Doublets 5 and 6 correspond to α- and β-glucose, respectively

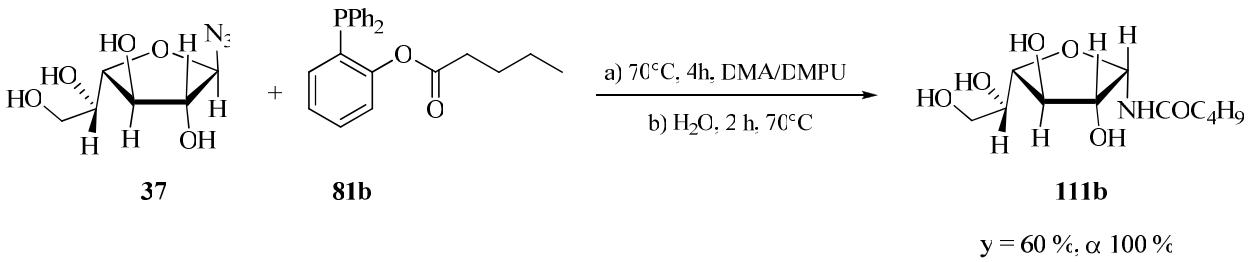
On the contrary, traceless Staudinger ligation of **36** with 2-diphenylphosphanyl-phenyl valeroate **81b** in a 98:2 DMA:DMPU solvent mixture for 4h at 70°C [4, 5, 9] afforded the corresponding tetra-*O*-acetyl-galactofuranosylamide **112b** in 60% yield as a single anomer (Scheme 7). The structure and anomeric configuration of **112b** were confirmed by NMR after deacetylation (cat. MeONa in MeOH) to yield the unprotected amide **113b**. The chemical shift of C4 in **113b** at 83.6 ppm is diagnostic for the furanose form and NOESY experiments (D_2O) showed the expected crosspeak between protons H1 and H3. The deprotection step was found to be critical to preserve the configurational integrity of the anomeric carbon. While 0.05 M MeONa solutions and short contact times (1h) were used successfully, exposure of **112b** to more concentrated (0.1 M) MeONa in MeOH caused partial anomeric epimerization to **111b**, presumably via MeONa induced deprotonation of the amide nitrogen.



a) 70°C, 4h, DMA/DMPU; b) H_2O, 2 h, 70°C; c) 0.05M MeONa, MeOH

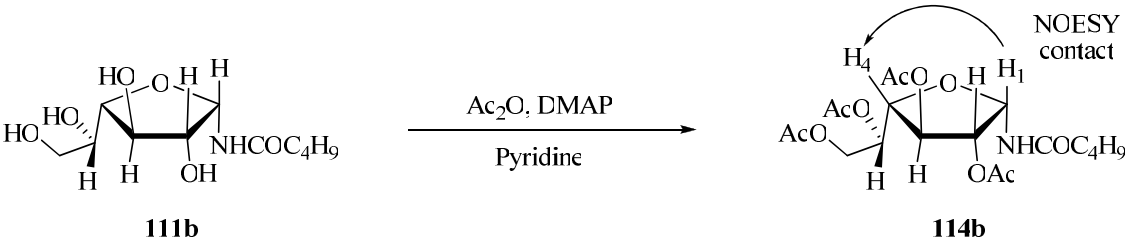
Scheme 7. Synthesis of β-furanosyl amides **112b** by ligation of **36**

Surprisingly ligation of unprotected β-Gal-*f*-azide **37** with **81b** under the same conditions used for **36** afforded only amide **111b** with *complete inversion* of anomeric configuration (Scheme 8). Products from the Staudinger ligation of **37** were isolated by water extraction from the crude reaction mixtures and purified by silica gel chromatography. Anomeric ratio were evaluated on the crude by ^{1}H-NMR spectroscopy. The structure of **111b** was confirmed by the C4 chemical shift (84.4 ppm) and by the absence of crosspeaks between protons H1 and H3 in the NOESY spectrum (CD_3OD; H1 and H3 of **111b** are isochronous in D_2O at 400 MHz).



Scheme 8. Synthesis of α-furanosyl amides **111b** by ligation of **37**

Compound **111b** was fully characterized as the 1,2-*cis* isomer after acetylation to **114b** (Scheme 9), by the presence of crosspeaks between protons H1 and H4 in the NOESY spectrum ($CDCl_3$ at 400 MHz).

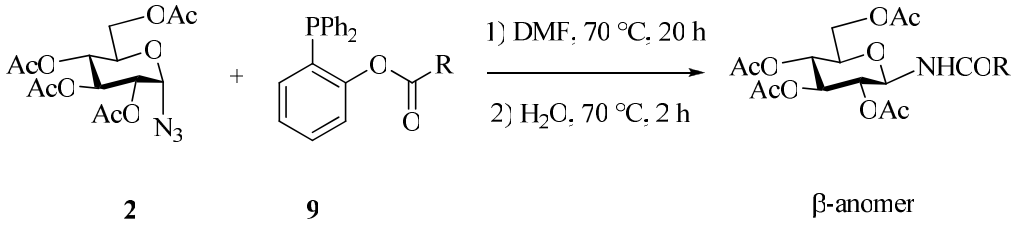


111b **114b**

Scheme 9. Acetylation of **111b** and NOESY characterization of **114b**

6.3 Synthesis of arabinofuranosyl and ribofuranosyl amides

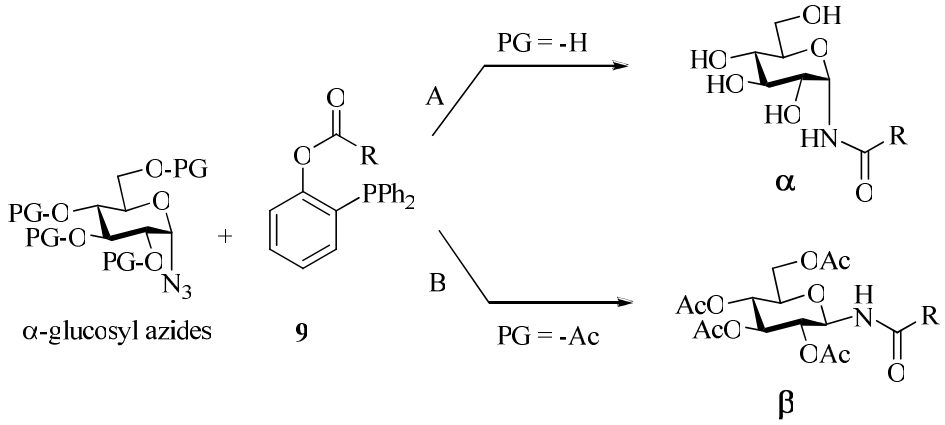
In Chapter 1 we showed that the reaction of phosphines **9** with tetra-*O*-acetyl-α-glucopyranosyl azides **2** was non-stereoconservative [9] (Scheme 10), and that β-glycosyl amides are obtained in good yield and complete stereoselectivity starting from both α- and β-azides.



2 **9** β-anomer

Scheme 10. Staudinger ligation of tetra-*O*-acetyl azides is non-stereoconservative

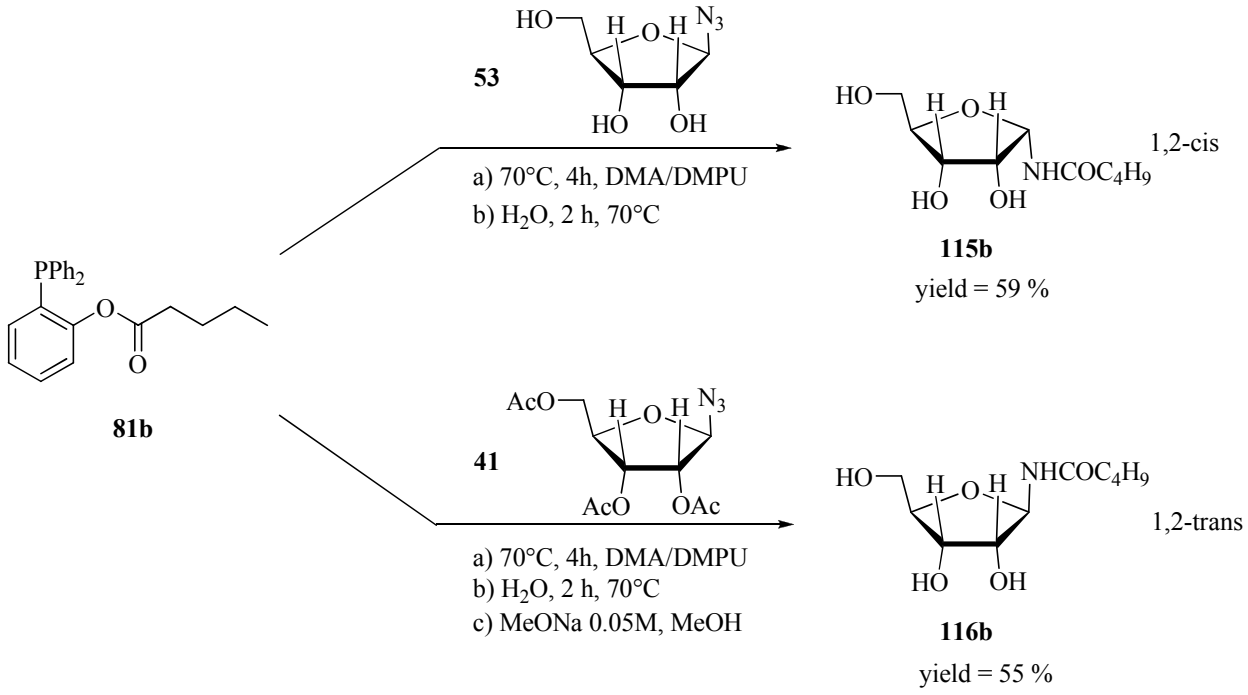
Usually tetra-*O*-acetyl-pyranosyl azides give β-anomerization, while unprotected pyranosyl azides give complete retention of the anomeric configuration (Scheme 11).



Scheme 11. β-anomerization of the tetra-*O*-acetyl-α-pyranosyl azides

It was surprising, therefore, to establish that tetra-*O*-acetyl-β-furanosyl azides **36** do not anomerize, but unprotected furanosyl azides **37** do, and afford 1,2-*cis* compounds with total inversion on the anomeric carbon. The inversion of configuration during the reaction of unprotected galactofuranosyl azides suggests that an unexpected mechanism may be involved in the formation of α-galactofuranosyl amides.

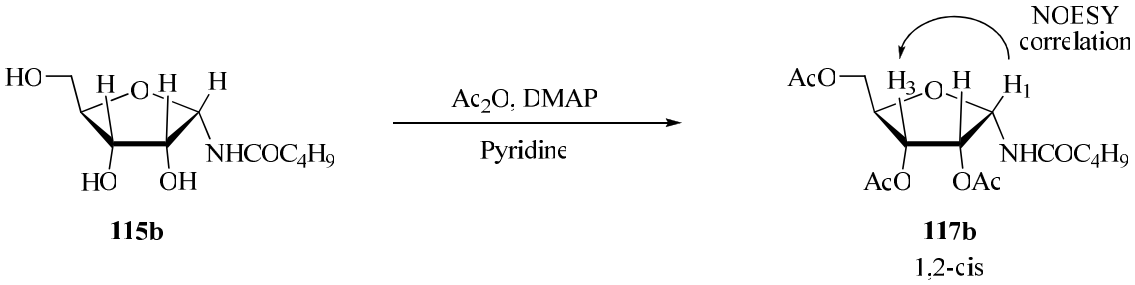
To further explore the scope of this reaction and to investigate the reasons of this unexpected result, a new set of experiments were designed using phosphine **81b** as the standard substrate. The ribofuranosyl azides **41** and **53** (Scheme 12) and the arabinofuranosyl azides **40** and **49** (Scheme 14) were subjected to traceless ligation with compound **81b** in the same reaction conditions used above (70°C for 4h in 98:2 DMA:DMPU). Both ribofuranosyl and arabinofuranosyl azides present a 1,2-*trans* configuration but with the opposite absolute configuration at C2.



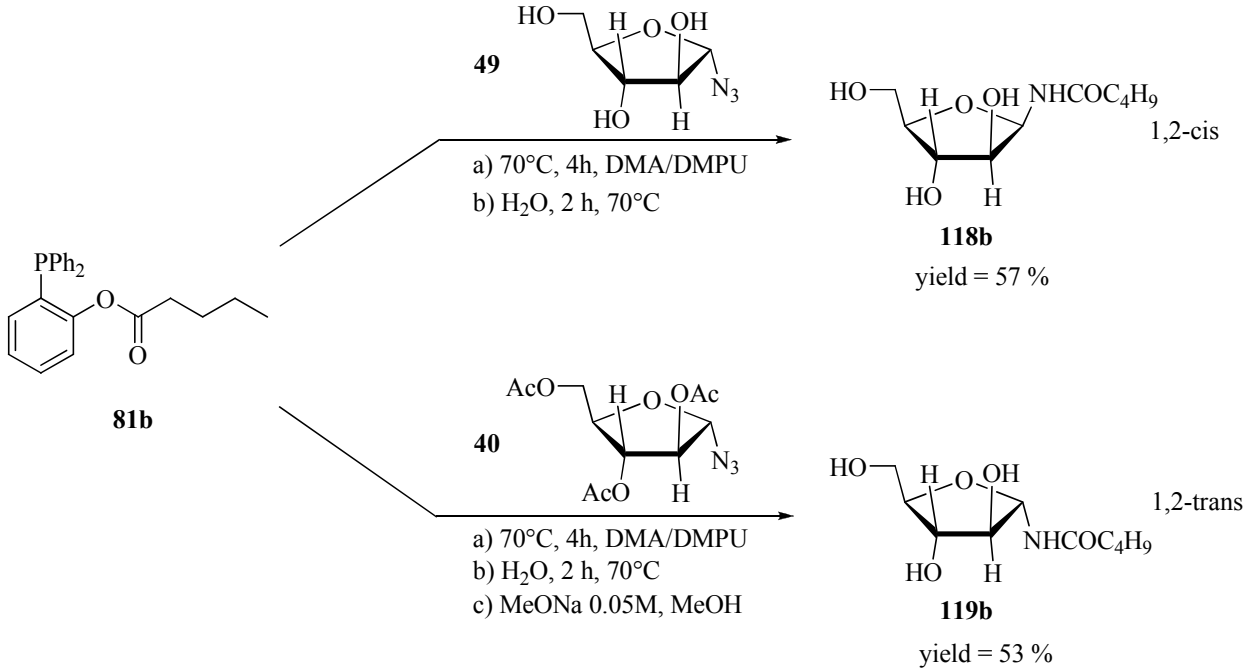
Scheme 12. Staudinger ligation of **81b** with β-ribofuranosyl azides **41** and **53**

Reaction of the β-tetra-*O*-acetyl ribofuranosyl azide **41** with **81b** yielded a β-amide. Its anomeric configuration of was characterized after Zemplen's deacetylation (Scheme 12), which afforded the unprotected 1,2-trans ribofuranosyl amide **116b**. NOESY correlation between protons H1

and H4, and the high value of C4 (83.5 ppm) in **116b** are diagnostic for a β-furanosyl ring. Reaction of the unprotected β-ribofuranosyl azide **53** gave the α-anomer **115b**. After acetylation (Scheme 13) of **115b** the structure of **117b** was assigned on the basis of strong crosspeak for the H1 and H3 protons in the NOESY spectrum (CD_3OD).



Scheme 13. Acetylation of the *N*-pentanoyl-α-D-ribofuranosylamide **115b**



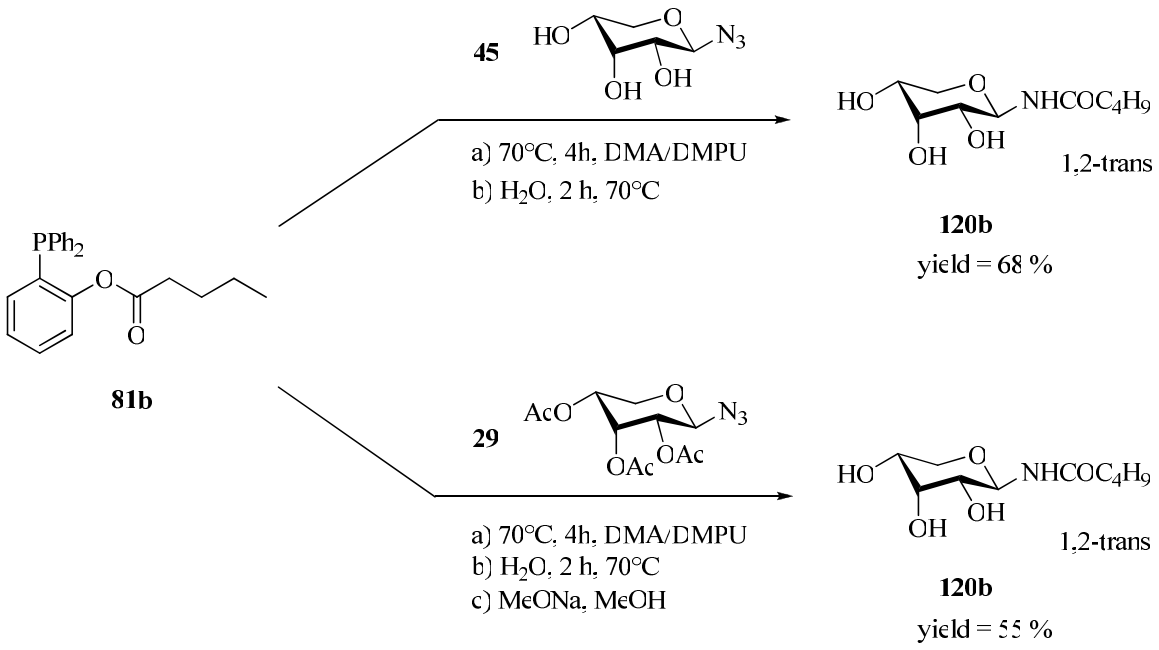
Scheme 14. Staudinger ligation of **81b** with α-arabinofuranosyl azides **40** and **49**

Similarly inversion of configuration was observed upon reaction of the unprotected arabinofuranosyl azide **49** to give **118b**. NOESY experiments on **118b**, showed a clear

correlation between protons H1 and H4, and also the chemical shift of C4 in **118b** at 85.4 ppm is diagnostic for the furanose form. On the contrary no inversion was observed upon ligation of the tetra-*O*-acetyl azide **40**, which gave **119b**.

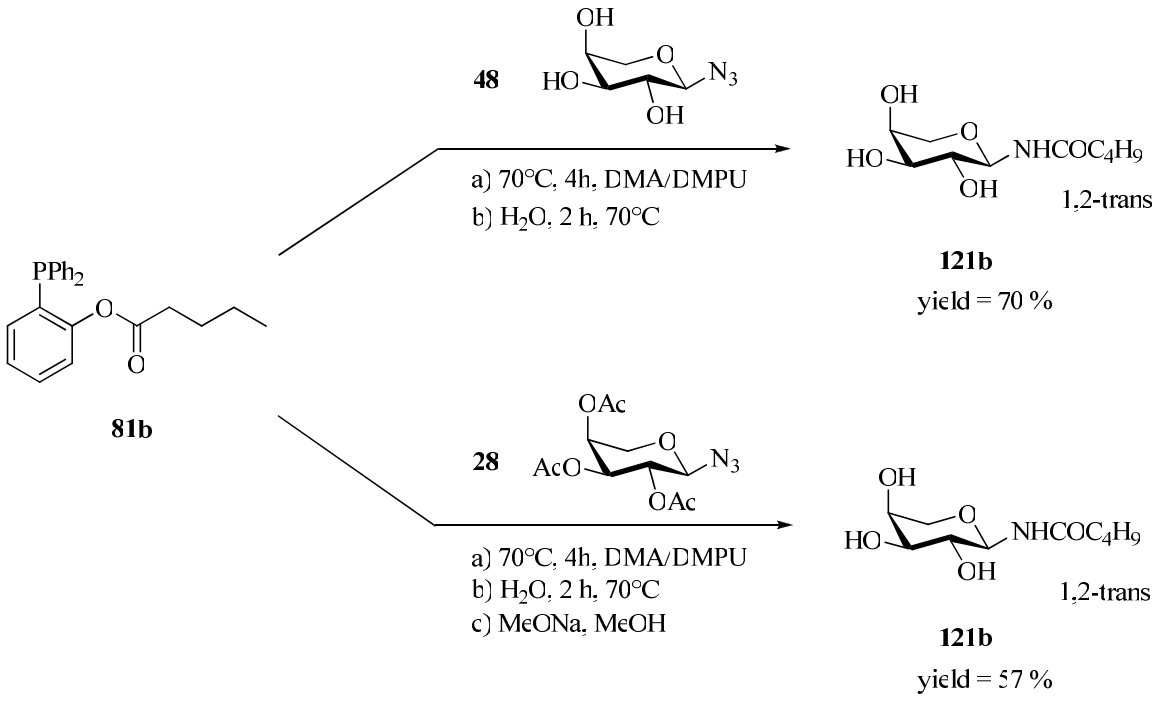
Thus, under the conditions developed all reactions occurred with selective inversion on the anomeric carbon to afford 1,2-*cis* compounds for the unprotected azides **53, 49** and **37** while they were stereoconservative for the *O*-acetyl azides **41**, **40** and **36**, leading to 1,2-*trans* amides. Products from the Staudinger ligation of the unprotected ribofuranosyl azides **53** and arabinofuranosyl **49** were isolated by water extraction from the crude reaction mixtures and purified by silica gel chromatography ($CHCl_3$/MeOH 85:15). Also in this case to facilitate products isolation from phosphine oxide, the tetra-*O*-acetyl ribofuranosyl and tetra-*O*-acetyl arabinofuranosyl amides were not directly isolated, but first deacetylated to afford the corresponding deprotected amides **116b** and **119b**. Finally the compounds were isolated by water extraction and purified by flash chromatography on silica gel. Under this conditions the 1,2-*cis* (**115b** and **118b**) and 1,2-*trans* (**116b** and **119b**) furanosyl amides were generally obtained in moderate yields and excellent anomeric ratio.

For comparison purpose and to fully characterize the ligation products, the corresponding pyranosyl azides β-ribopyranosyl azide **29** (tri-*O*-acetyl) and **45** (unprotected) (Scheme 15) and β-arabinopyranosyl azide **28** (tri-*O*-acetyl) and **48** (unprotected) (Scheme 16) were subjected to Staudinger ligation with **81b**. They afforded the corresponding 1,2-*trans* glycopyranosyl amides **120b** and **121b** in good yields and without anomeric inversion. ^{1}H NMR analysis of the crude reaction mixtures showed no trace of isomeric azides also starting from unprotected 1,2-*trans* azides **45** and **48**. The anomeric configuration of **120b** was confirmed by the typical high value of the trans-diaxial coupling constant ($J_{1,2}$ = 8 Hz) for the β compounds and also the chemical shift of C4 (68.8 ppm) is diagnostic for the pyranose form.



Scheme 15. Staudinger ligation of **81b** with β-ribopyranosyl azides **29** and **45**

The structure of **121b** was established on the basis of the chemical shift of the C4 carbon at 68.8 ppm, which is consistent with the pyranose form and of the $J_{1,2}$ of 8.8 Hz consistent with a *trans* configuration of C1-C2.



