DEVELOPMENT OF NOVEL METHODS FOR ATTACHMENT OF CARBOHYDRATES TO PROTEINS

AND

SYNTHESIS OF THE LEWIS B HEXASACCHARIDE

Progress report by

Helle Christensen

Department of chemistry

Aarhus University

September 2011
GRAPHICAL ABSTRACT

Part I: Development of novel methods for attachment of carbohydrates to proteins

The purpose is to develop of a novel procedure for synthesis of glycoproteins. By utilizing an aldehyde tagged protein, allyl bromide functionalised carbohydrate structures, such as 1, would be attracted