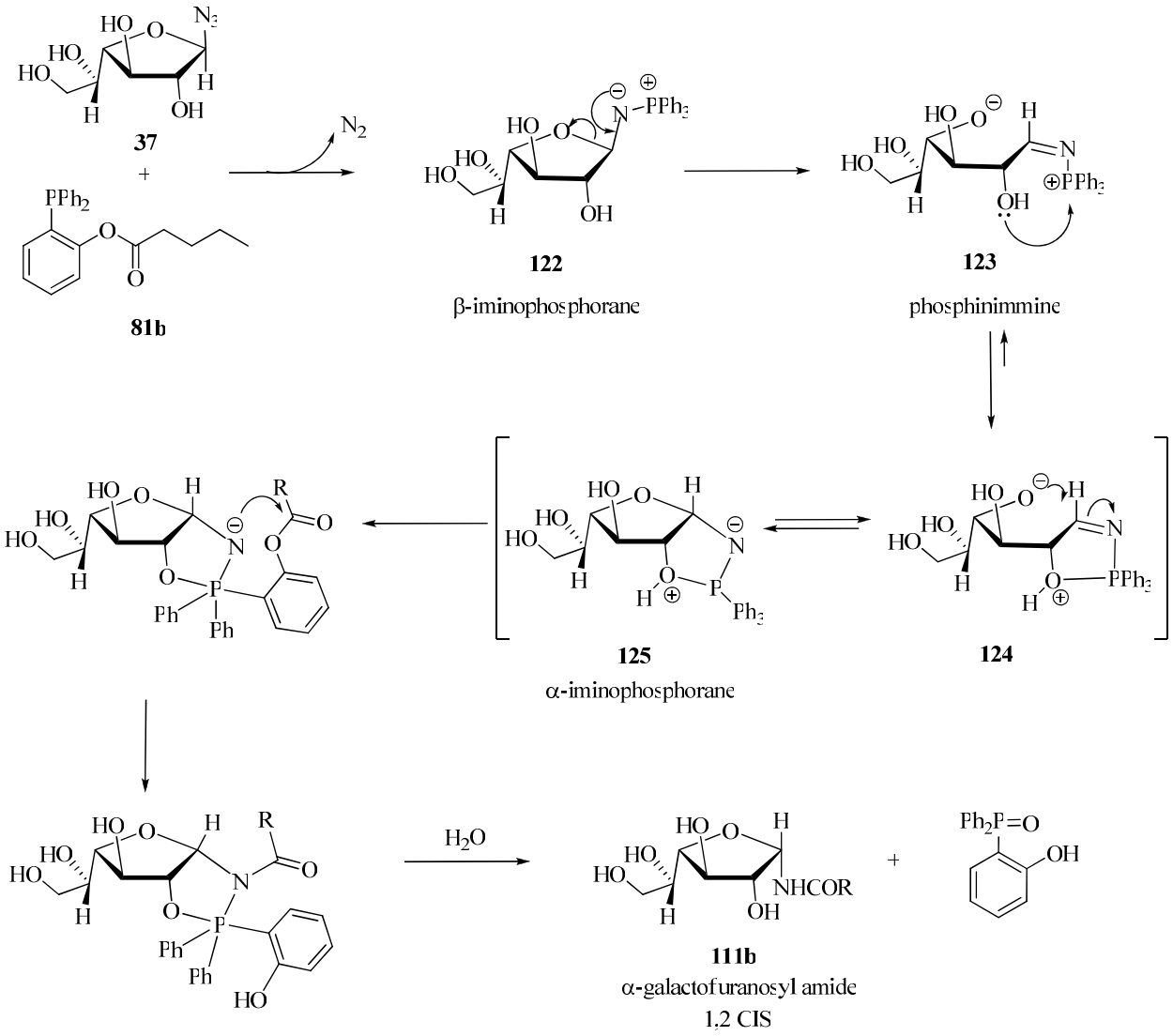
Scheme 16. Staudinger ligation of **81b** with β-arabinopyranosyl azides **28** and **48**

In conclusion, the ligation of **81b** with α-arabinopyranosyl and β-ribopyranosyl azides confirm the previous observation on the stereoselectivity for the intramolecular Staudinger ligation of glucopyranosyl azide:[9]

6.4 Proposed mechanism for the α-furanosylamide formation

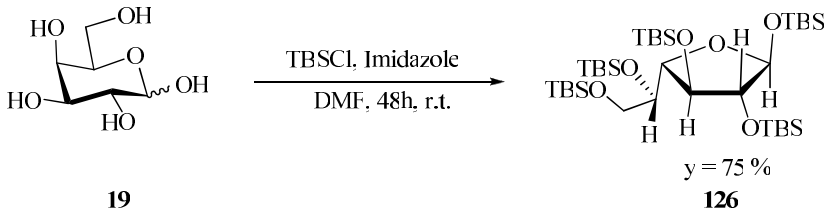
The ligation of unprotected 1,2-*trans* furanosyl azides **37**, **53** and **49** with phosphines **81b** afforded glycofuranosyl amides with the opposite 1,2-*cis* anomeric configuration respect to the starting azide precursor. This anomeric epimerization must derive from a ring-opening process occurring after the azide reduction step. A likely mechanism is shown in Scheme 17 for the reaction of **81b** with β-galactofuranosyl azide **37**. Upon azide reduction, the iminophosphorane **122** (Scheme 17) is formed and ring opening of **122** affords phosphinimine **123**. From this open-chain intermediate, the ring clousure step could be biased by the unprotected hydroxyl group at position 2, which can trap the phosphorous atom of **123** as the cyclic oxazaphospholane intermediate **124**, thus enforcing, after ring clousure, the formation of the α-iminophosphorane **125**. In other words, the anomeric carbon of the sugar is blocked by chelation of phosphorous in an intermediate **124** which can only afford α-amides upon ring closure. Finally, intramolecular acyl transfer and subsequent hydrolysis give α-galactofuranosyl amide **111b** with total inversion on the anomeric carbon.

This mechanism implies that ligation of the unprotected furanosyl azides will enforce a 1,2-*cis* configuration in the resulting amides by coordination of the free OH in positon 2 with the phosphorous atom of the iminophosphorane and can be used to clarify also the epimerizations observed during the ligation of **81b** with unprotected arabinofuranosyl **49** and ribofuranosyl azides **53**.



Scheme 17. Proposed mechanism for the anomeric epimerization of **37**

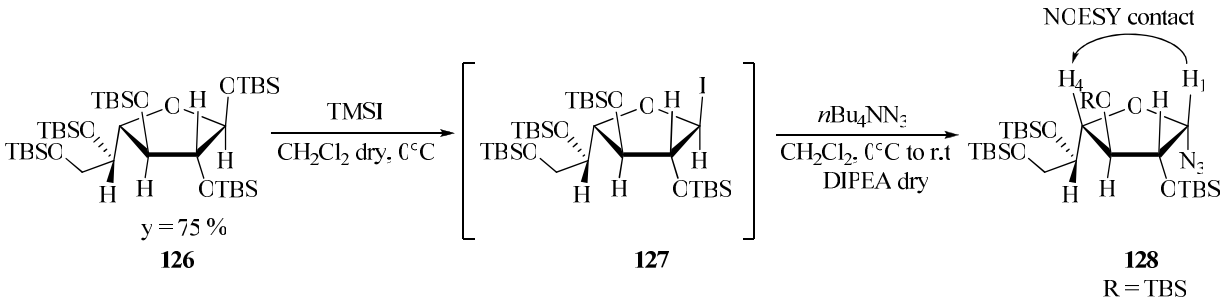
To support this mechanistic picture, we attempted to examine the ligation of the isomeric 1,2-*cis* α-galactofuranosyl azide **46** (Scheme 20), with the expectation that anomeric inversion should be no longer observed on this isomer. This compound has never been described. However, the conversion of free galactopyranose **19** into 2,3,5,6-tetra-*O*-*tert*-butyldimethylsilyl-β-galactofuranosyl iodide[10] **127** (Scheme 19) has been recently reported by Baldoni and Marino. The authors treated the free hexose sugar with an excess of *tert*-butyldimethylsilyl chloride in dimethylformamide and in the presence of imidazole at room temperature obtaining 1,2,3,5,6-penta-*O*-*tert*-butyldimethylsilyl-β-galactofuranose **126** which was isolated from the reaction mixture in 75 % yield by crystallization from methanol (Scheme 18).



19 **126**

Scheme 18. Stereoselective synthesis of 1,2,3,5,6-penta-*O*-*tert*-butyldimethylsilyl-β-galactofuranose **126**

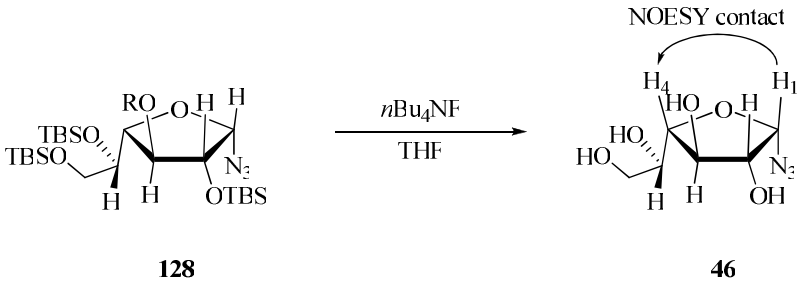
Treating **126** with 1.2 equiv. of tri-methylsilyliodide (TMSI) in anhydrous 1,2-dichloromethane at 0°C for 30 minutes, the anomeric iodide is formed.[10] When TLC examination showed a total consumption of the starting material **126**, tetra-butylammonium azide [11] (nBu$_4$NN$_3$) was added and the mixture was stirred overnight at room temperature. The reaction afforded good yields and an optimal stereoselectivity for the 2,3,4,6-tetra-*O*-*tert*-butyldimethylsilyl-α-galactofuranose azide **128**, the structure was confirmed by NOESY correlation between H1-H4 (Scheme 19).



126 **127** **128**
R = TBS

Scheme 19. Synthesis of α-galactofuranosyl azide **128** via in situ formed galactofuranosyl iodide

Removal of the TBS groups from **128** was accomplished with tetra-butylammonium floride (nBu$_4$NF) in THF [12] and afforded unprotected α-galactofuranose azide **46** (Scheme 20). The anomeric configuration of **46** was confirmed by NOESY experiments that showed a clear correlation between protons H1 and H4 (Scheme 20, Figure 2), and also the chemical shift of C4 in **46** at 84.5 ppm is diagnostic for the furanose form.



128 **46**

Scheme 20. Deprotection of **128**

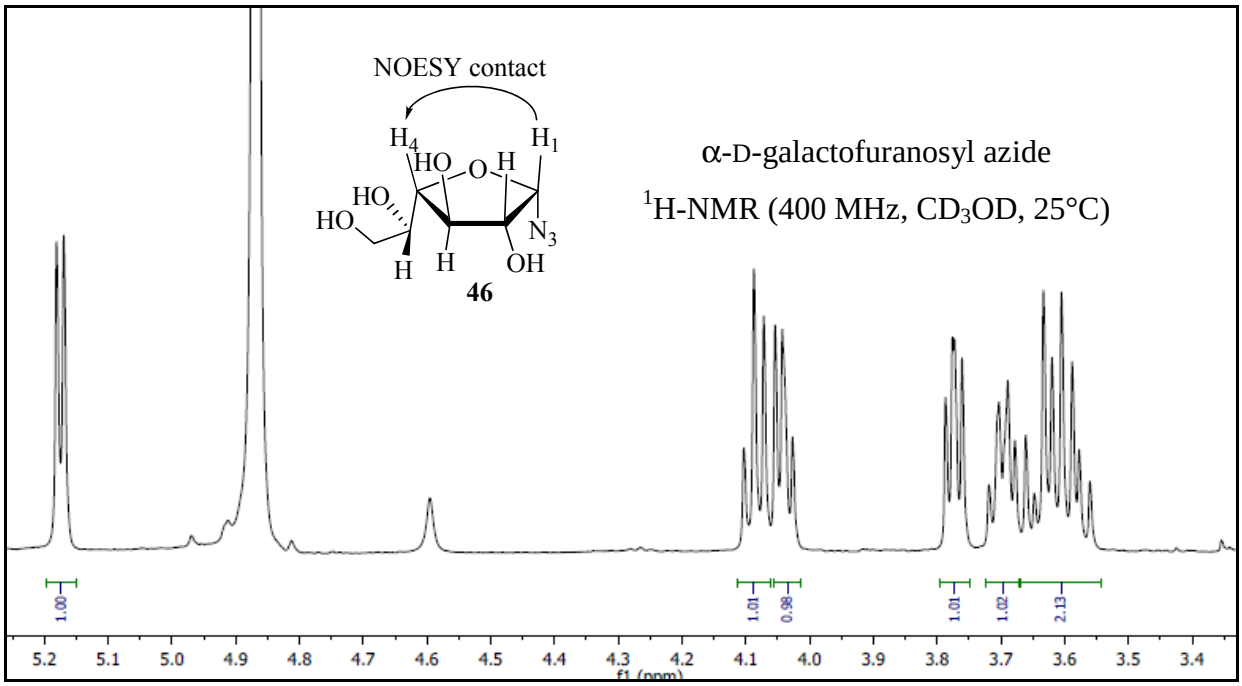
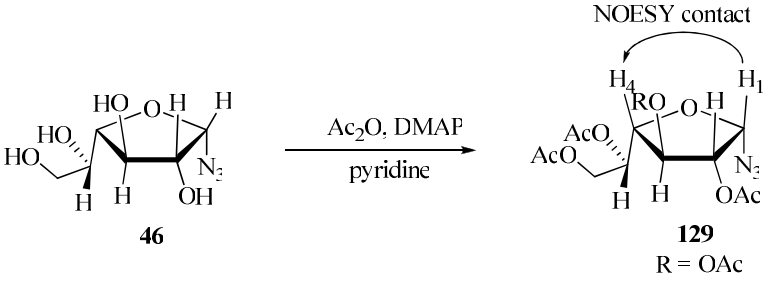


Figure 2. ^{1}H-NMR (CD_3OD) spectra of α-D-galactofuranosyl azide **46**

Acetylation of the unprotected α-galactofuranosyl azide **46** (Scheme 21, Figure 3), with classical procedure (acetic anhydride in pyridine) afforded the 2,3,5,6-tetra-acetyl-α-galactofuranosyl azide 1**29**. Also in this case we observed a NOESY contact between H1-H4 ($CDCl_3$).



46 **129**
R = OAc

Scheme 21. Acetylation of unprotected α-galactofuranosyl azide **46**

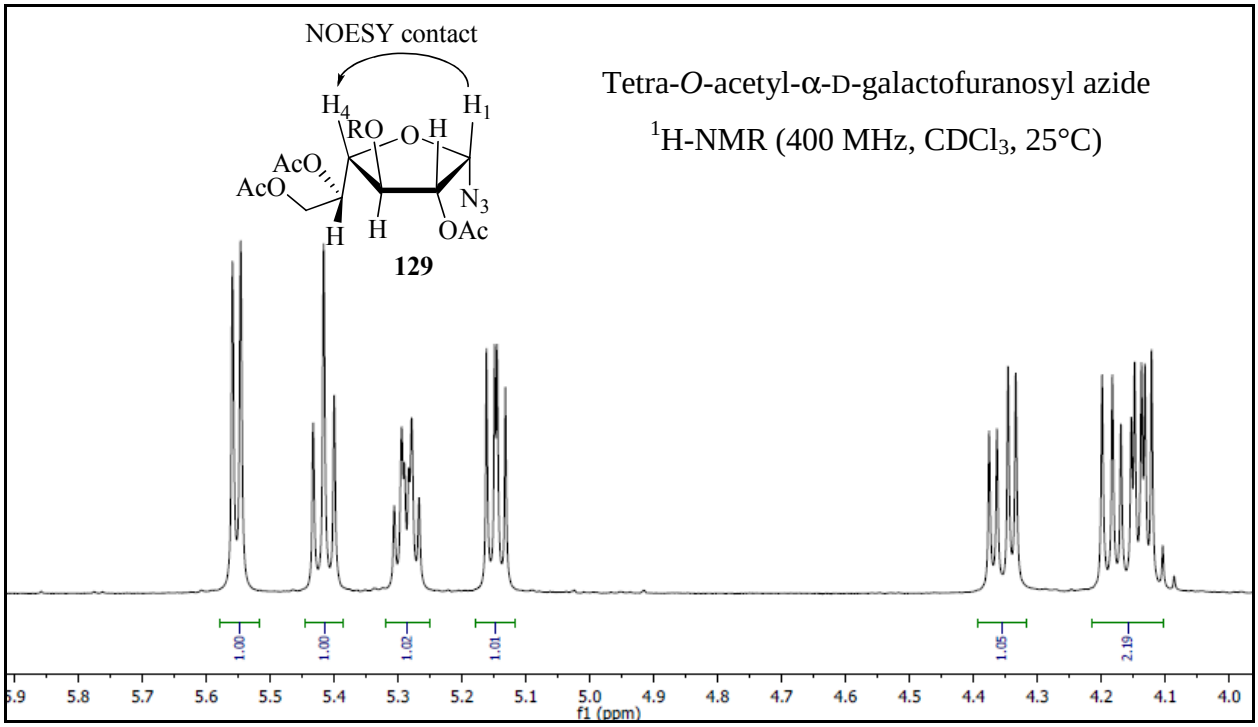
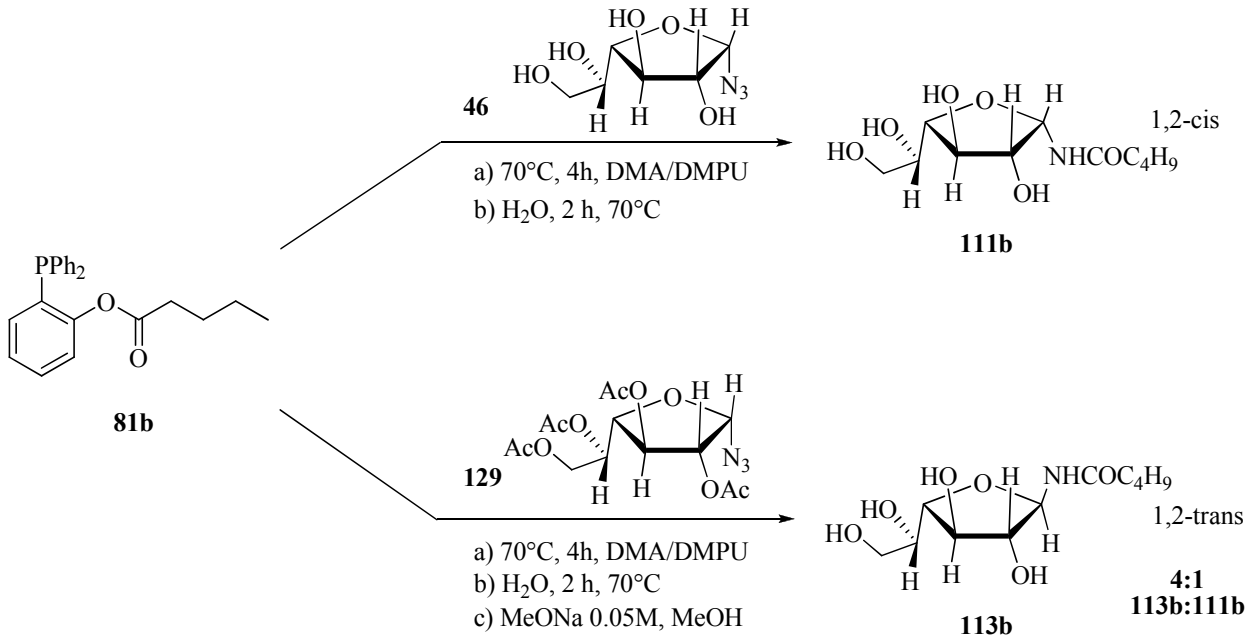


Figure 3. ^{1}H-NMR ($CDCl_3$) spectra of tetra-*O*-acetyl-α-D-galactofuranosyl azide **129**

The unprotected and tetra-*O*-acetyl-α-galactofuranosyl azides **46** and **129** (Scheme 22) were subjected to traceless ligation with compound **81b** in the same reaction conditions used for the ligation of protected and unprotected β-galactofuranosyl azides **36** and **37** (70°C for 4h in solvent mixture DMA:DMPU 98:2).

The ligation of unprotected α-galactofuranosyl azide **46** with phosphines **81b** afforded the amide **130b** with the same anomeric configuration of the starting azide precursor, in a stereoconservative process. Unprotected α-furanosyl azides **46**, thus, did not anomerize but afforded the 1,2-*cis* azide, as it would be expected based on the proposed mechanism. Surprisingly, ligation of the of tetra-*O*-acetyl-α-furanosyl azides **129** afforded a 4:1 β:α ratio, thus revealing that an equilibration process is taking place for this acetylated azide, which favors the 1,2-*trans* amide **113b** with inversion on the anomeric carbon (Scheme 22). The inversion of configuration during the reaction of protected galactofuranosyl azide **129** suggests that this configuration may be more stable for the tetra-acetylated compound.



Scheme 22. Staudinger ligation of **81b** with α-galactofuranosyl azide **46** and **129**

After these observations the mechanism proposed in Scheme 17 for α-furanosylamide formation appears totally confirmed. The full implications of this proposed mechanism relative to the ligation of furanosyl and pyranosyl azides will be discussed in Chapter 7.

6.5 Synthesis of small library of α and β galactofuranosylamides as inhibitors of *Mycobacteria*

The arabinogalactan constituent of mycobacterial cell walls contains a galactan polymer made of galactose residues in their furanose form. In particular galactofuranose (D-Gal*f*) was found to be an important coustituent of the cell wall of mycobacteria the causative agent of tuberculosis (TB).[7, 8] The development of new drugs for tuberculosis has taken on some urgency in the recent years. The World Health Organization reports that the global incidence rate ot TB is rising, and more than 4000 people die of TB everyday. [13] More new cases are expected since TB is also the main cause of death in AIDS patients. Inhibition of the incorporation of D-galactofuranose (D-Gal*f*) has become a potential therapeutic strategy since Gal*f*-containing oligosaccharides are critical for survival and infectivity [14, 15] of mycobacteria. Incorporation of Gal*f* into

oligosaccharides involves two steps. First, the activated precursor UDP-galactofuranose (UDP-Gal*f* 1) is synthesized from UDP-galactopyranose (UDP-Galp 2) in a reaction catalyzed by UDP-galactopyranose mutase (UGM). Subsequently, Gal*f* is transferred from UDP-Gal*f* 1 onto acceptors to form various Gal*f*-containing oligosaccharides by UDP-galactofuranosyltransferase (Gal*f*T).[16, 17]

The furanose form of D-galactose, Gal*f*, is uknown in mammals and UDP-galactopyranose (UDP-Galp 2) mutase has become an interesting target for the development of drugs against the many pathogens which contain Gal*f*, most prominently *Mycobacterium tuberculosis*. UDP-Galp 2 mutase inhibitors have been reported and shown to block mycobacterial growth.[18, 19, 20, 21, 22, 23]

Various molecules containing a structural unit mimicking the Gal*f* monosaccharide have been synthesised, including iminosugars, thiosugars, galactofuranosyl thioglycosides and C-glycosides (Figure 4), but no galactofuranosylamides have been described so far. The ability of some of these compounds to inhibit bacterial growth makes galactofuranose mimics interesting biological targets with potential therapeutic value.[7, 24, 25, 26]

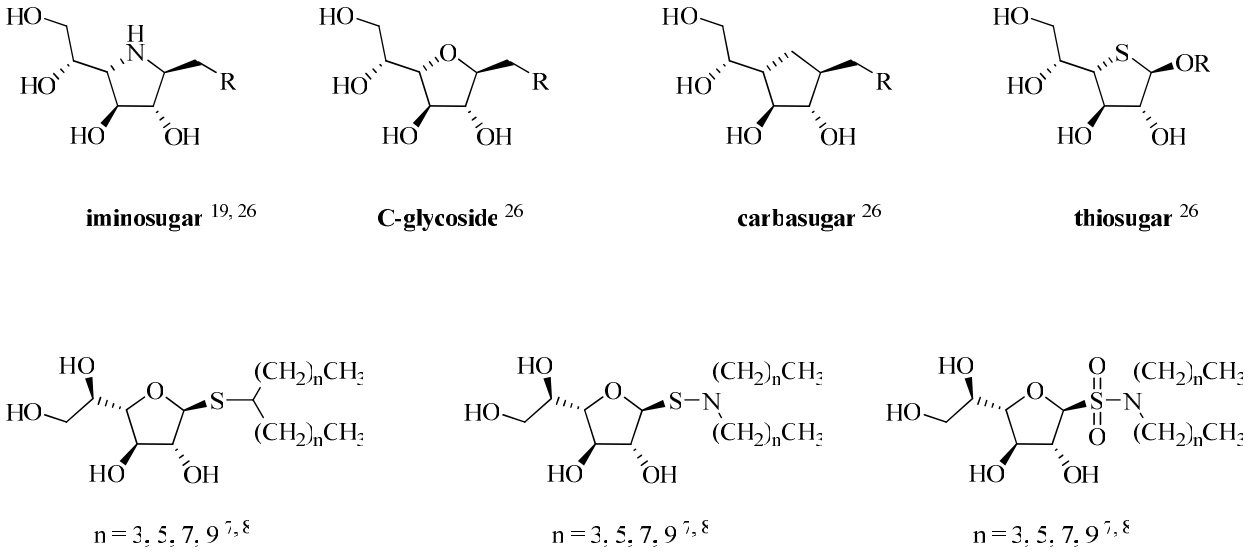
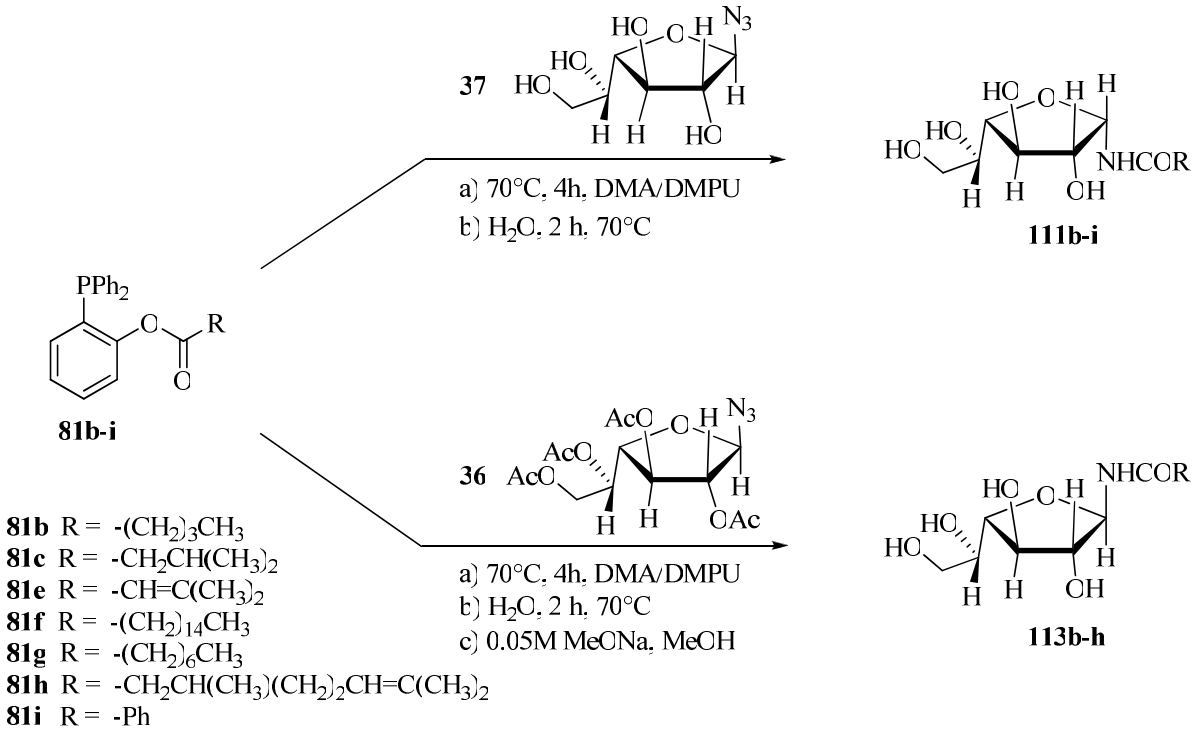


Figure 4. Some analogues of β-galactofuranose which have shown activity against *Mycobacterium tubercolosis*

To explore the scope of galactofuranosyl amides as bacterial growth inhibitors, we used the stereodivergent ligation of furanosyl azides just discovered and applied it to a set of phosphines

variously functionalized, to obtain a small library of α- and β-galactofuranosyl amides (Scheme 23).



Scheme 23. Traceless Staudinger ligation of **36** and **37** with phosphines **81b-i**

The results of this screening are collecetd in Table 2. Transfer of linear aliphatic (**81b, f, g**), branched aliphatic (**81c, e**), unsaturated (**81e, h**) and aromatic (**81i**) amide chains was performed with uniform results. All reactions gave the expected products in moderate yields. As previously reported for unprotected glycopyranosyl azides,[3] microwaves were found to accelerate the ligation of **37** (entries 7 and 11). The stereoselectivity was complete, as determined by ^{1}H-NMR of the crudes. In all cases, β-anomers were obtained from the tetra-*O*-acetyl azide **36** and α-anomers from the unprotected azide **37**. Experimental procedures, purification and characterization data were performed as described before in *par*. 6.2.

Table 2. Ligation of **36** and **37** with phosphines **81b-i**.[a]

Entry	Azide	Phosphine	R	Product	α/β [b]	Yields
1	**36**	**81b**	$-(CH_2)_3CH_3$	**113b**	2:98	60
2	**36**	**81c**	$-CH_2CH(CH_3)_2$	**113c**	≥ 1:99	53
3	**36**	**81e**	$-CH=C(CH_3)_2$	**113e**	2:98	48
4	**36**	**81f**	$-(CH_2)_{14}CH_3$	**113f**	≥ 1:99	60
5	**36**	**81g**	$-(CH_2)_6CH_3$	**113g**	≥ 1:99	56
6	**36**	**81h**	$-CH_2CH(CH_3)(CH_2)_2CH=C(CH_3)_2$	**113h**	≥ 1:99	51
7	**37**	**81b**	$-(CH_2)_3CH_3$	**111b**	≥ 99:1[c]	60
8	**37**	**81c**	$-CH_2CH(CH_3)_2$	**111c**	≥ 99:1	57
9	**37**	**81e**	$-CH=C(CH_3)_2$	**111e**	≥ 99:1	51
10	**37**	**81f**	$-(CH_2)_{14}CH_3$	**111f**	≥ 99:1	55
11	**37**	**81g**	$-(CH_2)_6CH_3$	**111g**	≥ 99:1 [c]	63
12	**37**	**81h**	$-CH_2CH(CH_3)(CH_2)_2CH=C(CH_3)_2$	**111h**	≥ 99:1	56
13	**37**	**81i**	-Ph	**111i**	≥ 99:1	62

a) Unless otherwise stated, all reactions were performed in 98:2 DMA:DMPU mixtures for 4h at 70°C. Then water was added and the solution stirred for additional 2h at 70°C before work up. b) ^{1}H-NMR ratio of the crude. c) Reaction performed under microwave: irradiation at 30 min, 70°C.

All these compounds were tested in minimal inhibition concentration (MIC) tests against a panel of bacteria. One compound, the α-galactofuranosyl amides **111f** displayed a modest activity against *Mycobacterium bovis* with a MIC value of 64 μg/ml. The β-anomer **113f** showed no activity.

This finding may represent the discovery of a new potential class of antibacterial agents, because the totality of the molecules reported so far which contain a structural unit mimicking Gal*f* are all in the β-configuration. More work is required to verify this finding and to optimize the structure and activity of these compounds.

6.6 Experimental Section

Solvents were dried by standard procedures: dichloromethane, methanol, *N*,*N*-diisopropylethylamine and triethylamine were dried over calcium hydride; *N*,*N*-dimethylacetamide (DMA), 1,3-dimethyltetrahydro-2(1*H*)pyrimidinone (DMPU), chloroform and pyridine were dried over activated molecular sieves. Reactions requiring anhydrous conditions were performed under nitrogen. ^{1}H, ^{13}C and ^{31}P-NMR spectra were recorded at 400 MHz on a Bruker AVANCE-400 instrument. Chemical shifts (δ) for ^{1}H and ^{13}C spectra are expressed in ppm relative to internal Me_4Si as standard. Signals were abbreviated as s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained with a Bruker ion-trap Esquire 3000 apparatus (ESI ionization) and FT-ICR Mass Spectrometer APEX II & Xmass software (Bruker Daltonics) - 4.7 Magnet. Thin layer chromatography (TLC) was carried out with pre-coated Merck F_{254} silica gel plates. Flash chromatography (FC) was carried out with Macherey-Nagel silica gel 60 (230-400 mesh).

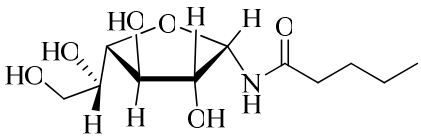
General procedure for stereoselective ligation of protected and unprotected glycosyl azides in DMA:DMPU mixtures.

The phosphine (1.2 eq) was added, at room temperature, to a 0.1 M solution of glycosyl azide (1 eq) in 98:2 *N*,*N*-dimethylacetamide and DMPU. The solution was stirred for 4 h at 70 °C, then water was added and the mixture was stirred for an additional 2 h at the same temperature. The solvent was evaporated under reduced pressure, and the residue was purified as indicated below for each compound.

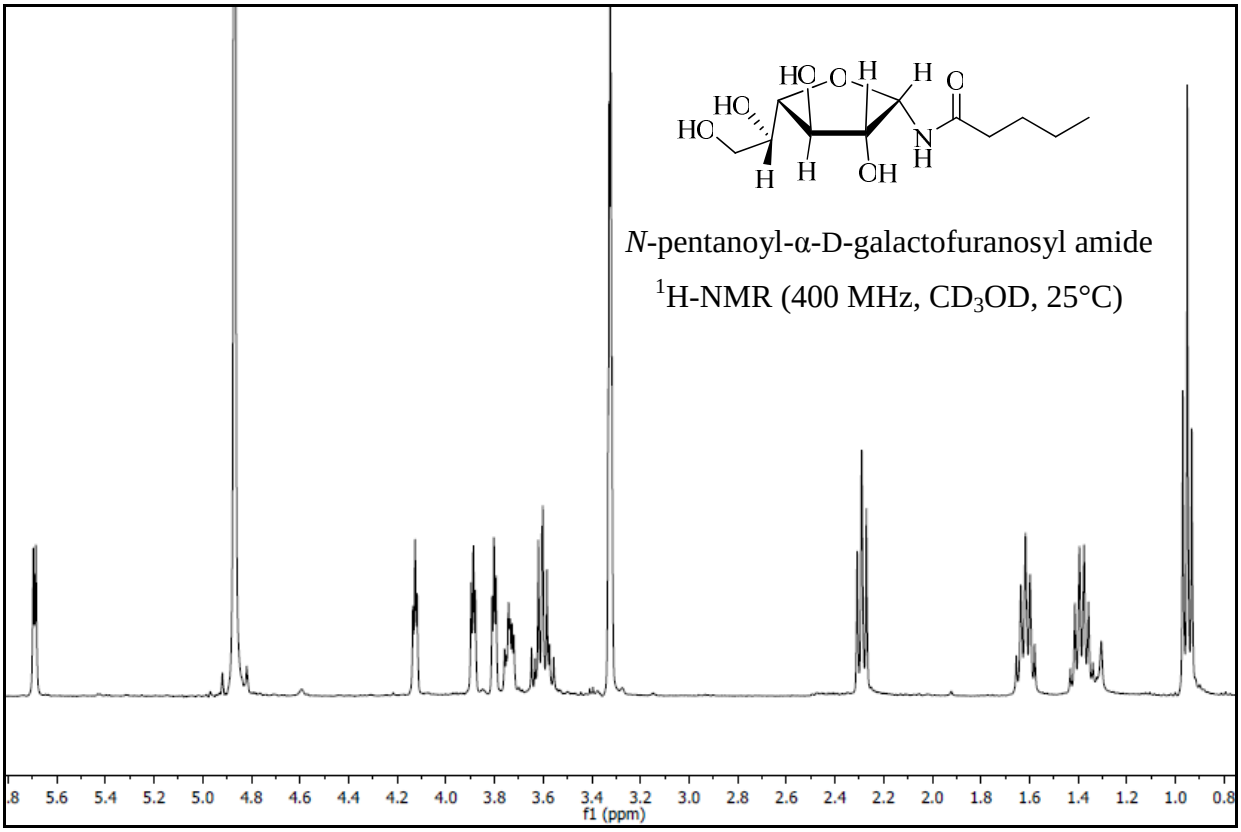
General procedure for acetylation of unprotected glycosyl amides.

Acetic anhydride (10 eq) and a catalytic amount of *N*,*N*-dimethylaminopyridine were added, at room temperature, to a solution of substrate (1 eq) in pyridine dried on molecular sieves (0.1 M). The solution was stirred for 24 h and then was concentrated in vacuo. The residue was dissolved in AcOEt and washed with aqueous 5 % HCl, aqueous 5 % $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated to give the product in quantitative yield**.**

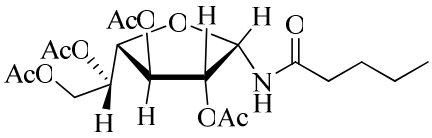
_N_-pentanoyl-α-D-galactofuranosyl amide (111b)



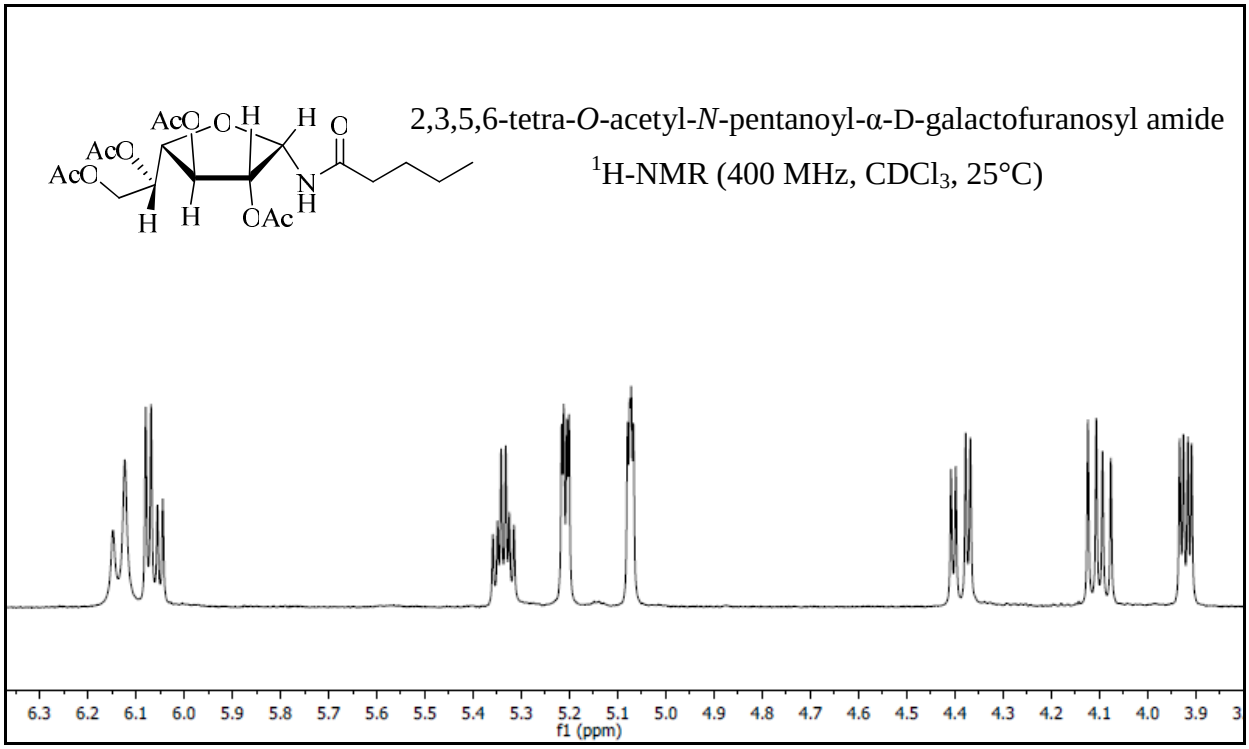
The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20) yield = 60 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.69 (d, $J_{1,2}$ = 4.4 Hz, 1H, H-1), 4.13 (t, $J_{2,3}$ = 3.4, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 3.88 (dd, $J_{1,2}$ = 4.4, $J_{2,3}$ = 3.4 Hz, 1H, H-2), 3.80 (dd, $J_{3,4}$ = 3.6, $J_{4,5}$ = 3 Hz, 1H, H-4), 3.74 (ddd, $J_{4,5}$ = 3, $J_{5,6}$ = 6, $J_{5,6'}$ = 6.8 Hz, 1H, H-5), 3.62 (dd, $J_{5,6}$ = 6, $J_{6,6'}$ = 11.6 Hz, 1H, H-6), 3.57 (dd, $J_{5,6'}$ = 6.8, $J_{6,6'}$ = 11.6 Hz, 1H, H-6'), 2.29 (t, J = 7.6 Hz, 2H, CH_2), 1.64-1.56 (m, 2H, CH_2), 1.43-1.32 (m, 2H, CH_2), 0.95 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 176.7, 84.4 (C-4), 82.2 (C-1), 78.5 (C-3), 77.4 (C-2), 73 (C-5), 64.3 (C-6), 37 (CH_2), 28.9 (CH_2), 23.5 (CH_2), 14.3 (CH_3). $[\alpha]^D_{25}$ = +18.1 (c 1, MeOH). FT-ICR (ESI) calcd. for $C_{11}H_{21}N_1O_6$ $[M+Na]^+$ 286.12611; found 286.12597.



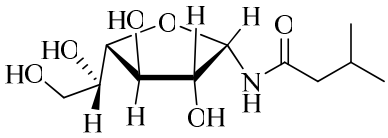
2,3,5,6-tetra-*O*-acetyl-*N*-pentanoyl-α-D-galactofuranosyl amide (114b)



^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 6.14 (d, $J_{NH,1}$ = 10 Hz, 1H, NH), 6.06 (d, $J_{1,2}$ = 4.4 Hz, $J_{NH,1}$ = 10, 1H, H-1), 5.34 (ddd, $J_{4,5}$ = 6.8, $J_{5,6}$ = 3.6, $J_{5,6'}$ = 6.8 Hz, 1H, H-5), 5.21 (dd, $J_{1,2}$ = 4.4, $J_{2,3}$ = 1.8 Hz, 1H, H-2),5.07 (dd, $J_{2,3}$ = 1.8, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 4.38 (dd, $J_{5,6}$ = 3.6, $J_{6,6'}$ = 12 Hz, 1H, H-6), 4.09 (dd, $J_{5,6'}$ = 6.8, $J_{6,6'}$ = 12 Hz, 1H, H-6'), 3.92 (dd, $J_{3,4}$ = 3.2, $J_{4,5}$ = 6.8 Hz, 1H, H-4), 2.23 (t, J = 7.6 Hz, 2H, CH_2), 2.18 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.61-1.56 (m, 2H, CH_2), 1.41-1.32 (m, 2H, CH_2), 0.92 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 176.7, 173, 170.7, 169.8, 169.1, 79.9 (C-1), 79.7 (C-4), 76.7 (C-3), 75.1 (C-2), 70.3 (C-5), 62.9 (C-6), 36.7 (CH_2), 29.9 (CH_2), 22.5 (CH_2), 21.1-20.8 (4xOAc), 14.3 (CH_3). NOESY (400 MHz, $CDCl_3$, 25°C): contact between H-1/H-4.

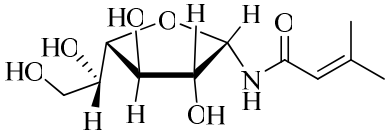


***N*-(3-Methylbutanoyl)-α-D-galactofuranosyl amide (111c)**



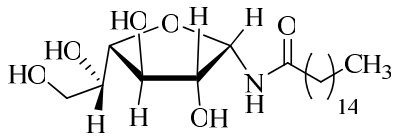
The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20) yield = 57 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.72 (d, $J_{1,2}$ = 4.4 Hz, 1H, H-1), 4.16 (t, $J_{2,3}$ = 3.2, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 3.92 (dd, $J_{1,2}$ = 4.4, $J_{2,3}$ = 3.2 Hz, 1H, H-2), 3.84 (dd, $J_{3,4}$ = 3.6, $J_{4,5}$ = 3.2 Hz, 1H, H-4), 3.78 (ddd, $J_{4,5}$ = 3.2, $J_{5,6}$ = 5.6, $J_{5,6'}$ = 6.8 Hz, 1H, H-5), 3.65 (dd, $J_{5,6}$ = 5.6, $J_{6,6'}$ = 11.2 Hz, 1H, H-6), 3.61 (dd, $J_{5,6'}$ = 6.8, $J_{6,6'}$ = 11.2 Hz, 1H, H-6'), 2.18 (d, J = 6.4 Hz, 2H, CH_2), 2.16-2.08 (m, 1H, CH), 1.01 (d, J = 2.4 Hz, 3H, CH_3), 0.99 (d, J = 2.4 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 176, 85.6 (C-4), 84.5 (C-1), 82.2 (C-3), 77.4 (C-2), 73 (C-5), 64.3 (C-6), 46.5 (CH_2), 27.3 (CH), 22.9 (CH_3). $[\alpha]^D_{25}$ = +33.4 (c 1, MeOH). FT-ICR (ESI) calcd. for $C_{11}H_{21}N_1O_6$ $[M+Na]^+$ 286.12611; found 286.12591.

***N*-3-methyl-2-butenoyl-α-D-galactofuranosyl amide (111e)**



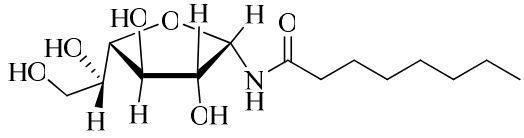
The compound was purified by flash chromatography ($CHCl_3$:MeOH 85:15) yield = 51 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.86 (s, 1H, CH), 5.77 (d, $J_{1,2}$ = 4 Hz, 1H, H-1), 4.17 (t, $J_{2,3}$ = 3.2, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 3.94 (dd, $J_{1,2}$ = 4, $J_{2,3}$ = 3.2 Hz, 1H, H-2), 3.85 (bt, $J_{3,4}$ = 3.6, $J_{4,5}$ = 3.2 Hz, 1H, H-4), 3.79 (ddd, $J_{4,5}$ = 3.2, $J_{5,6}$ = 6, $J_{5,6'}$ = 6.8 Hz, 1H, H-5), 3.68 (dd, $J_{5,6}$ = 6, $J_{6,6'}$ = 11.2 Hz, 1H, H-6), 3.62 (dd, $J_{5,6'}$ = 6.8, $J_{6,6'}$ = 11.2 Hz, 1H, H-6'), 2.20 (s, 3H, CH_3), 1.94 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 169.5, 154.2, 119.4 (CH), 84.4 (C-4), 82.1 (C-1), 78.7 (C-3), 77.5 (C-2), 73.1 (C-5), 64.4 (C-6), 27.4 (CH_3), 20.3 (CH_3). $[\alpha]^D_{25}$ = +30.2 (c 0.8, MeOH). FT-ICR (ESI) calcd. for $C_{11}H_{21}N_1O_6$ $[M+Na]^+$ 284.11046; found 284.11044.

_N_-palmitoyl-α-D-galactofuranosyl amide (111f)



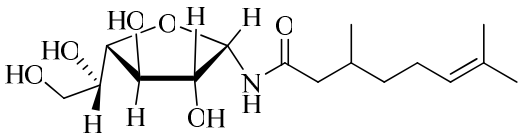
The compound was purified by flash chromatography ($CHCl_3$:MeOH 90:10) yield = 55 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.74 (d, $J_{1,2}$ = 4 Hz, 1H, H-1), 4.18 (t, $J_{2,3}$ = 3.2, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 3.94 (dd, $J_{1,2}$ = 4, $J_{2,3}$ = 3.2 Hz, 1H, H-2), 3.85 (bt, $J_{3,4}$ = 3.6, $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.79 (ddd, $J_{4,5}$ = 2.8, $J_{5,6}$ = 6, $J_{5,6'}$ = 6.8 Hz, 1H, H-5), 3.67 (dd, $J_{5,6}$ = 6, $J_{6,6'}$ = 11.2 Hz, 1H, H-6), 3.63 (dd, $J_{5,6'}$ = 6.8, $J_{6,6'}$ = 11.2 Hz, 1H, H-6'), 2.33 (t, J = 7.2 Hz, 2H, CH_2), 1.68 (bt, J = 7.2 Hz, 2H, CH_2), 1.44-1.28 (m, 24H), 0.96 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 176.7, 84.4 (C-4), 82.2 (C-1), 78.5 (C-3), 77.4 (C-2), 73 (C-5), 64.4 (C-6), 37.3, 33.2, 30.9, 30.8, 30.6, 30.4, 26.8, 23.9, 14.6 (CH_3). $[\alpha]^D_{25}$ = +9 (c 1, MeOH). FT-ICR (ESI) calcd. for $C_{22}H_{43}N_1O_6$ $[M+Na]^+$ 440.29826; found 440.29797.

_N_-capryloyl-α-D-galactofuranosyl amide (111g)



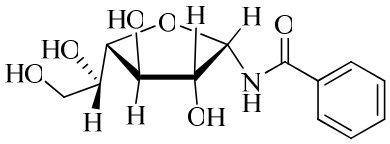
The compound was purified by flash chromatography ($CHCl_3$:MeOH 85:15) yield = 63 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.68 (d, $J_{1,2}$ = 4.4 Hz, 1H, H-1), 4.12 (t, $J_{2,3}$ = 3.6, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 3.87 (dd, $J_{1,2}$ = 4.4, $J_{2,3}$ = 3.6 Hz, 1H, H-2), 3.79 (t, $J_{3,4}$ = 3.2, $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.73 (ddd, $J_{4,5}$ = 2.8, $J_{5,6}$ = 6, $J_{5,6'}$ = 6.8 Hz, 1H, H-5), 3.61 (dd, $J_{5,6}$ = 6, $J_{6,6'}$ = 11.2 Hz, 1H, H-6), 3.57 (dd, $J_{5,6'}$ = 6.8, $J_{6,6'}$ = 11.2 Hz, 1H, H-6'), 2.27 (t, J = 7.2 Hz, 2H, CH_2), 1.62 (bt, J = 7.2 Hz, 2H, CH_2), 1.40-1.24 (m, 8H, 4xCH_2), 0.91 (t, J = 6.4, J = 7.2 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 176.7, 84.4 (C-4), 82.2 (C-1), 78.5 (C-3), 77.3 (C-2), 72.9 (C-5), 64.3 (C-6), 37.3 (CH_2), 33.0, 30.4, 30.3 (3xCH_2), 26.8 (CH_2), 23.8 (1xCH_2), 14.6 (CH_3). $[\alpha]^D_{25}$ = +12.2 (c 1, MeOH). FT-ICR (ESI) calcd. for $C_{14}H_{27}N_1O_6$ $[M+Na]^+$ 328.17306; found 328.17314.

_N_-citronelloyl-α-D-galactofuranosyl amide (111h)



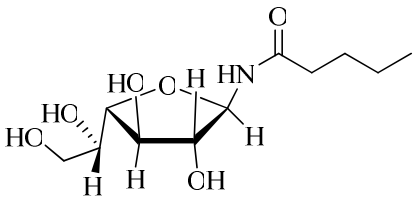
The compound was purified by flash chromatography ($CHCl_3$:MeOH 90:10) yield = 56 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.74 (d, $J_{1,2}$ = 4 Hz, 1H, H-1), 5.17 (t, J = 5.8 Hz, 1H, CH) 4.18 (t, $J_{2,3}$ = 3.4, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 3.93 (dd, $J_{1,2}$ = 4, $J_{2,3}$ = 3.4 Hz, 1H, H-2), 3.85 (dd, $J_{3,4}$ = 3.6, $J_{4,5}$ = 3.2 Hz, 1H, H-4), 3.79 (ddd, $J_{4,5}$ = 3.2, $J_{5,6}$ = 5.6, $J_{5,6'}$ = 6.8 Hz, 1H, H-5), 3.68 (dd, $J_{5,6}$ = 5.6, $J_{6,6'}$ = 11.2 Hz, 1H, H-6), 3.63 (dd, $J_{5,6'}$ = 6.8, $J_{6,6'}$ = 11.2 Hz, 1H, H-6'), 2.34 (dd, J = 13.6, J = 6 Hz, 1H, Ha, CH_2), 2.18-1.98 (m, 4H), 1.73 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.48-1.40 (m, 1H), 1.33-1.22 (m, 1H), 0.95 (d, J = 6.8 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 175.9, 132.4, 125.7 (CH), 84.5 (C-4), 82.3 (C-1), 78.5 (C-3), 77.4 (C-2), 73 (C-5), 64.4 (C-6), 44.9, 38.1, 31.7, 26.6, 26, 19.9, 17.9. $[\alpha]^D_{25}$ = +13.2 (c 1, MeOH). FT-ICR (ESI) calcd. for $C_{16}H_{29}N_1O_6$ $[M+Na]^+$ 354.18871; found 354.18864.

_N_-benzoyl-α-D-galactofuranosyl amide (111i)



The compound was purified by flash chromatography ($CHCl_3$:MeOH 85:15) yield = 62 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 7.91 (m, 2H, H_{orto}), 7.60 (m, 1H, H_{para}), 7.53 (m, 2H, H_{meta}), 5.94 (d, $J_{1,2}$ = 4.8 Hz, 1H, H-1), 4.27 (t, $J_{2,3}$ = 4.2, $J_{3,4}$ = 4.4 Hz, 1H, H-3), 4.13 (t, $J_{1,2}$ = 4.8, $J_{2,3}$ = 4.2 Hz, 1H, H-2), 3.93 (dd, $J_{3,4}$ = 3.6, $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.84 (ddd, $J_{4,5}$ = 2.8, $J_{5,6}$ = 5.4, $J_{5,6'}$ = 6.8 Hz, 1H, H-5), 3.70 (dd, $J_{5,6}$ = 5.6, $J_{6,6'}$ = 11.2 Hz, 1H, H-6), 3.66 (dd, $J_{5,6'}$ = 6.8, $J_{6,6'}$ = 11.2 Hz, 1H, H-6'). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 169.8, 135.4, 133.2, 129.8, 128.6, 84.5 (C-4), 82.7 (C-1), 78.2 (C-3), 77.9 (C-2), 72.7 (C-5), 64.4 (C-6). $[\alpha]^D_{25}$ = +7.9 (c 1, MeOH). FT-ICR (ESI) calcd. for $C_{13}H_{17}N_1O_6$ $[M+Na]^+$ 306.09481; found 306.09465.

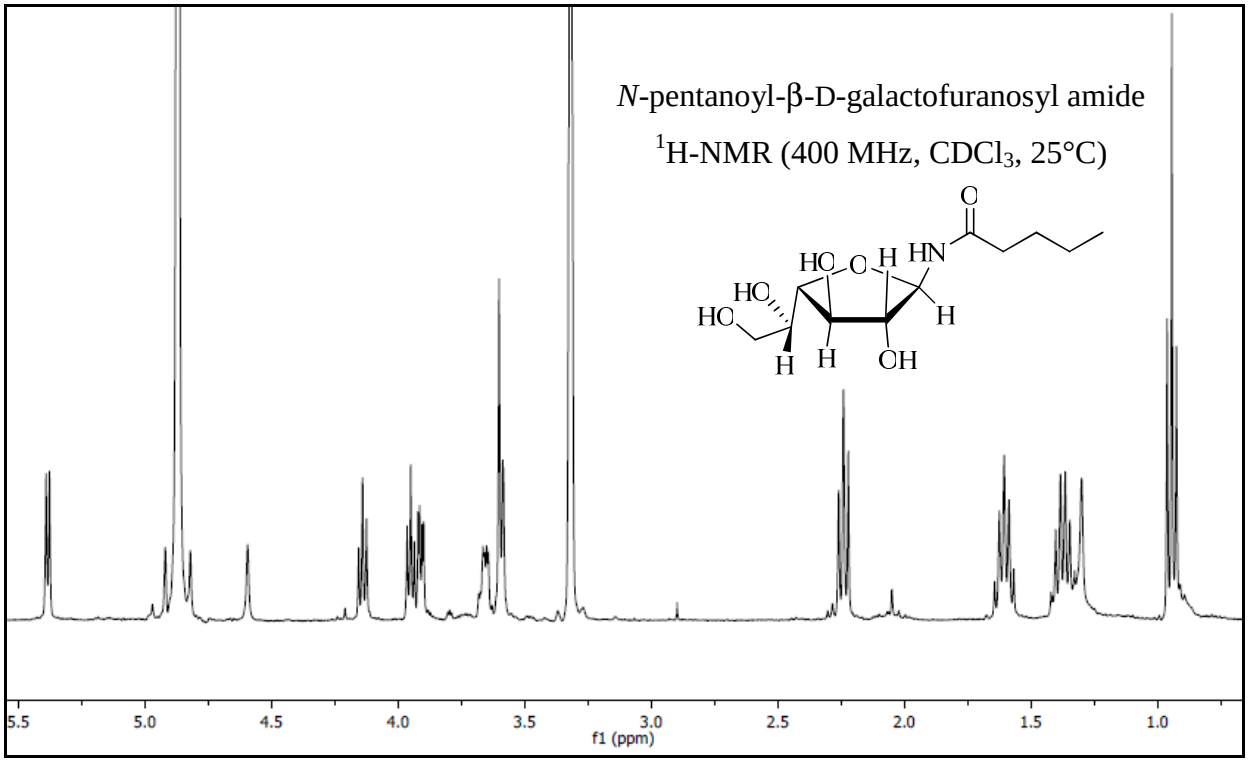
_N_-pentanoyl-β-D-galactofuranosyl amide (113b)



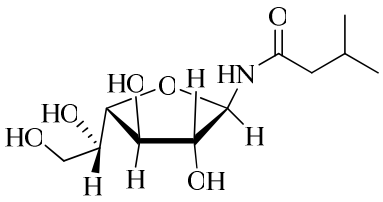
After Zemplen deacetylation the compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20) yield = 60 %.

^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.38 (d, $J_{1,2}$ = 5.2 Hz, 1H, H-1), 4.14 (t, $J_{2,3}$ = 5.8, $J_{3,4}$ = 6.4 Hz, 1H, H-3), 3.95 (t, $J_{1,2}$ = 5.2, $J_{2,3}$ = 5.8 Hz, 1H, H-2), 3.90 (dd, $J_{3,4}$ = 6.4, $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.68-3.63 (m,1H, H-5), 3.62-3.57 (m, 2H, H-6, H-6'), 2.22 (t, J = 7.4 Hz ,2H, CH_2), 1.71-1.54 (m, 2H, CH_2), 1.48-1.32 (m, 2H, CH_2), 0.95 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 177.4, 85.5 (C-1), 83.6 (C-4), 81.1 (C-2), 77.5 (C-3), 72.7 (C-5), 64.4 (C-6), 37.1 (CH_2), 28.9 (CH_2), 23.5 (CH_2), 14.3 (CH_3). NOESY (400 MHz, CD_3OD, 25°C): contact between H-1/H-3.

$[\alpha]^D_{25}$ = -17.9 (c 0.2, MeOH). FT-ICR (ESI) calcd. for $C_{11}H_{21}N_1O_6$ $[M+Na]^+$ 286.12611; found 286.12586.



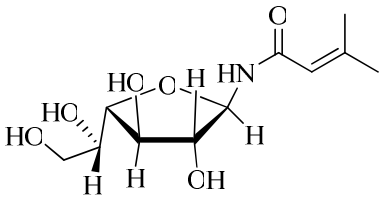
***N*-(3-Methylbutanoyl)-β-D-galactofuranosyl amide (113c)**



After Zemplen deacetylation the compound was purified by flash chromatography ($CHCl_3$:MeOH 85:15) yield = 53 %.

^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.40 (d, $J_{1,2}$ = 5.2 Hz, 1H, H-1), 4.15 (t, $J_{2,3}$ = 5.8, $J_{3,4}$ = 6.4 Hz, 1H, H-3), 3.97 (t, $J_{1,2}$ = 5.2, $J_{2,3}$ = 5.8 Hz, 1H, H-2), 3.92 (dd, $J_{3,4}$ = 6.4, $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.71-3.65 (m,1H, H-5), 3.63-3.58 (m, 2H, H-6, H-6'), 2.35 (d, J = 7.2 Hz, 2H, CH_2), 1.01 (d, J = 6.4 Hz , 3H, CH_3), 0.97 (d, J = 6 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 176.6, 85.6 (C-1), 83.7 (C-4), 81.2 (C-2), 77.5 (C-3), 72.7 (C-5), 64.4 (C-6), 46.5 (CH_2), 22.9 (CH_3), 22.7 (CH_3). $[\alpha]^D_{25}$ = -24.5 (c 1, MeOH). FT-ICR (ESI) calcd. for $C_{11}H_{21}N_1O_6$ $[M+Na]^+$ 286.12611; found 286.12584.

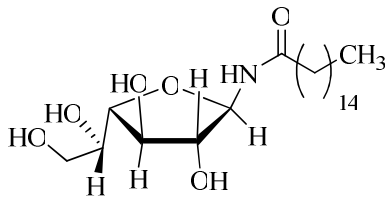
***N*-3-methyl-2-butenoyl-β-D-galactofuranosyl amide (113e).**



After Zemplen deacetylation the compound was purified by flash chromatography ($CHCl_3$:MeOH 85:15) yield = 48 %.

^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.74 (s, 1H, CH), 5.41 (d, $J_{1,2}$ = 5.6 Hz, 1H, H-1), 4.15 (t, $J_{2,3}$ = 5.8, $J_{3,4}$ = 6.4 Hz, 1H, H-3), 3.96 (t, $J_{1,2}$ = 5.6, $J_{2,3}$ = 5.8 Hz, 1H, H-2), 3.90 (dd, $J_{3,4}$ = 6.4, $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.68-3.63 (m,1H, H-5), 3.61-3.57 (m, 2H, H-6, H-6'), 2.15 (s, 3H, CH_3), 1.89 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 170.2, 154.3, 119.3 (CH), 85.4 (C-1), 83.4 (C-4), 81.1 (C-2), 77.5 (C-3), 72.8 (C-5), 64.4 (C-6), 27.5 (CH_3), 20.3 (CH_3). $[\alpha]^D_{25}$ = -27.8 (c 0.2, MeOH). FT-ICR (ESI) calcd. for $C_{11}H_{21}N_1O_6$ $[M+Na]^+$ 284.11046; found 284.11027.

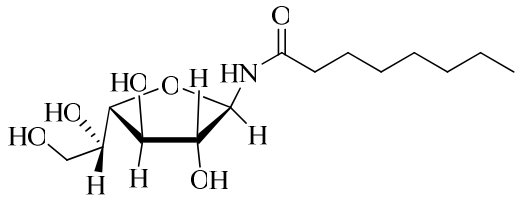
_N_-palmitoyl-β-D-galactofuranosyl amide (113f).



After Zemplen deacetylation the compound was purified by flash chromatography ($CHCl_3$:MeOH 90:10) yield = 60 %.

^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.40 (d, $J_{1,2}$ = 5.2 Hz, 1H, H-1), 4.15 (t, $J_{2,3}$ = 6, $J_{3,4}$ = 6 Hz, 1H, H-3), 3.96 (t, $J_{1,2}$ = 5.2, $J_{2,3}$ = 5.6 Hz, 1H, H-2), 3.93 (dd, $J_{3,4}$ = 6.4, $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.70-3.65 (m, 1H, H-5), 3.63-3.58 (m, 2H, H-6, H-6'), 2.24 (t, J = 8 Hz, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.41-1.25 (m, 24 H, 12x CH_2), 0.92 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 177.1, 85.7 (C-1), 83.8 (C-4), 81.2 (C-2), 77.6 (C-3), 72.8 (C-5), 64.4 (C-6), 37.4, 33.2, 30.9, 30.7, 30.6, 30.4, 26.8, 23.8, 14.6 (CH_3). $[\alpha]^D_{25}$ = -37.3 (c 1, MeOH). FT-ICR (ESI) calcd. for $C_{22}H_{43}N_1O_6$ $[M+Na]^+$ 440.29826; found 440.29844.

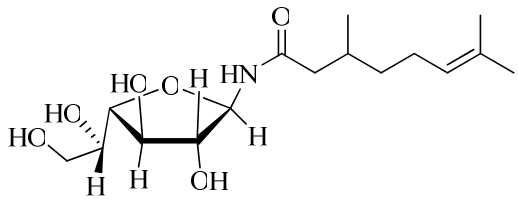
_N_-capryloyl-β-D-galactofuranosyl amide (113g)



After Zemplen deacetylation the compound was purified by flash chromatography ($CHCl_3$:MeOH 85:15) yield = 56 %.

^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.42 (d, $J_{1,2}$ = 5.2 Hz, 1H, H-1), 4.17 (t, $J_{2,3}$ = 5.8, $J_{3,4}$ = 6.4 Hz, 1H, H-3), 3.98 (t, $J_{1,2}$ = 5.2, $J_{2,3}$ = 5.8 Hz, 1H, H-2), 3.95 (dd, $J_{3,4}$ = 6.4, $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.73-3.68 (m, 1H, H-5), 3.65-3.59 (m, 2H, H-6, H-6'), 2.28 (t, J = 7.2, J = 8 Hz, 2H, CH_2), 1.65 (m, 2H, CH_2), 1.47-1.27 (m, 8H, 4xCH_2), 0.94 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 177.1, 85.4 (C-1), 83.6 (C-4), 81 (C-2), 77.3 (C-3), 72.6 (C-5), 64.2 (C-6), 37.2, 32.8, 30.2, 30.1, 26.7, 23.6, 14.4 (CH_3). $[\alpha]^D_{25}$ = -50.4 (c 0.9, MeOH). FT-ICR (ESI) calcd. for $C_{14}H_{27}N_1O_6$ $[M+Na]^+$ 328.17306; found 328.17317.

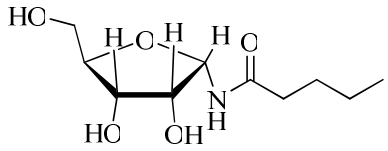
_N_-citronelloyl-β-D-galactofuranosyl amide (113h)



After Zemplen deacetylation the compound was purified by flash chromatography ($CHCl_3$:MeOH 90:10) yield = 51 %.

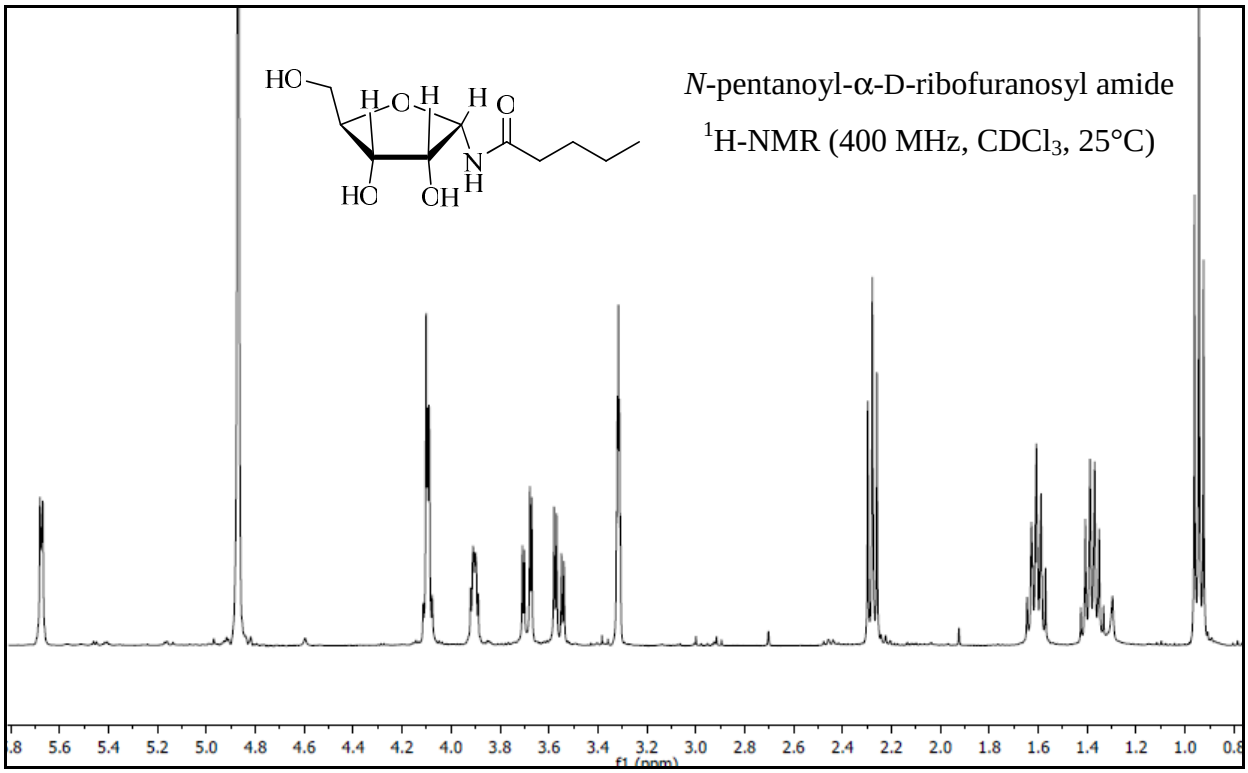
^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.45 (d, $J_{1,2}$ = 5.2 Hz, 1H, H-1), 5.16 (t, J = 6.8 Hz, 1H, CH) 4.20 (t, $J_{2,3}$ = 5.6, $J_{3,4}$ = 6.2 Hz, 1H, H-3), 4.01 (t, $J_{1,2}$ = 5.2, $J_{2,3}$ = 5.6 Hz, 1H, H-2), 3.97 (dd, $J_{3,4}$ = 6.2, $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.71 (ddd, $J_{4,5}$ = 2.8, J = 6, J = 6.8 Hz, 1H, H-5), 3.68-3.63 (m, 2H, H-6, H-6'), 2.29 (dd, J = 13.6, J = 6 Hz, 1H, Ha, CH_2), 2.18-1.98 (m, 4H), 1.73 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.48-1.40 (m, 1H), 1.33-1.22 (m, 1H), 1.01 (d, J = 6.4 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ =176.6, 132.4, 125.7, 85.7 (C-1), 83.8 (C-4), 81.2 (C-2), 77.6 (C-3), 72.8 (C-5), 64.4 (C-6), 44.9, 38.1, 30.9, 26.6, 26, 19.9, 17.9. $[\alpha]^D_{25}$ = -55.8 (c 0.9, MeOH). FT-ICR (ESI) calcd. for $C_{16}H_{29}N_1O_6$ $[M+Na]^+$ 354.18871; found 354.18866.

_N_-pentanoyl-α-D-ribofuranosyl amide (115b)

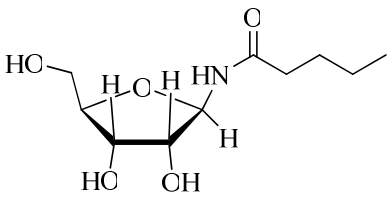


The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20) yield = 59 %.

^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.67 (d, $J_{1,2}$ = 4.8 Hz, 1H, H-1), 4.12-4.06 (m, 2H, H-2, H-3), 3.93-3.87 (q, $J_{4,5}$ = 3.2 Hz, $J_{4,5'}$= 4.4 Hz, 1H, H-4), 3.69 (dd, $J_{4,5}$ = 3.2 Hz, $J_{5,5'}$= 12 Hz, 1H, H-5), 3.55 (dd, $J_{4,5'}$ = 4.4 Hz, $J_{5,5'}$= 12 Hz, 1H, H-5') 2.24 (t, J = 7.2 Hz ,2H, CH_2), 1.65-1.57 (m, 2H, CH_2), 1.43-1.33 (m, 2H, CH_2), 0.95 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 176.6, 84.2 (C-4), 81.5 (C-1), 72.6, 71.8, 62.9 (C-5), 36.9 (CH_2), 28.8 (CH_2), 23.3 (CH_2), 14.1 (CH_3).

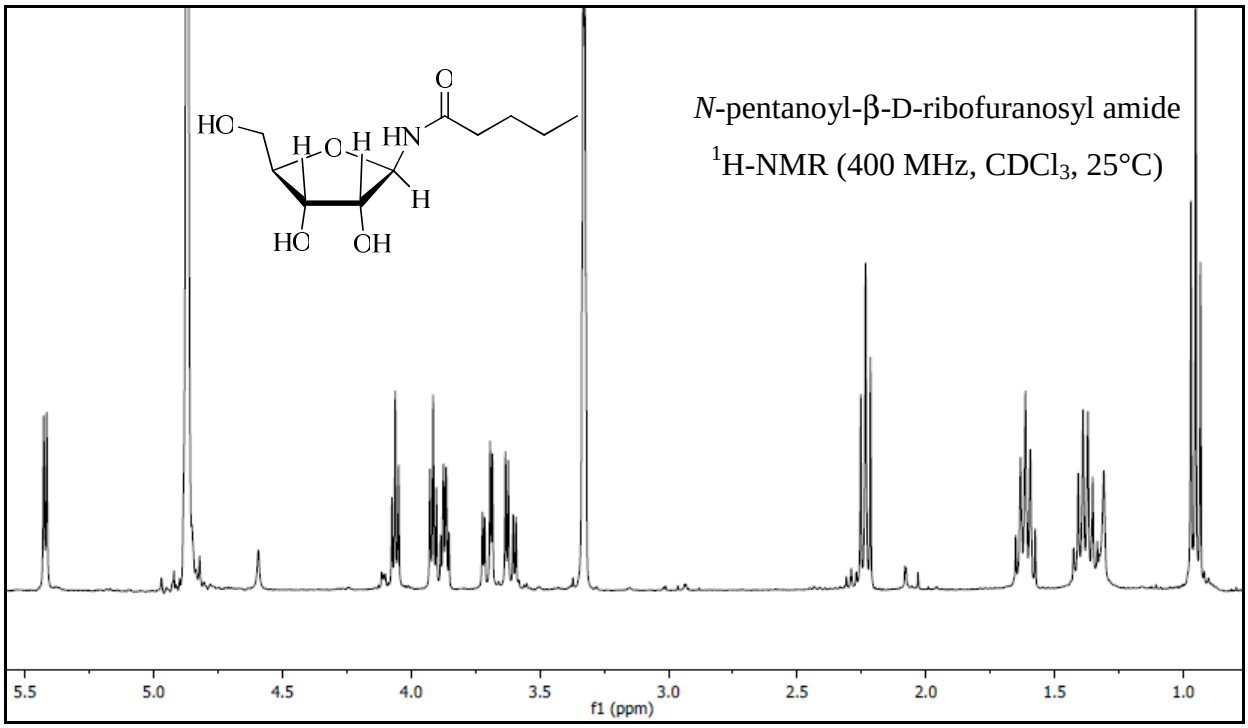


_N_-pentanoyl-β-D-ribofuranosyl amide (116b)

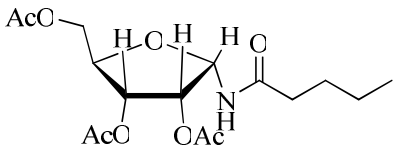


After Zemplen deacetylation the compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20) yield = 55 %.

^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.38 (d, $J_{1,2}$ = 4.8 Hz, 1H, H-1), 4.06 (t, $J_{2,3}$ = 5.2, $J_{3,4}$ = 5.2 Hz, 1H, H-3), 3.91 (t, $J_{1,2}$ = 4.8, $J_{2,3}$ = 5.2 Hz, 1H, H-2), 3.88-3.83 (q, $J_{4,5}$ = 3.6 Hz, $J_{4,5'}$= 4.4 Hz, 1H, H-4), 3.70 (dd, $J_{4,5}$ = 3.6 Hz, $J_{5,5'}$= 12 Hz, 1H, H-5), 3.61 (dd, $J_{4,5'}$ = 4.4 Hz, $J_{5,5'}$= 12 Hz, 1H, H-5') 2.23 (t, J = 7.2 Hz ,2H, CH_2), 1.65-1.57 (m, 2H, CH_2), 1.43-1.33 (m, 2H, CH_2), 0.95 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 176.8, 85.8 (C-1), 85.1 (C-4), 75.9 (C-2), 71.9 (C-3), 63.3 (C-5), 37.1 (CH_2), 28.9 (CH_2), 23.5 (CH_2), 14.3 (CH_3). NOESY (400 MHz, CD_3OD, 25°C): contact between H-1/H-4.

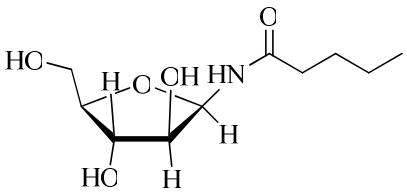


Tetra-*O*-acetyl *N*-pentanoyl-β-D-ribofuranosyl amide (117b)

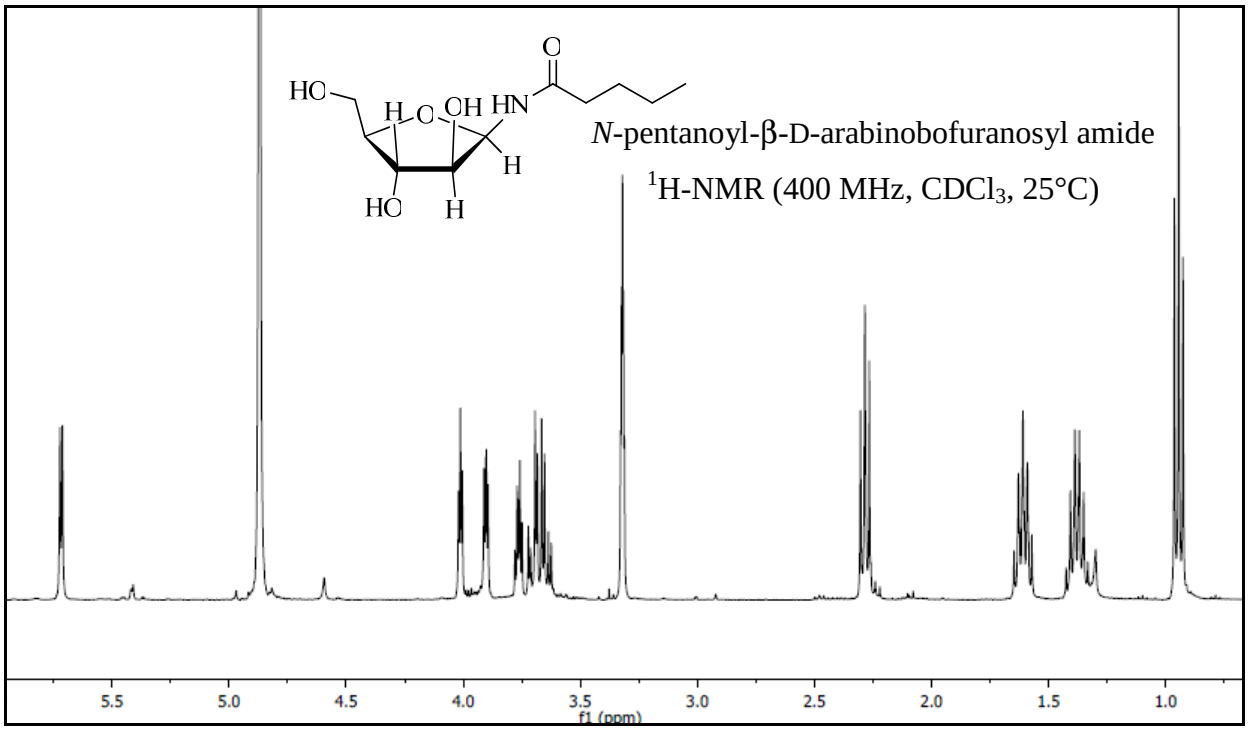


^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 6.06 (d, $J_{1,2}$ = 4.8 Hz, 1H, H-1), 5.45 (t, $J_{1,2}$ = 4.8, $J_{2,3}$ = 5.6 Hz, 1H, H-2), 5.36 (t, $J_{2,3}$ = 5.6 Hz, 1H, H-3), 4.36-4.31 (m, 2H, H-4, H-5), 4.21 (dd, $J_{4,5'}$ = 4.8, $J_{5,5'}$= 12 Hz, 1H, H-5') 2.23 (t, J = 7.2 Hz ,2H, CH_2), 2.19 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.12 (s, 3H, OAc), 1.67-1.59 (m, 2H, CH_2), 1.44-1.35 (m, 2H, CH_2), 0.99 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 176.1, 172.2, 171.5, 171.3, 80.8 (C-1), 79.2 (C-4), 72.9 (C-3), 71.9 (C-2), 64.8 (C-5), 36.8 (CH_2), 29 (CH_2), 23.4 (CH_2),20.7-20.5 (3xOAc), 14.2 (CH_3). NOESY (400 MHz, CD_3OD, 25°C): contact between H-1/H-3 and H-1/H-5.

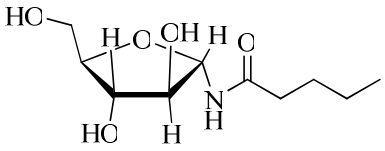
_N_-pentanoyl-β-D-arabinobofuranosyl amide (118b)



The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20) yield = 57 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.72 (d, $J_{1,2}$ = 4.4 Hz, 1H, H-1), 4.01 (t, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$= 3.6 Hz, 1H, H-3), 3.90 (dd, $J_{1,2}$= 4.4 Hz, $J_{2,3}$ = 3.6 Hz, 1H, H-2), 3.76 (ddd, $J_{3,4}$= 3.6 Hz, $J_{4,5}$ = 3.6 Hz, $J_{4,5'}$= 5.2 Hz, 1H, H-4), 3.70 (dd, $J_{4,5}$ = 3.6 Hz, $J_{5,5'}$= 11.6 Hz, 1H, H-5), 3.64 (dd, $J_{4,5'}$ = 5.2 Hz, $J_{5,5'}$= 11.6 Hz, 1H, H-5') 2.28 (t, J = 7.6 Hz ,2H, CH_2), 1.65-1.57 (m, 2H, CH_2), 1.43-1.33 (m, 2H, CH_2), 0.94 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 176.8, 84.4 (C-4), 82.2 (C-1), 78.3 (C-3), 77.3 (C-2), 63.5 (C-5), 36.9 (CH_2), 28.9 (CH_2), 23.5 (CH_2), 14.3 (CH_3). NOESY (400 MHz, CD_3OD, 25°C): contact between H-1/H-4.

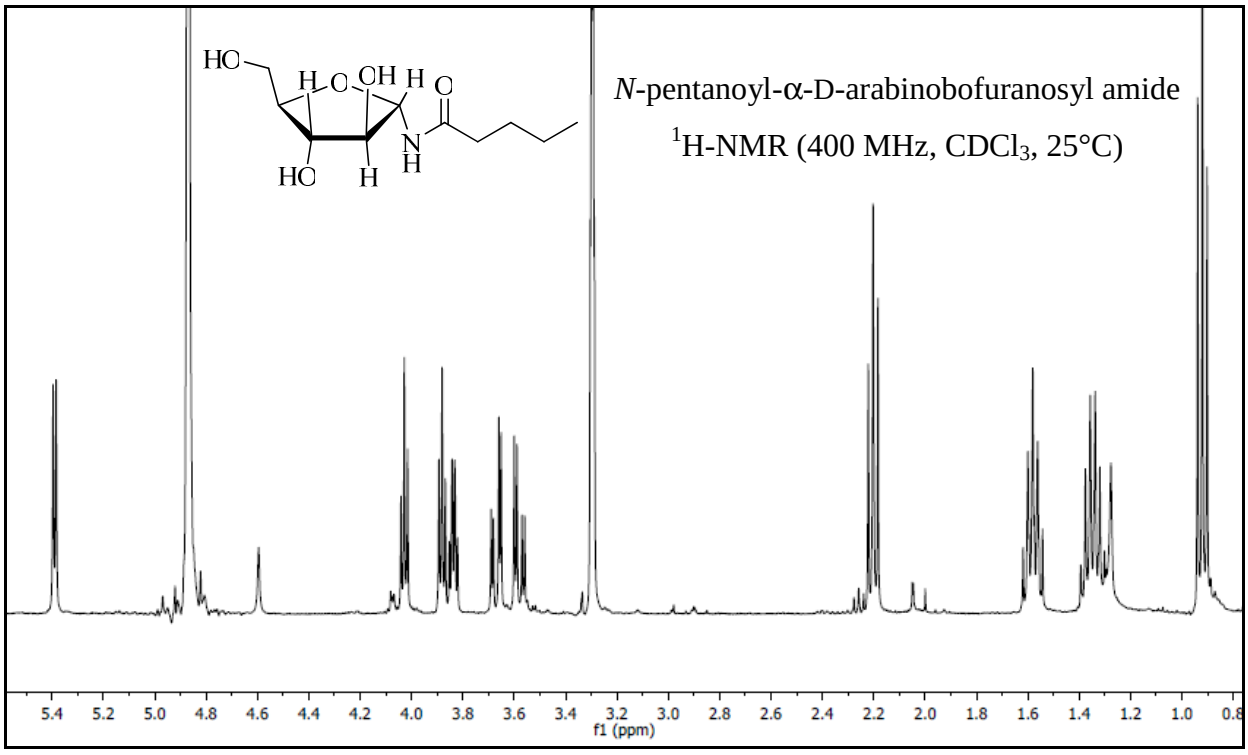


_N_-pentanoyl-α-D-arabinobofuranosyl amide (119b)

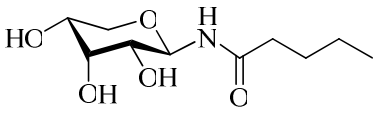


After Zemplen deacetylation the compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20) yield = 53 %.

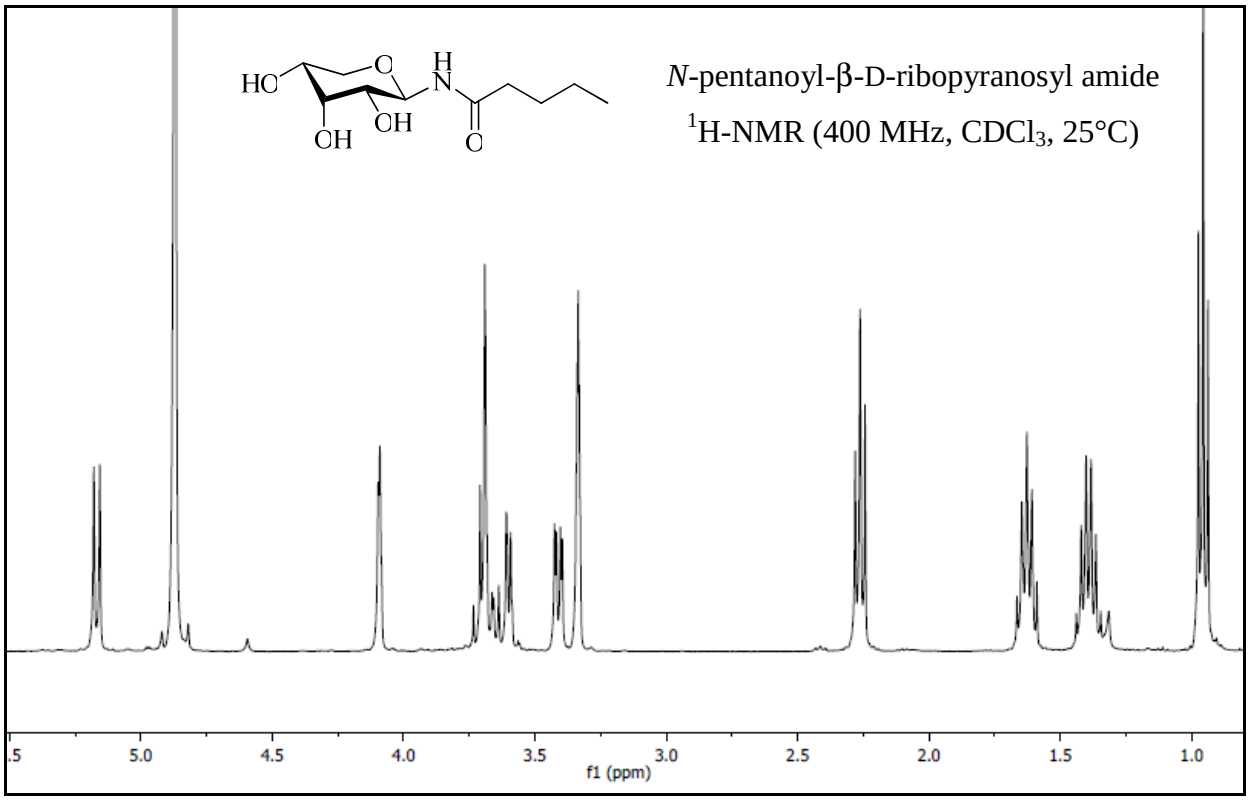
^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.39 (d, $J_{1,2}$ = 4.8 Hz, 1H, H-1), 4.03 (t, $J_{2,3}$ = 5.2 Hz, 1H, H-3), 3.88 (t, $J_{1,2}$ = 4.8, $J_{2,3}$ = 5.2 Hz, 1H, H-2), 3.84 (q, $J_{4,5}$ = 3.6 Hz, $J_{4,5'}$= 4.4 Hz, 1H, H-4), 3.67 (dd, $J_{4,5}$ = 3.6, $J_{5,5'}$= 12 Hz, 1H, H-5), 3.55 (dd, $J_{4,5'}$ = 4.4 Hz, $J_{5,5'}$= 12 Hz, 1H, H-5') 2.20 (t, J = 7.6 Hz ,2H, CH_2), 1.60-1.54 (m, 2H, CH_2), 1.40-1.29 (m, 2H, CH_2), 0.92 (t, J = 7.2 Hz, 3H, CH_3).



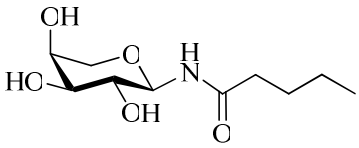
_N_-pentanoyl-β-D-ribopyranosyl amide (120b)



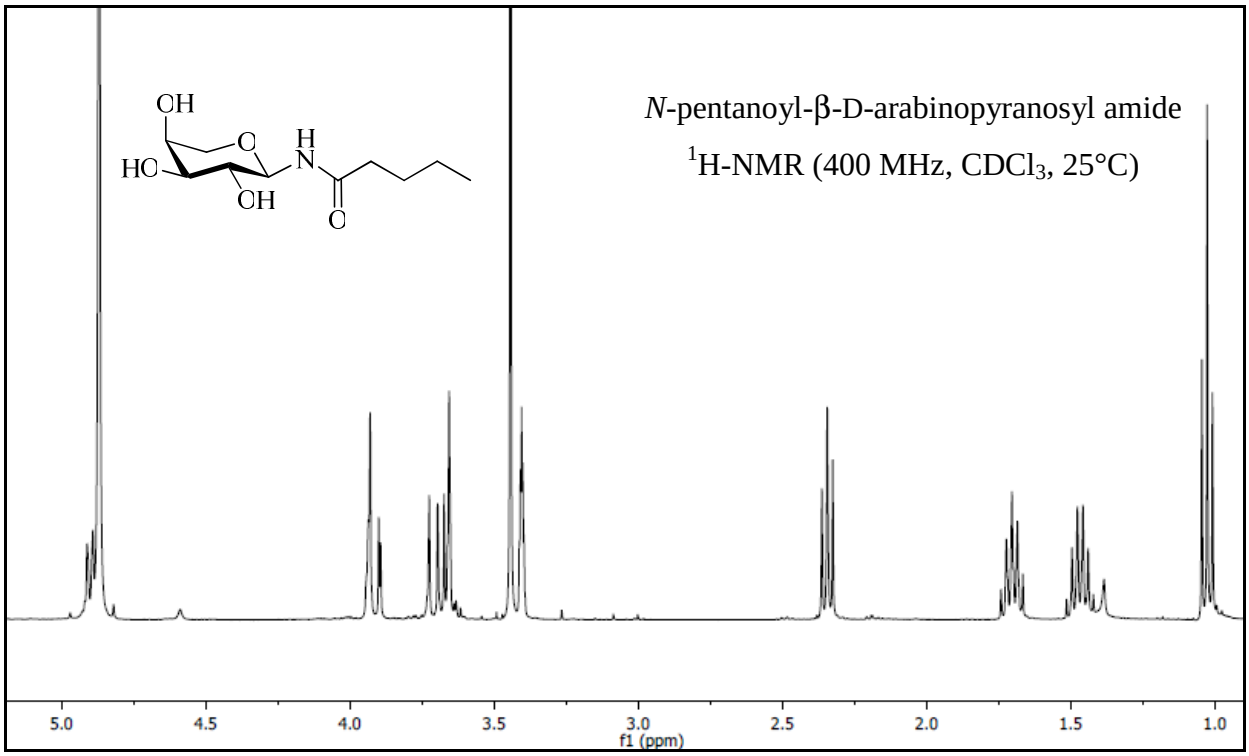
The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20) yield = 68 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.15 (d, $J_{1,2}$ = 9.2 Hz, 1H, H-1), 4.07 (d, $J_{2,3}$ = 2.8 Hz, 1H, H-3), 3.73-3.57 (m, 3H, H-4, H-5, H-5'), 3.39 (dd, $J_{1,2}$ = 9.2, $J_{2,3}$ = 2.8 Hz, 1H, H-2), 2.22 (t, J = 7.6 Hz ,2H, CH_2), 1.64-1.56 (m, 2H, CH_2), 1.43-1.32 (m, 2H, CH_2), 0.94 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 177.5, 78 (C-1), 72.4 (C-3), 71.3 (C-2), 68.8 (C-4), 65.7 (C-5), 37 (CH_2), 28.9 (CH_2), 23.5 (CH_2), 14.3 (CH_3).



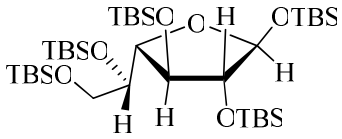
_N_-pentanoyl-β-D-arabinopyranosyl amide (121b)



The compound was purified by flash chromatography ($CHCl_3$:MeOH 80:20) yield = 70 %. ^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 4.90 (d, $J_{1,2}$ = 8 Hz, 1H, H-1), 3.95-3.88 (m, 2H, H-3, H-5), 3.73-3.66 (m, 3H, H-2, H-4, H-5'), 2.34 (t, J = 7.6 Hz ,2H, CH_2), 1.74-1.67 (m, 2H, CH_2), 1.53-1.42 (m, 2H, CH_2), 1.03 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ = 177.2, 81.8 (C-1), 75.3 (C-2), 71.4 (C-4), 70.3 (C-3), 68.9 (C-5), 37.1 (CH_2), 29 (CH_2), 23.5 (CH_2), 14.2 (CH_3).



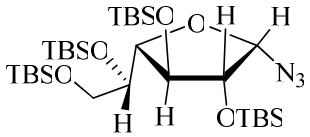
Synthesis of 1,2,3,5,6-penta-*O-tert*-butyldimethylsislyl-β-D-galactofuranose (126)



To a solution of D-galactose (0.5 g, 2.77 mmol) in dry DMF (14mL), imidazole (2.74 g, 18.25 mmol) and TBSCl (3 g, 20.08 mmol) were added, and the reaction mixture was stirred at room temperature. After 48h TLC (Hexane/AcOEt 10:1) monitoring showed total consumption of the starting material. The solution was poured into ice/water and was diluted with CH_2Cl_2. The organic layer was washed with HCl (5 %), water, $NaHCO_3$ (ss), and water, dried (Na_2SO_4), and concentrated. Addition of MeOH (5 mL) to the syrup afforded crystalline compound **126**.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 4.41 (d, J = 7.1 Hz, 1H, H-1), 4.00 (d, J = 2.3 Hz, 1H, H-4), 3.85 (dd, J = 10.0, 5.8, 2.9 Hz, 1H, H-6), 3.71 (dd, J = 10.0, 5.8 Hz, 1H, H-6'), 3.57 (m, 2H, H-2, H-3), 3.38 (ddd, J = 9.3, 5.4, 2.9 Hz, 1H, H-5), 0.93-0.87 (m, 45H, $SiC(CH_3)_3$), 0.14-0.04 (m, 30H, $Si(CH_3)_2$). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 98.7, 76.2, 75.2, 74.1, 69.2, 61.6, 26.3-18.0, -3.2 to -4.45.

Synthesis of 2,3,5,6-tetra-*O-tert*-butyldimethylsislyl-α-D-galactofuranosyl azide (128)



A solution of **126** (200 mg, 0.26 mmol) in anhydrous CH_2Cl_2 (10 mL) was cooled to 0°C and stirred for 10 min. under nitrogen. Then, iodotrimethylsilane (1.2 eq.,0.042 mL, 0.32 mmol) was added, and the solution was stirred at 0°C until TLC (Hexane/AcOEt 10:1) monitoring showed complete transformation of 129 in two products R_f = 0.70 and R_f = 0.54. In parallel a solution of tetrabutylammonium hydroxide was prepared by addition 280 μL of 10M sodium hydroxide solution (an excess) to 383.3 mg (0.2 mol) of tetrabutylammonium hydrogene sulphate in 560 μL of water.

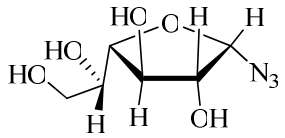
A solution of 147 mg (0.4 mol) of sodium azide in 280 μL of water was added and tetrabutylammonium azide extracted with 1 mL of dichloromethane. The organic layer, was separated off and the aqueous phase again extracted with 1 mL of dichloromethane. The

combined organic phases were evaporated *in vacuo* at 40°C to yield a crude tetrabutylammonium azide as a colourless oil. The solutions thus obtained were used directly in the following step.

Then $EtN(iPr)_2$ (0.054 mL, 0.032 mmol) and a solution of the azide were added, and the stirring was continued until consumption of the components of $R_f = 0.70$ and $R_f = 0.54$. The solution was diluted with CH_2Cl_2 washed with HCl 5 %, $NaHCO_3$ (ss) and water, dried (Na_2SO_4), and concentrated. The syrup obtained was purified by flash column chromatography (Hexane:AcOEt 98:2). Yield = 75 %

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.11 (d, $J_{1,2}$ = 4 Hz, 1H, H-1), 4.24 (t, $J_{2,3}$ = 4.4, $J_{3,4}$ = 4.6 Hz, 1H, H-3), 3.99 (t, $J_{1,2}$ = 4, $J_{2,3}$ = 4.4 Hz, 1H, H-2), 3.87 (t, $J_{3,4}$ = 4.6, $J_{4,5}$ = 4.4 Hz, 1H, H-4), 3.79 (ddd, $J_{4,5}$ = 4.4, $J_{5,6}$ = 6, $J_{5,6'}$ = 5.6 Hz, 1H, H-5), 3.66 (dd, $J_{5,6}$ = 6, $J_{6,6'}$ = 10 Hz, 1H, H-6), 3.60 (dd, $J_{5,6'}$ = 5.6, $J_{6,6'}$ = 10 Hz, 1H, H-6'), 0.92-0.84 (m, 36H, $SiC(CH_3)_3$), 0.18-0.06 (m, 24 H, $Si(CH_3)_2$). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 90.9 (C-1), 85.5 (C-4), 79.5 (C-2), 76.3 (C-3), 73 (C-5), 65 (C-6), 26.2, 26.1, 26, 25.9, - 0.25 to -0.5. NOESY (400 MHz, $CDCl_3$, 25°C): contact between H-1/H-4.

Synthesis of α-D-galactofuranosyl azide (46)

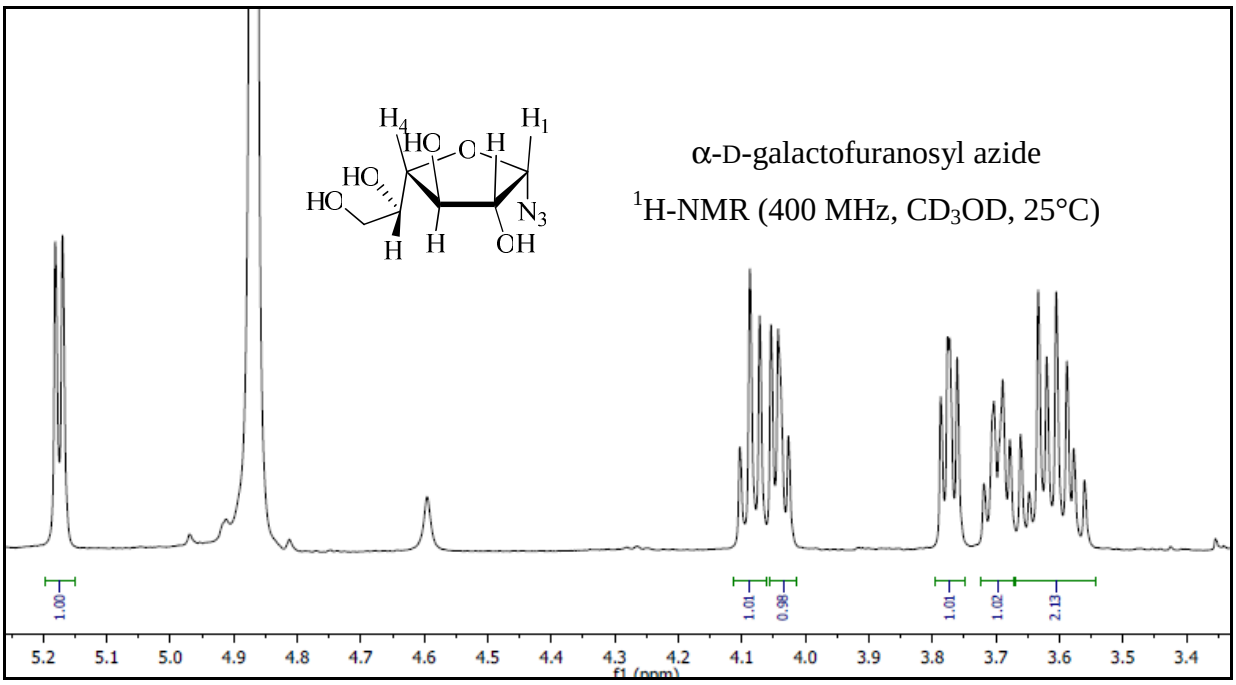


A solution of **128** (60 mg, 0.09 mmol, 1 eq.) in anhydrous THF dry (180 μL,) was cooled to 0°C and stirred for 10 min. under nitrogen. Then, tetrabutylammonium fluoride 1M in THF (157 μL, 0.54 mmol, 5 eq.) was added, and the solution was stirred at room temperature until TLC (Hexane/AcOEt 60:40 and $CHCl_3$/MeOH 80.20) monitoring showed complete transformation of **128** in **46**.

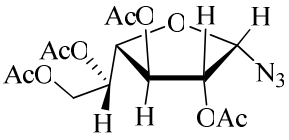
The solution was diluted with water washed with dichloromethane and AcOEt and then concentrated.The syrup obtained was purified by flash column chromatography ($CHCl_3$:MeOH 90:10). Quantitative yield

^{1}H-NMR (400 MHz, CD_3OD, 25°C): δ = 5.17 (d, $J_{1,2}$ = 4.8 Hz, 1H, H-1), 4.08 (t, $J_{2,3}$ = 6.4, $J_{3,4}$ = 6 Hz, 1H, H-3), 4.04 (t, $J_{1,2}$ = 4.8, $J_{2,3}$ = 6.4 Hz, 1H, H-2), 3.77 (dd, $J_{3,4}$ = 6, $J_{4,5}$ = 4.4 Hz, 1H, H-4), 3.69 (ddd, $J_{4,5}$ = 4.4, $J_{5,6}$ = 5.2, $J_{5,6'}$ = 6.8 Hz, 1H, H-5), 3.66 (dd, $J_{5,6}$ = 5.2, $J_{6,6'}$ = 11.2 Hz, 1H, H-6), 3.60 (dd, $J_{5,6'}$ = 6.8, $J_{6,6'}$ = 11.2 Hz, 1H, H-6'). ^{13}C-NMR (100 MHz, CD_3OD, 25°C): δ =

92.8 (C-1), 84.5 (C-4), 79 (C-2), 76.2 (C-3), 73.5 (C-5), 64.1 (C-6). NOESY (400 MHz, CD_3OD, 25°C): contact between H-1/H-4.



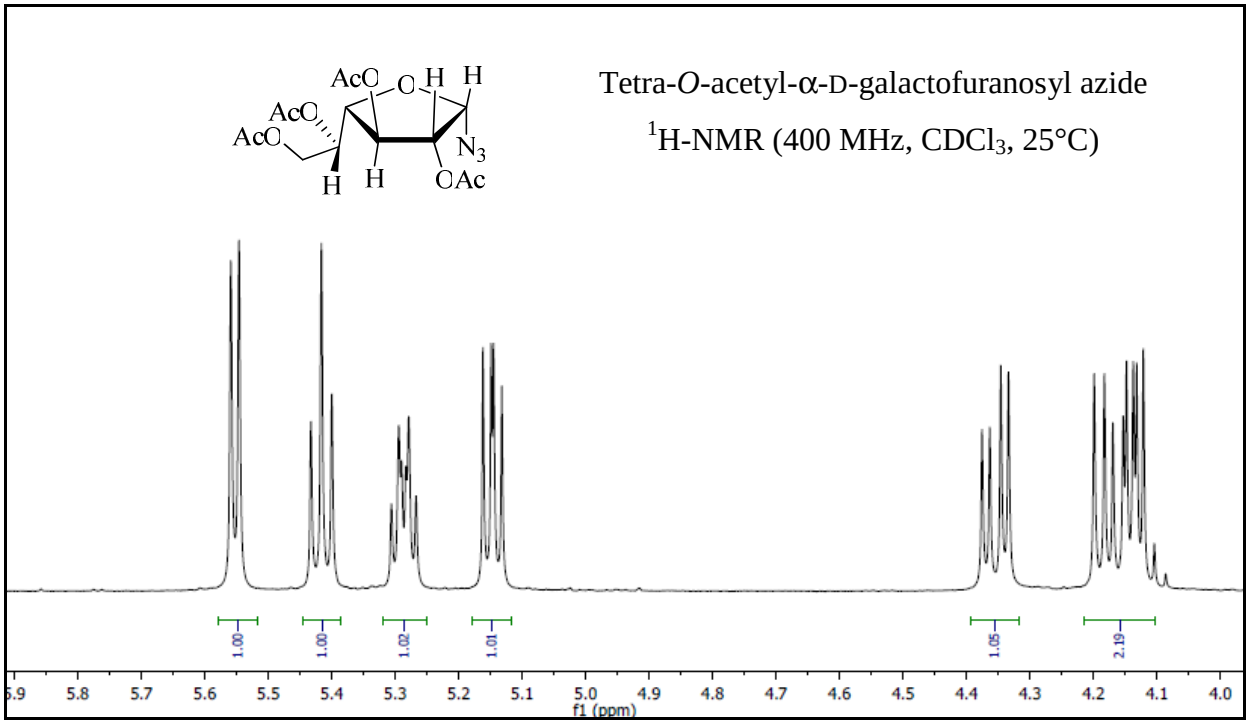
Synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-galactofuranosyl azide (129)



Acetic anhydride (10 eq) and a catalytic amount of *N*,*N*-dimethylaminopyridine were added, at room temperature, to a solution of substrate **46** (1 eq) in pyridine dried on molecular sieves (0.1 M). The solution was stirred for 24 h and then was concentrated in vacuo. The residue was dissolved in AcOEt and washed with aqueous 5 % HCl, aqueous 5 % $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated to give the product **129** in quantitative yield**.**

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.55 (d, $J_{1,2}$ = 5.6 Hz, 1H, H-1), 5.41 (t, $J_{2,3}$ = 6.8, $J_{3,4}$ = 6.4 Hz, 1H, H-3), 5.28 (ddd, $J_{4,5}$ = 2, $J_{5,6}$ = 4.8, $J_{5,6'}$ = 6.4 Hz, 1H, H-5), 5.14 (t, $J_{1,2}$ = 5.6, $J_{2,3}$ = 6.8 Hz, 1H, H-2), 4.35 (dd, $J_{5,6}$ = 4.8, $J_{6,6'}$ = 12 Hz, 1H, H-6), 4.17 (dd, $J_{5,6'}$ = 6.4, $J_{6,6'}$ = 12 Hz, 1H, H-6'), 4.13 (dd, $J_{3,4}$ = 6, $J_{4,5}$ = 4.4 Hz, 1H, H-4), 2.13 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.08

(s, 3H, OAc), 2.05 (s, 3H, OAc). ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): δ = 170.6, 170.2, 170.1, 169.9, 88.8 (C-1), 79.1 (C-4), 75.9 (C-2), 73.6 (C-3), 69.6 (C-5), 62.4 (C-6) 20.9-20.6 (4xOAc). NOESY (400 MHz, $CDCl_3$, 25°C): contact between H-1/H-4.



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Chapter 7

Summary, discussion, perspectives

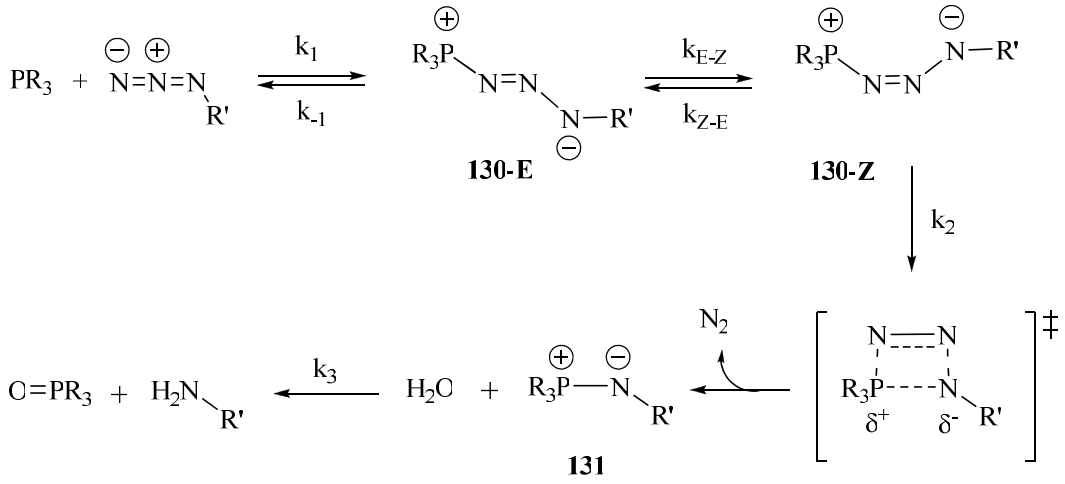
7.1 Mechanistic considerations

Although the Staudinger reaction has been known from 1919, and was actually described before the Wittig counterpart, the mechanism of this reaction is still poorly understood. The number of the intermediates and of the possible reaction pathways connecting them to each other and to the starting and final products is high and the potential energy surface extremely complex.

The mechanism of the Staudinger reaction has been extensively studied by using computational[1, 2, 3] and experimental methods.[4, 5]

Kinetic studies of the classical Staudinger reaction have shown that the process can be first- or second-order overall depending on the exact nature of the reactants. For example, the rate-limiting step in the reaction between triphenylphosphine and benzenesulfonyl azide (R' = -SO_2Ph) is the unimolecular decomposition (k_2, Scheme 1) of the phosphazide complex **130**.[6]

In contrast, the reaction of benzoyl azide (R' = -COPh) with triphenylphosphine follows second order kinetics, indicating that the bimolecular step leading to the formation of the phosphazide **130** (k_1, Scheme 1) is rate-limiting.[7] In reactions between substituted phenyl azides and substituted triphenylphosphines, deviations from second-order kinetics are observed, presumably due to the reversibility of formation of the phosphazide intermediate **130** (k_{-1}, Scheme 1).[7]



Scheme 1. Schematic mechanism of the classical Staudinger reaction

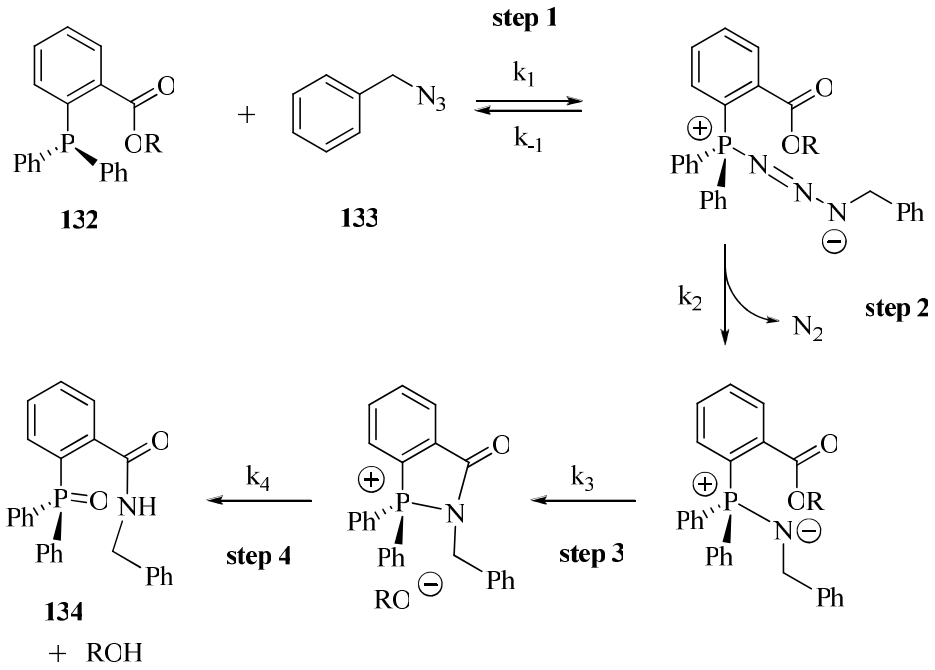
Phosphotriazadienes have been isolated from Staudinger azide reductions: these intermediates are stabilised by electron-donating groups on the phosphine, or electron-withdrawing groups on the azide, or sterically hindering groups on both phosphine and azide.[1] Some

phosphotriazadienes have been characterised by X-ray crystallography and other techniques:[1, 4, 8, 9, 10] they all have been found to be consistent with a zwitterionic structure corresponding to **130** (Scheme 1) displaying a partial double bond character of the central N-N linkage. The E configuration of the N-N double bond (Scheme 1, **130-E**) is generally observed,[8] while the *cis*-configuration has rarely been isolated.[8a]

In the reaction mixture **130-E** can isomerise to the Z isomer **130-Z**, which spontaneously decomposes to afford N_2 and the aza-ylide **131**.

Iminophosphoranes **131** are relatively stable species and have been isolated in a number of instances.[1] In particular, glycosyl iminophosphoranes from *O*-acetyl protected sugars are rather stable, and many have been isolated and characterised by NMR spectroscopy.[9, 11, 12]

Mechanistic investigations of the traceless Staudinger ligation with phosphine **132** (Scheme 2) were recently reported by the Bertozzi group.[13] They determined the kinetic parameters of the Staudinger ligation of phosphine **132** and benzyl azide **133** using ^{31}P-NMR spectroscopy. When **132** (0.041 M) and **133** (0.41 M) were combined in CD_3CN with 5 % water (v/v) (2.78 M), consumption of **132** followed pseudo-first-order kinetics. Under these conditions, the only species detectable by ^{31}P-NMR were the starting material **132** and the ligation product **134**.



Scheme 2. Mechanistic investigations of the traceless Staudinger ligation [13]

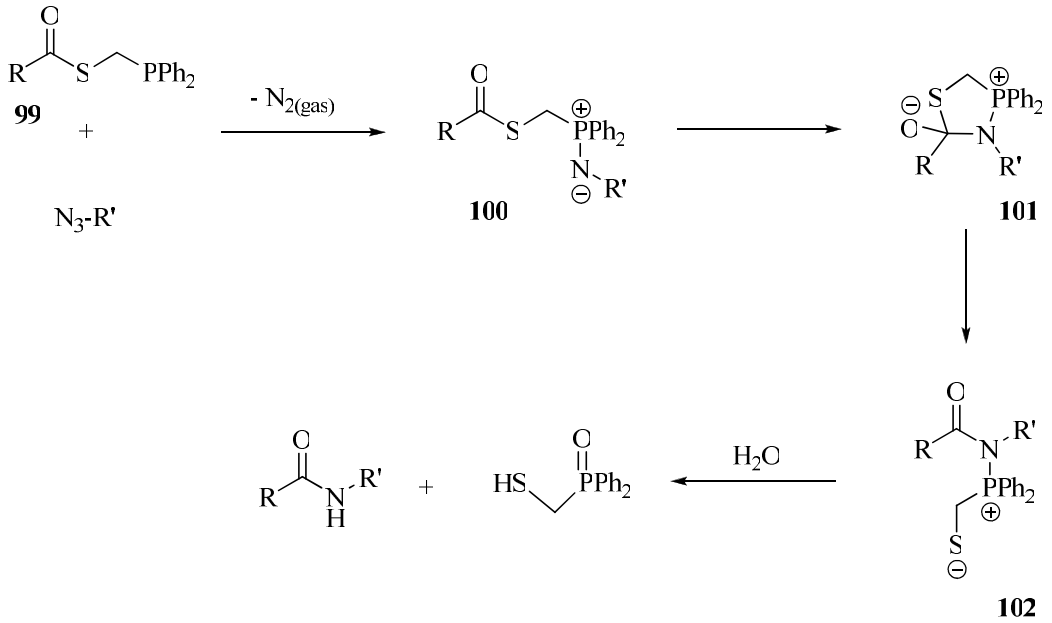
For this reaction the results can be summarised as follows:

1. The reaction proceeded faster in solvents with higher dielectric constants. These observations suggest that the rate-limiting step involves a polar transition state that can be stabilised by polar solvents.
2. The kinetic studies suggest that the rate-limiting step of the reaction occurs prior to the intramolecular reaction of the aza-ylide group with the ester functionality and consists of the initial bimolecular reaction between the phosphine and the benzyl azide. The acyl transfer step, however, competes with the hydrolysis of the aza-ylide if the ester leaving group is bulky (R = *t*-Bu).
3. The overall rate of the reaction increases when the phosphine is substituted with electron-donating groups.

Different conclusions can be drawn from the reaction between **132** and phenyl azide. In this instance, a stable aza-ylide intermediate (^{31}P-NMR signal +13 ppm) is formed and only slowly converted to the ligation product. Thus, the rate-determining step in the Staudinger ligation of aryl azides appears to be the intramolecular amide bond formation. This difference is likely associated to the lower basicity / nucleophilicity of the *N* atom in the iminophosphorane intermediate.[13]

More recently, Raines and coworkers discussed the mechanistic aspects of the Staudinger ligation of peptides mediated by (diphenylphosphino)methanethiol.[14, 15]

The mechanism proposed by Raines and co-workers (Scheme 3)[15] for the ligation mediated by (diphenylphosphino)methanethiol **99**, show that the imminophosphorane **100** can form a tetrahedral intermediate **101**, which collapses to give an amidophosphonium salt **102** that is hydrolyzed in aqueous solution, to afford the final amide.



Scheme 3. Mechanism for the Traceless Staudinger ligation mediated by (diphenylphosphino)methanthiol

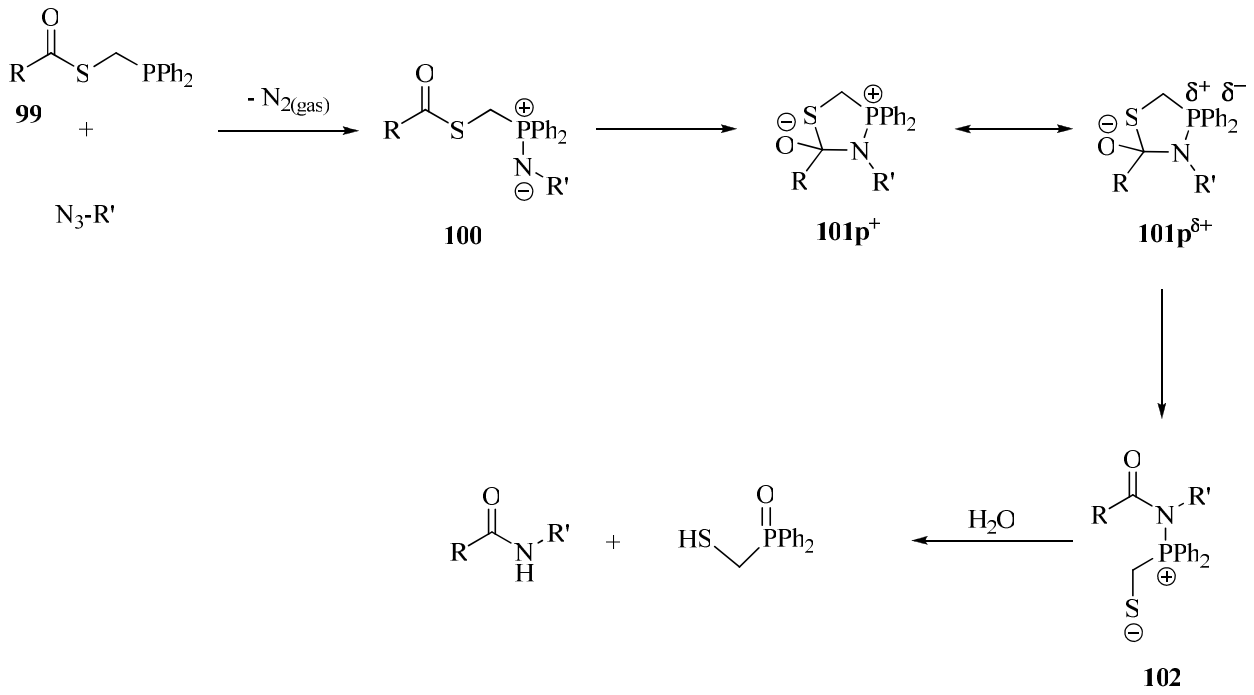
To obtain insight on the ability of nonpolar solvents to provide higher yields of the desired peptide product, the author performed DFT calculations on the effect of solvent on the electron density on phosphorus in tetrahedral intermediate **101** (R = R' = CH_3). The calculation indicated that this electron density increases in nonpolar solvents (Table 1), presumably due to the dispersal of charge into the phenyl groups.

Table 1. Effect of solvent polarity on the calculated electron density on P and N in **101**

Parameter	**H_2O**	**THF**
P, charge	+ 0.082	+ 0.028
N, charge	- 0.348	- 0.335

All of the experimental and theoretical results from reactions with different phenyl substituents and in solvents of different polarity are consistent. These results indicate that increasing the electron density on phosphorus leads to a higher yield of amide products. This finding can be depicted by the dissection of tetrahedral intermediate **101** into resonance forms **101p$^+$** (which has less charge dispersal) and **101p$^{\delta+}$**. To emphasize the importance of **101p$^+$** and **101p$^{\delta+}$** to the

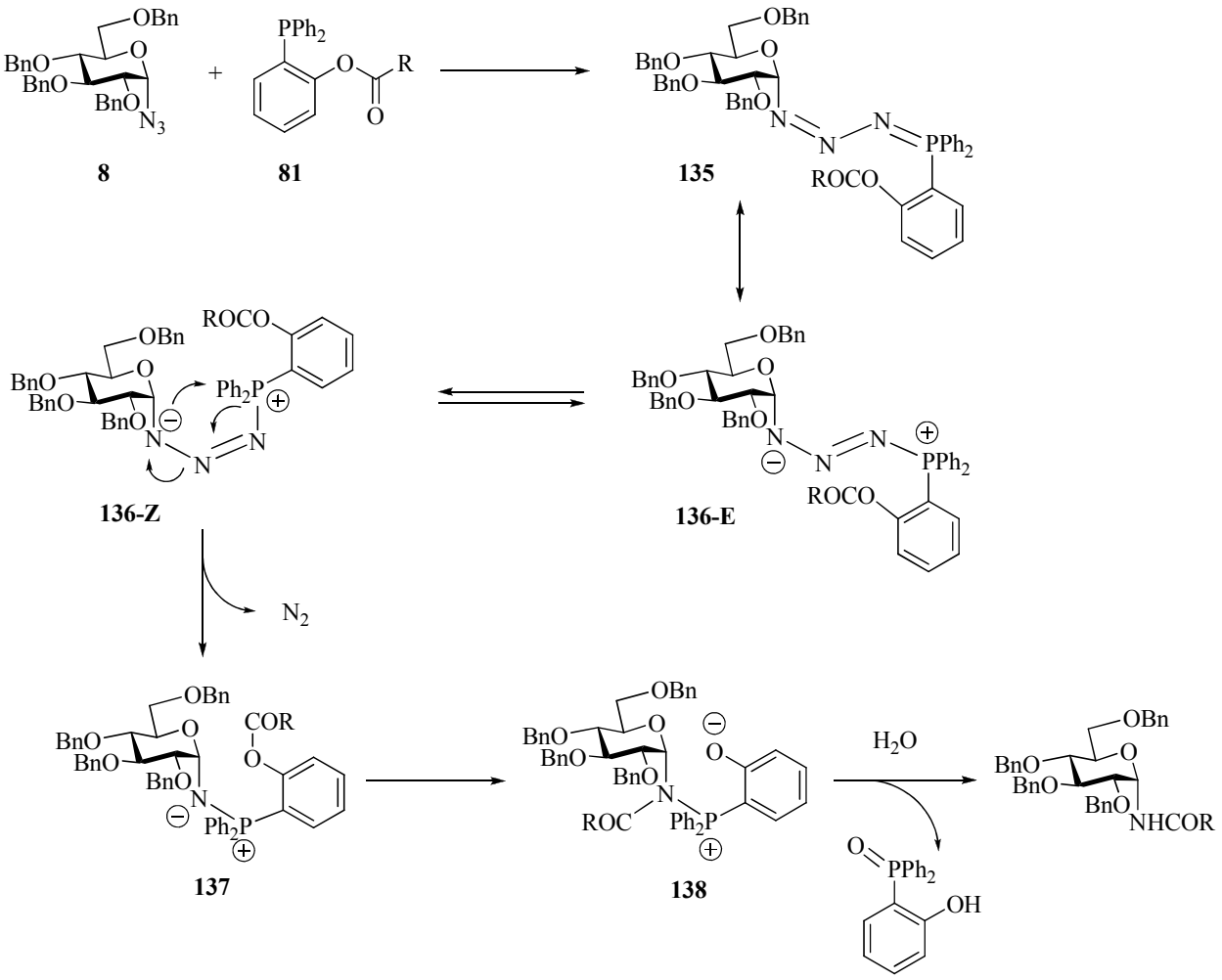
product distribution, Raines refined the mechanism of the Staudinger ligation as reported in Scheme 4.



Scheme 4. Refined mechanism including the resonance form **101p$^{\delta+}$**

Previous studies from our laboratory [16] examined the mechanism of the reaction of the α-tetra-*O*-benzyl glucosyl azide **8** with phosphines **81** (Scheme 5). The accepted mechanism for the Staudinger reduction of azides with phosphines **81** requires the formation of two sequential intermediates. The initial nucleophilic attack of the phosphine on the azide is expected to proceed through a phosphotriazadiene **135** (often called phosphotriazene or phosphazene or phosphazide), which should be present as an equilibrating *Z*-*E* mixture of zwitterions (**136-*Z*** and **136-*E***). This intermediate is stabilized by electron-donating groups on the phosphine, electron-withdrawing groups on the azide, or sterically hindering groups on both the phosphine and the azide.[17]

In the course of reactions performed in solvents of low polarity (toluene, CCl_4, $CHCl_3$) and starting from α-tetra-*O*-benzyl glucosyl azide **8**, intermediates that are consistent with the structures **136** were indeed isolated,[16] with a ratio that probably depends on the steric hindrance of the acyl group that is transferred (86:14 ratio from **81b**, and 50:50 from **81j**).



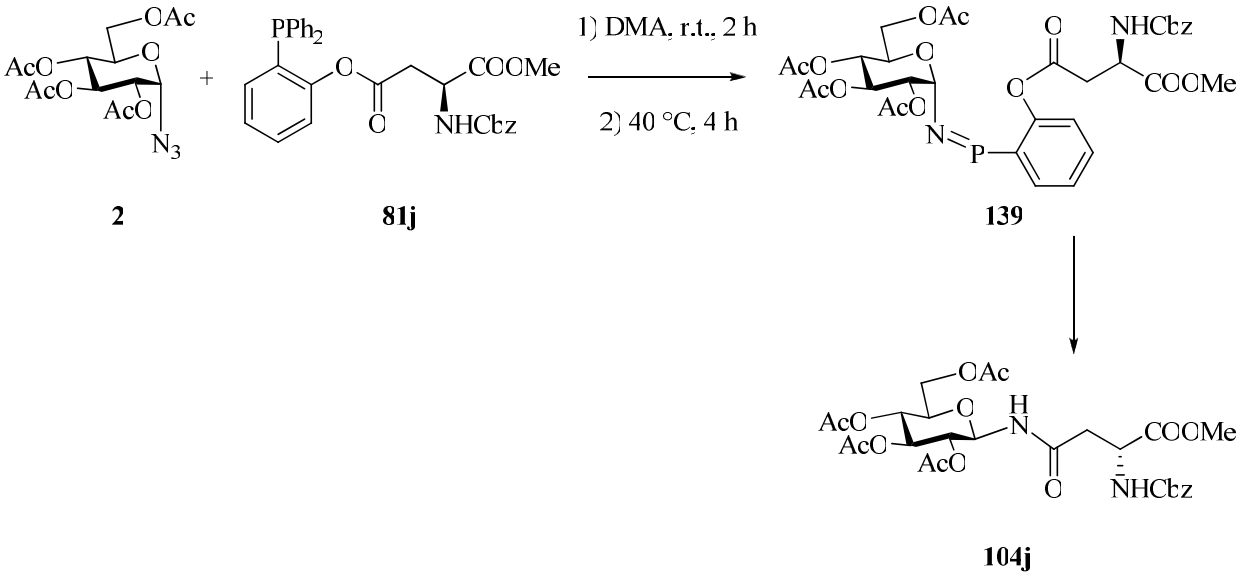
Scheme 5. Proposed mechanism for the traceless Staudinger ligation of **81** with α-tetra-*O*-benzyl-glucosyl azide **8**

The configuration of the two intermediates was not fully assigned, but it could be clearly differentiated analysing the coupling constant between the anomeric proton and the phosphorous atom. Unfortunately, these compounds, once isolated from the reaction mixture by chromatography, rapidly undergo nitrogen extrusion and are not stable enough to allow a complete characterisation. Furthermore, in apolar solvents, sunlamp irradiation appeared to accelerate the decomposition of some, but not all, of the intermediates. In any case, acyl transfer appeared to occur with retention of configuration at the anomeric carbon, leading to the formation of the corresponding α-tetra-*O*-benzyl-glucosyl amides.

Acceleration of the acyl transfer was obtained more reliably using aprotic dipolar solvents. The intermediates were isolated only for the reaction of **8** with **81j**, and found to be identical to those

isolated from toluene. The α/β selectivity was also improved in these solvents, but phosphine **81j** represents one exception, because the reaction became non-stereoconservative.

Finally, upon reaction of the α-tetra-*O*-acetyl glucosyl azide **2** with **81j** in *N*,*N*-dimethylacetamide at 40 °C formation of the phosphotriazadiene could not be characterized, but the corresponding aza-ylide (iminophosphorane) intermediate **139** was directly isolated and fully characterised by NMR spectroscopy (1H NMR, CDCl3, 400 MHz: H1, 5.29 ppm, dd, $J_{1\text{-}2}$ = 3.7 Hz, $J_{1\text{-}P}$ = 22.6 Hz, Scheme 6).[17] This is in agreement with previous reports on the Staudinger reaction of *O*-acetyl glycosyl azides.[9, 18, 19]



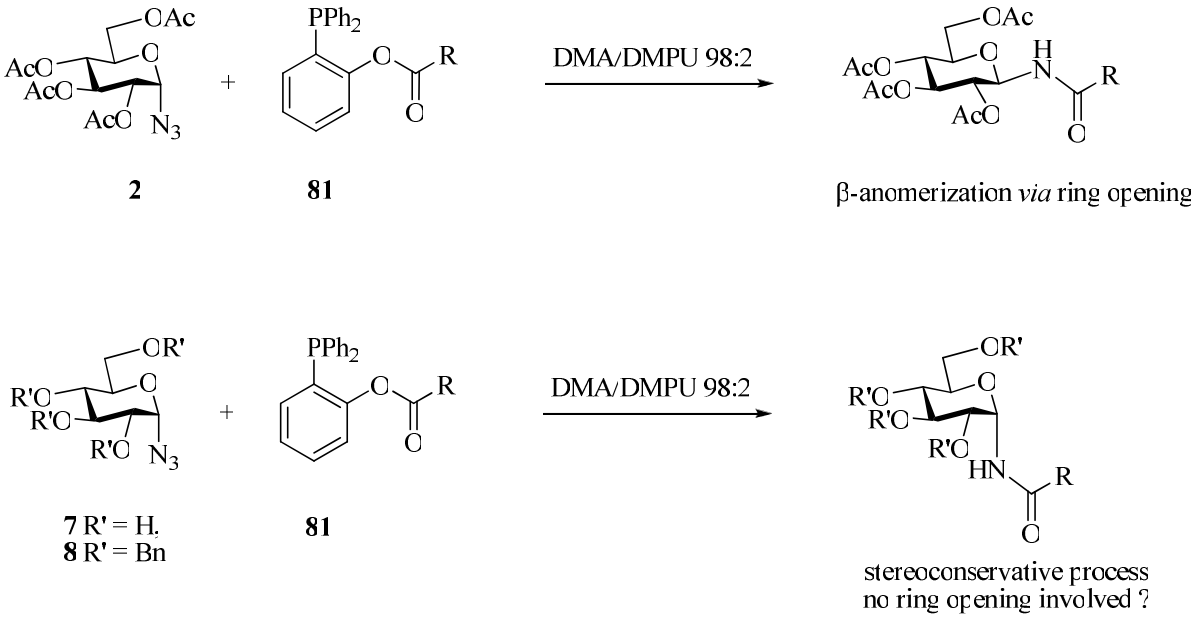
Scheme 6. Staudinger ligation of azide **2** with phosphine **81j**

Interestingly, upon water work up at 40 °C or 70 °C, this intermediate afforded the β-glucosyl amide **104j** only. This behavior is not limited to phosphine **81j**, but is a general feature of the ligation reaction of α-tetra-*O*-acetl-glycopyranosyl azides.

In general, the anomeric iminophosphoranes of *O*-acetyl pyranoses appear to react more sluggishly than the corresponding *O*-benzyl derivatives with acylating agents and to allow α to β anomerization to occur prior to acyl transfer. Unlike the ligation of tetra-*O*-benzyl-glycopyranosyl azides, the reduction-acylation of tetra-*O*-acetyl-glycopyranosyl azides is a nonstereoconservative process, and acyl transfer occurs more slowly than iminophosphorane anomerisation.

These results are in agreement with recent literature reports obtained by the Kiessling group using dialkylphosphino-(borane)methanethioesters. [20]

We speculated that tetra-*O*-acetyl-glycopyranosyl iminophosphoranes may be deactivated toward acyl transfer by the electron-withdrawing effect of the acetates, which delocalize the negative charge on the nitrogen atom and allow anomeric equilibration to occur before the acyl transfer step. On the contrary, the stereoconservative process obtained with tetra-*O*-benzyl and unprotected glycopyranoses was interpreted as the result of a stronger localization of the negative charge on the anomeric nitrogen, leading to faster acyl transfer and blocking the ring-opening anomerization process (Scheme 7).



2 **81**

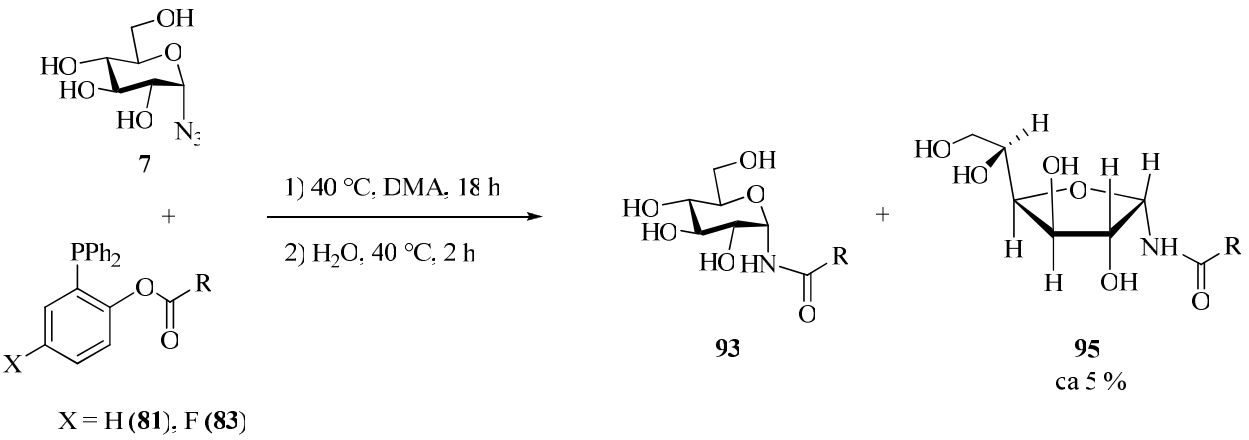
7 R' = H,
8 R' = Bn

81

Scheme 7. Stereochemical course of the staudinger ligation of tetra-*O*-acetyl-, tetra-*O*-benzyl- and unprotected α-glucopyranosyl azides **2**, **8**, and **7** with phosphines **81**

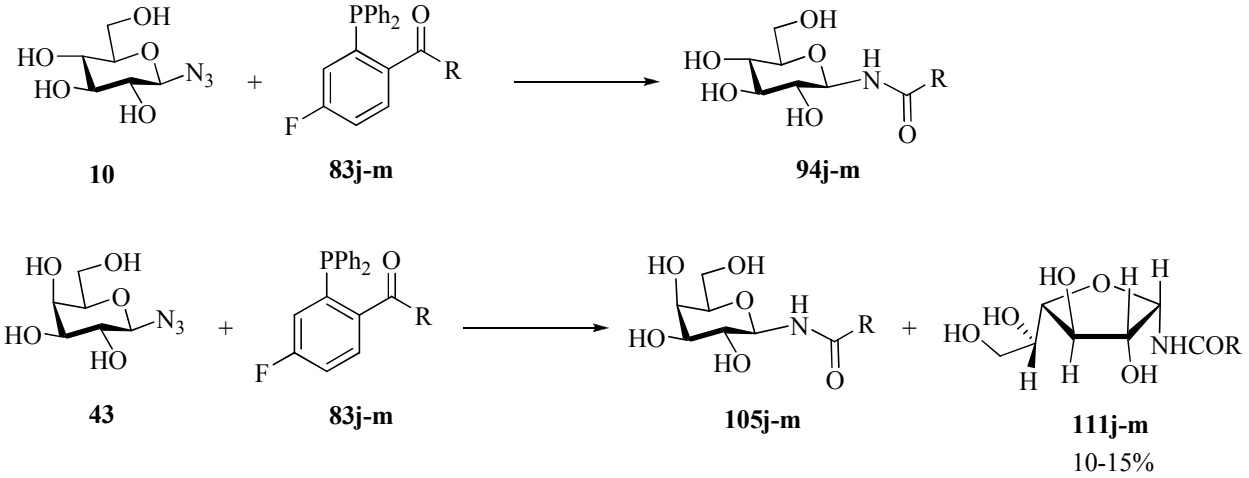
However, closer inspection of the results from unprotected α-glycopiranosyl azides and furanosyl azides revealed a more complex picture.

Indeed, formation of variable quantities of glucofuranosyl amide by-products from unprotected α-glucopyranosyl azide **7** both in the reaction with **81** and **83** (Scheme 8) suggests that the Staudinger intermediates must, also in these cases, undergo a ring-opening process, at least to some extent. This however, is not leading to α-β equilibration, but rather to a ring-contraction, which is most unusual for pyranoses particularly in the *gluco* configuration.



Scheme 8. Formation of α-glucofuranosyl amide **95** from α-glucopyranosyl azide **7**

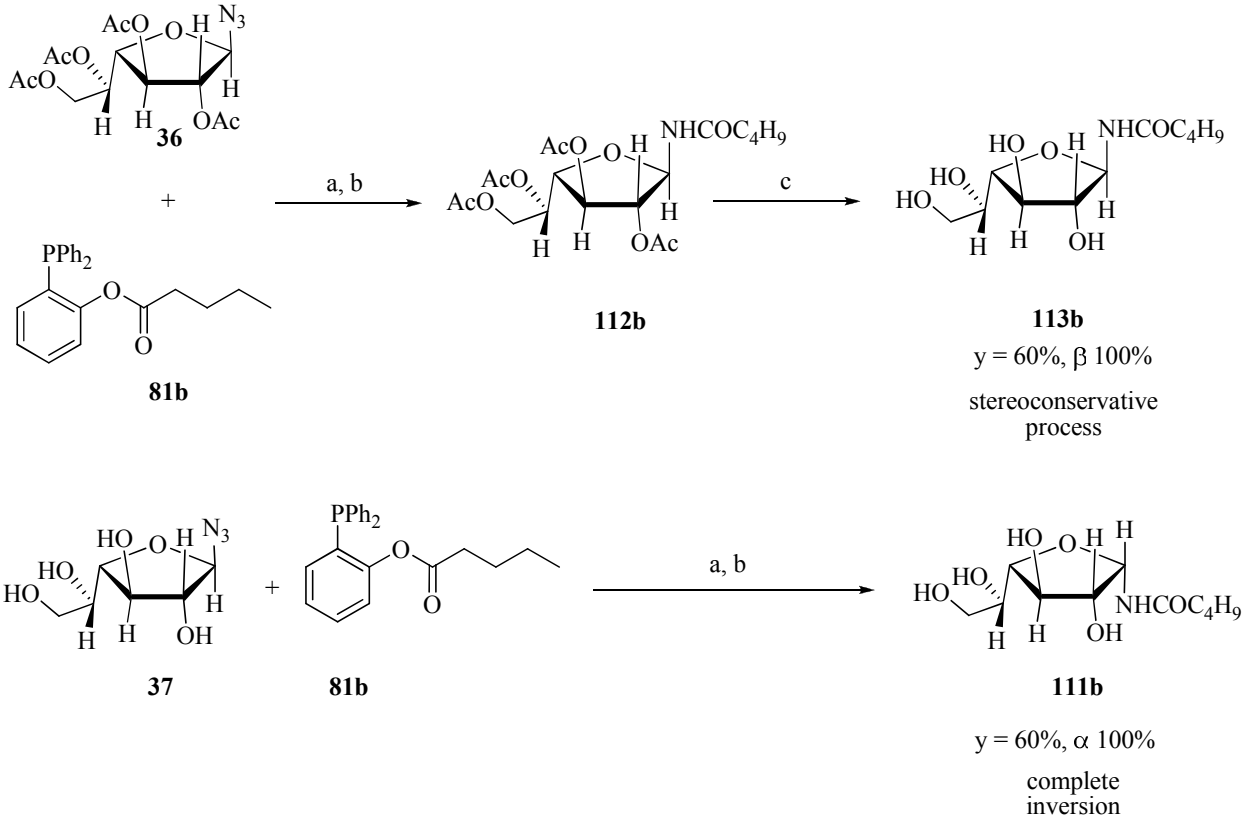
Other elements are gathered from the ligation reactions of β-glucopyranosyl azide **10** and β-galactopyranosyl azide **43** with phosphines **83j-m** (Scheme 9). β-glucopyranosyl azide **10** reacts with **83** to afford β-pyranosyl amides **94** only and no ring contraction products are identified in the crude reaction mixtures. However, α-galactofuranosyl amides **111** are formed as by-products in the ligation of the β-galactopyranosyl amide **43** with **83**. Again, the unusual and unstable α-anomer is the only furanose form isolated.



Scheme 9. β-glucopyranosyl amide **10** does not afford ring contraction products in the reaction with **83**; β-galactopyranosyl azide **43** does

Finally, and perhaps more strikingly, the reaction of pyranosyl and furanosyl azides appear, at first sight, to follow opposite stereochemical pathways. In fact 1,2-*trans* *O*-acetyl-furanosyl

azides of the galacto-, ribo- and arabino- series (**36**, **41** and **40**) do not isomerize upon ligation with **81b**, whereas the same 1,2-*trans* furanosyl azides do when they are unprotected (compounds **37**, **53** and **49**, respectively). This behaviour, exemplified in Scheme 10 for the β-galactofuranosyl azides **36** and **37**, is exactly the opposite as that described for **2** and **7** in Scheme 7**.**



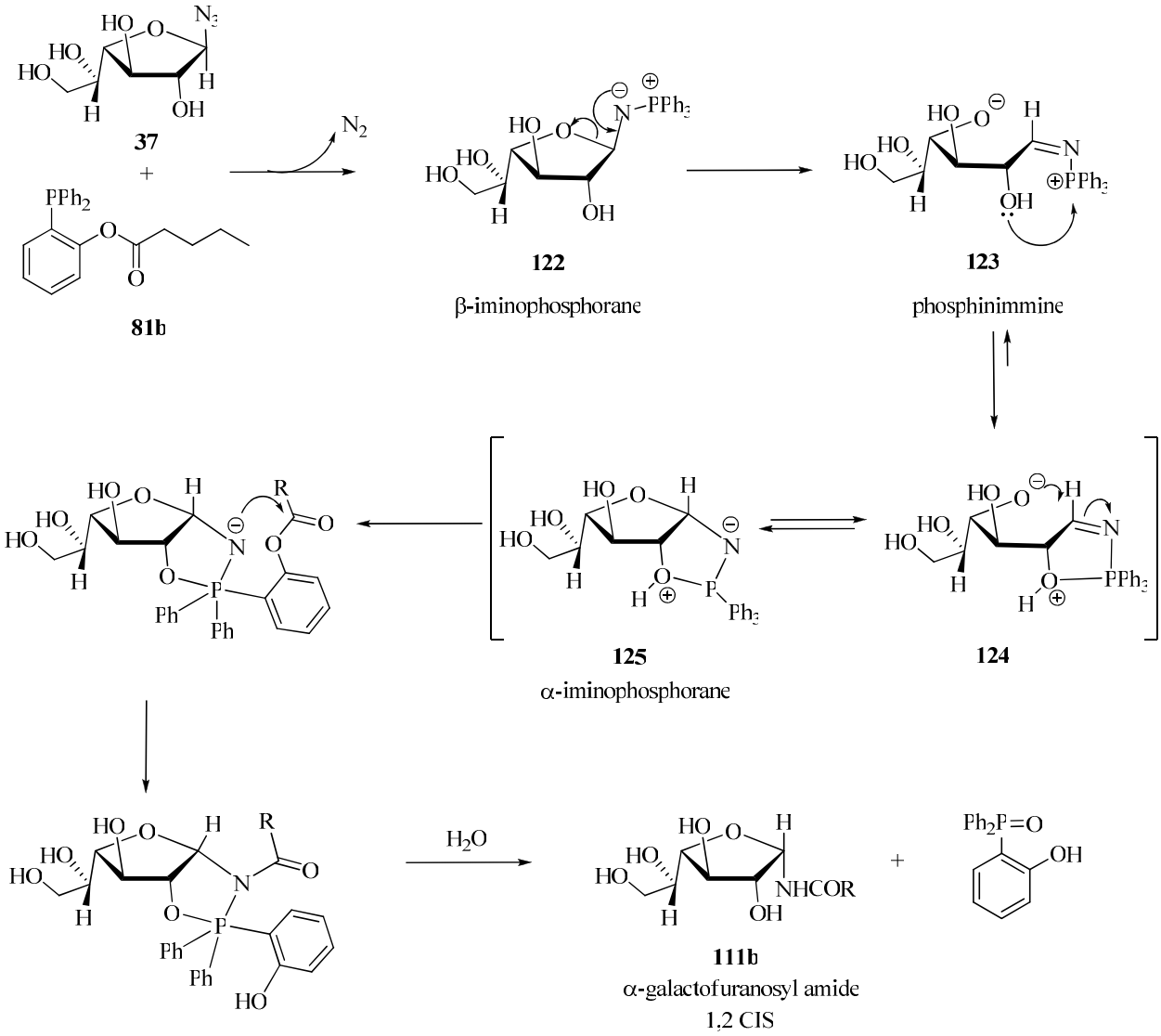
Scheme 10. Stereochemical course of the Staudinger ligation of tetra-*O*-acetyl and unprotected β-glycofuranosyl azides **36** and **37** with phopshines **81b**

The remaining sections of this chapter will be dedicated to a full discussion of the reaction selectivity for unprotected and *O*-acetyl-glycosyl azides in the context of a common mechanistic framework.

7.2 Mechanism of the ligation of unprotected glycosyl azides

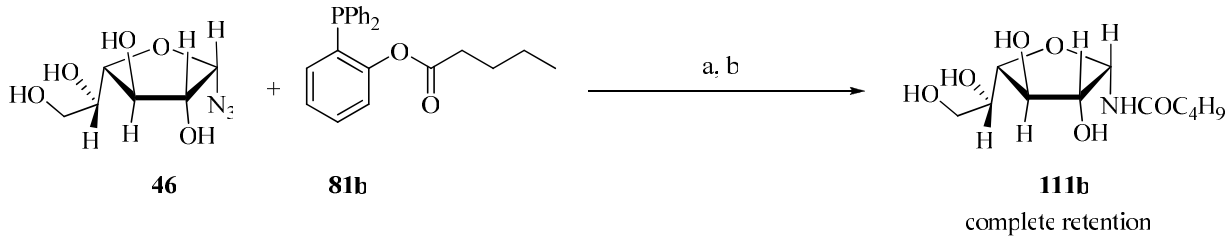
We have shown in this thesis that the behavior of the unprotected furanosyl azides may be traced

to ring opening of the intermediate iminophosphorane, leading to a phosphinimine **123** which allows coordination of the phosphorous atom by the hydroxy group in position 2 of the sugar to afford the oxaphospholane **124**, which leads to the 1,2-*cis* isomer after closure of the furanose ring (Scheme 11).



Scheme 11. Proposed mechanism for the anomeric epimerization of **37**

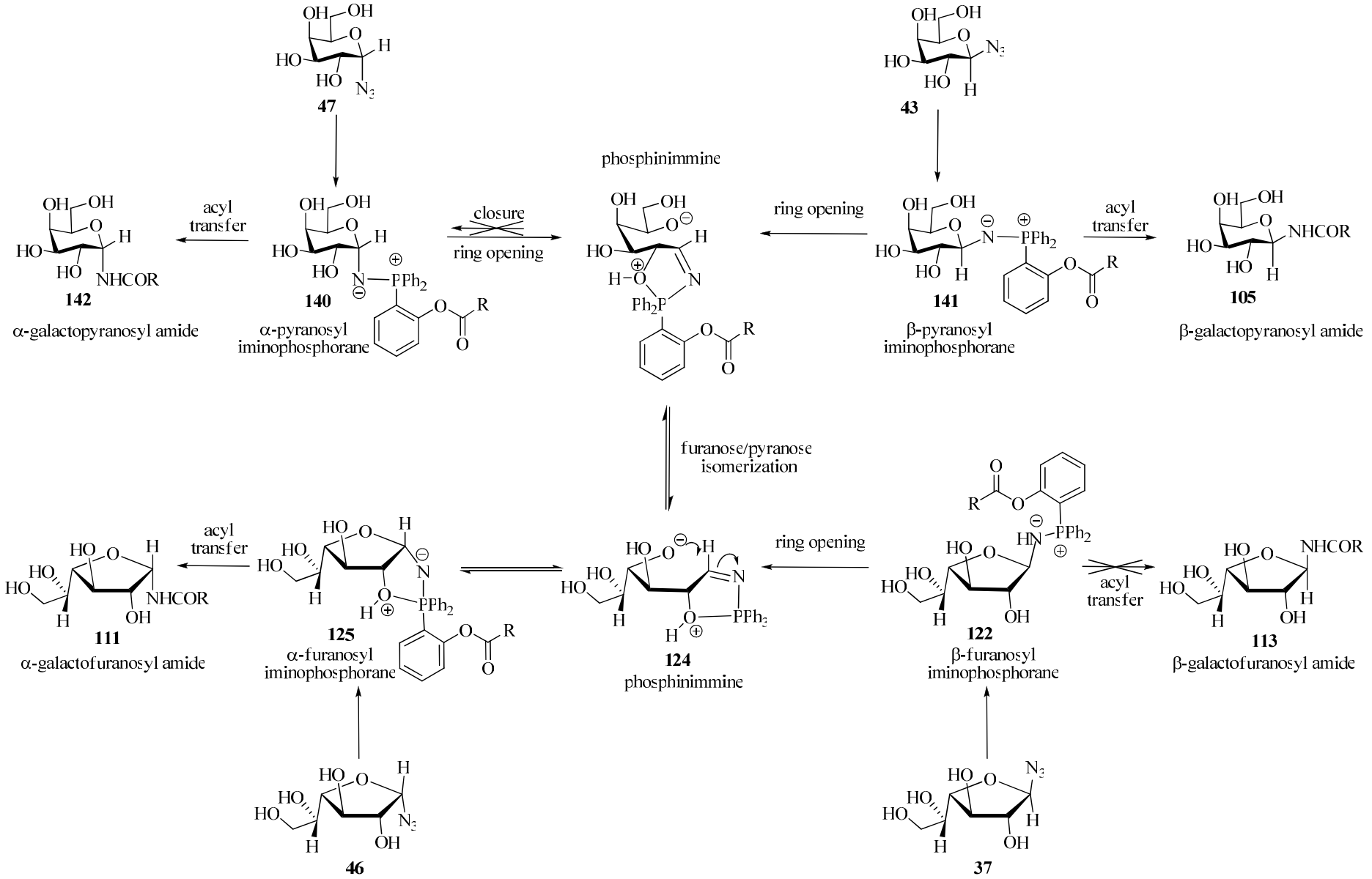
This hypothesis was confirmed by the observation that the unprotected 1,2-*cis* galactofuranosyl azide **46** reacted with **81b** with total retention of configuration (Scheme 12), thus showing that 1,2-*cis* compounds are formed from furanosyl azides regardless of the configuration of the starting material.



Scheme 12. Synthesis of α-galactofuranosyl amide **111b** by ligation of 46 with **81b**

The mechanistic path shown in Scheme 11 can be used to reconcile the apparently opposite behaviors of pyranosyl and furanosyl azides in the ligation process. The full mechanistic picture is shown in Scheme 13, using unprotected galactosyl azides as substrates.

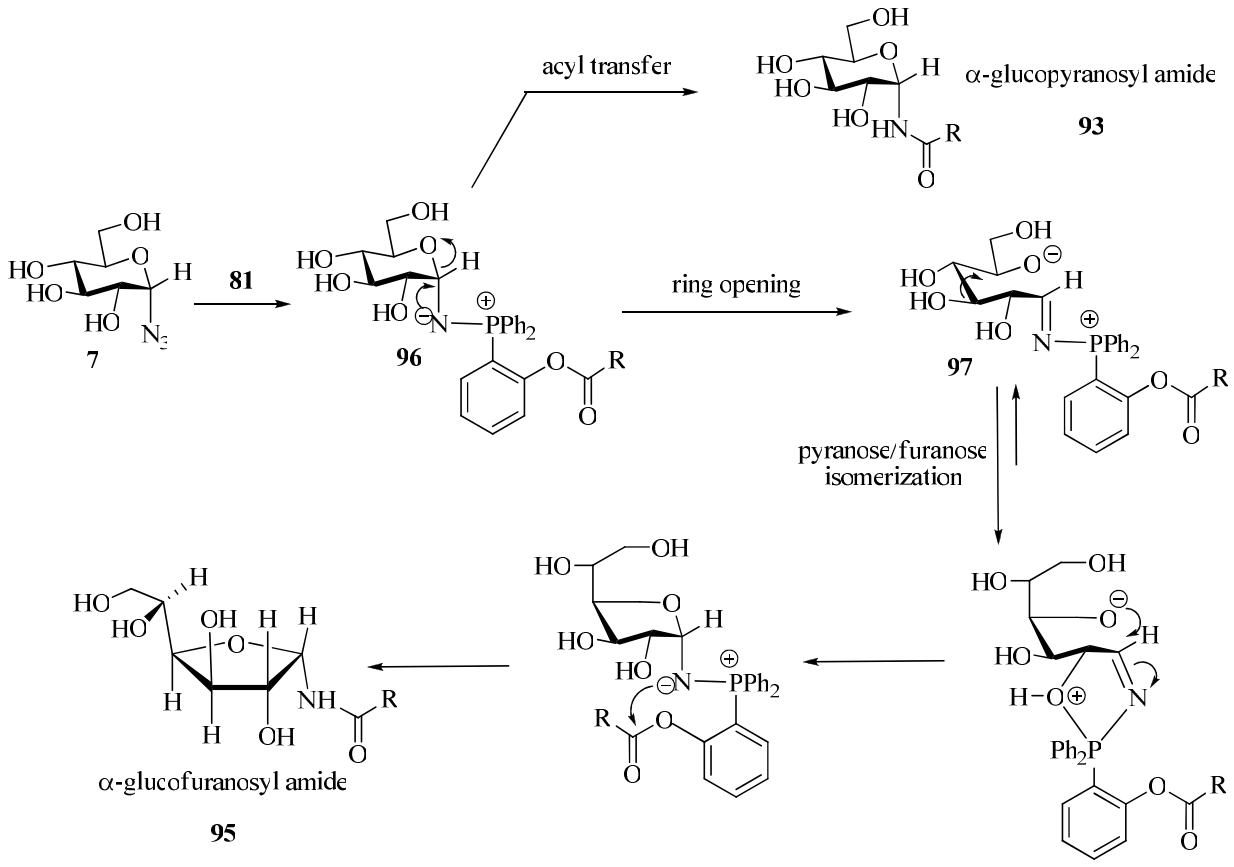
The reaction course is dominated by the competition between acyl transfer and ring opening process, as discussed in the opening sections of this thesis. However, for unprotected sugars the behavior of the intermediated phosphinimine is more complex than previously discussed. In fact, once the phosphinimine is formed, the phosphorous atom is probably trapped by the 2 hydroxy group of the sugar in a 5-membered oxazaphospholane ring, exemplified in **124**. Once this cycle is formed, the ring closure process can no longer revert to β-isomers of the iminophosphorane and only the left half side of the Scheme 13 is still available. In particular **124** can cyclize by attack of O4 to the anomeric carbon and form the α-furanosyl iminophosphorane **125**, which, upon acyl transfer leads to the α-furanosyl amides **111**. An alternative pathway for **124** is represented by O5 attack to the anomeric carbon, leading to the α-pyranosyl iminophosphorane **140**, from which acyl transfer would generate the α-galactopyranosyl amides **142**. This pathway does not appear to be followed, since no α-pyranosyl azide is observed in the ligation of the furanosyl azides **46** and **37**. But the reverse pathway, leading from **140** to **124,** is the source of α-furanosyl amide formation in the ligation of the α-pyranosyl azide **47** and of the corresponding azide in the gluco-series (**7**). Acyl transfer in the α-pyranosyl iminophosphoranes **140** is relatively slow, allowing for ring opening and phosphorous coordination by the 2 hydroxy group. The latter event prevents α-β isomerization of the pyranose ring to β-pyranosyl iminophosphorane **141**, and rather redirects the open sugar through the furanose pathway. Hence, O4 attack on the anomeric carbon, leads to **125** and thus to the α-furanosyl amide by-products both in the galacto- (**111**) and gluco- series.



Scheme 13. Full mechanistic pathway for the reaction of unprotected galactosyl azides

When the ligation is performed starting from β-pyranosyl azides, a more facile acyl transfer is expected. Thus, in the gluco series, β-pyranosyl amides are the only reaction product. However, if ring opening of the β-pyranosyl iminophosphorane **141** can occur, the reaction can again be funnelled through the cyclic oxazaphospholane **124**, thus providing a path for pyranose to α-furanose isomerization. This appears to be the case with β-galactopyranosyl azide **43** (Scheme 13). Finally, the β-furanosyl iminophosphorane **122** must initially be formed upon reduction of β-galacto furanosyl azide **37**. This, however, appears to be unable of direct acyl transfer but undergoes fast ring opening leading through **124** and **125** to the α-furanosyl amides **111**.

In this rather complex framework, the mechanistic scheme shown in Chapter 4 for the reaction of α-gluco pyranosyl azide **7** with **9** (Scheme 4, Chapter 4) can be riformulated as shown in Scheme 14. The iminophosphorane **96**, formed in the first steps of the ligation can either transfer the acyl group to the anomeric N leading to α-glucopyranosyl amide **93**, or can undergo ring opening to **97**. This is probably not an equilibrium reaction, because the resulting phosphinimine **97** is blocked in the oxazaphospholane which strongly favors the formation of 5-5-fused bicyclic systems and hence of the furanosyl amide **95**.



Scheme 14. Mechanism for the formation of furanosyl amide **95** in the ligation of **7** with **81**

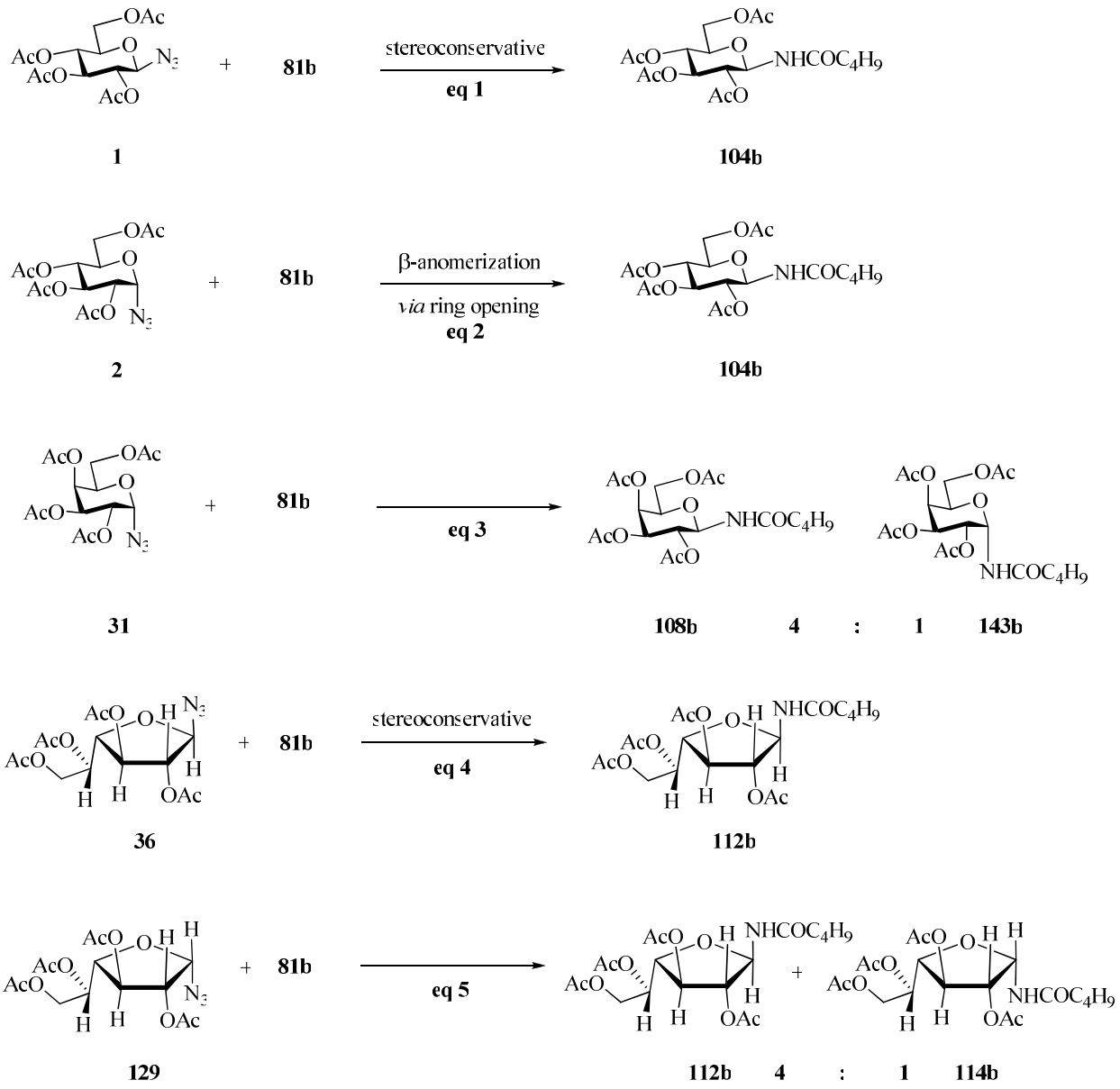
Indirect proof of this is the absence of pyranosyl amides in the ligation products of furanosyl azides. The amount of **95** formed in this reaction increases with the reaction temperature because higher temperatures favour ring opening of **96** and reduce the acyl transfer selectivity.

7.3 Mechanism of the ligation of *O*-acetyl-glycosyl azides

The results obtained in the ligation of tetra-*O*-acetyl-glycosyl azides **1**, **2**, **31**, **36** and **129** with the pentanoyl phosphine **81b** are summarized in Scheme 15.

Ligation of tetra-*O*-acetyl-β-glucopyranosyl azide **1** procedes uneventfully to afford the β-amide **104b** (Scheme 15, eq. 1). The same product is form by ligation of α-tetra-*O*-acetyl-glucopyranosyl azide **2** (Scheme 15, eq 2) As seen in Chapter 1 and previously reported [17] the complete inversion of configuration observed in this reaction can be explained by ring opening of the intermediate iminophosphorane, facilitated by the electron-withdrawing effect of the acetyl groups, which favors phosphinimine formation. (Ring contraction paths are not available to these *O*-acetyl substrates). A similar behaviour is observed for the α-tetra-*O*-acetyl-galactopyranosyl azide **31** (Scheme 15, eq 3), although the anomeric inversion is not complete and a 4:1 β:α mixture of anomeric amides **108b** and **143b** is formed. This suggests that either the acyl transfer is faster for the α-galactopyranosyl iminophosphorane than for the corresponding gluco- derivative, or that the anomeric equilibrium in the galactopyranose series is less shifted toward the β-isomer.

Given the propensity of acetylated glycosyl iminophosphoranes to undergo ring opening upon formation of the iminophosphorane, it is rather surprising to observe a totally stereoconservative process in the ligation of β-tetra-*O*-acetyl-galactofuranosyl azide **36** (Scheme 15, eq 4). Indeed, all literature data support small energetic differences for the α and β isomers of furanoses.[21] Ring opening would be expected to produce a mixture of isomer iminophosphoranes and there is no reason why **36** should be stabilized against it. Indeed, ligation of the α-tetra-*O*-acetyl-galactofuranosyl azide **129** (Scheme 15, eq 5) affords a 4:1 mixture of β- and α- isomers **112b** and **114b**, which must originate from a ring opening event. The partial inversion of the anomeric configuration observed in this reaction suggests that when ring opening occurs for these galactofuranose substrates, the subsequent ring closure is biased towards the formation of 1,2-*trans*-amides, presumably for steric reasons.



Scheme 15. Ligation of tetra-*O*-acetyl-glycosyl azides **1**, **2**, **31**, **36** and **129**

With these observations we think we have now begun to clarify the mechanistic picture of these ligation processes to the point where predictions can be made concerning the stereochemistry of the reaction on new substrates and with new combinations of reagents. Work is in progress in our laboratory to test hypotheses generated by the models described above.

7.4 Reference

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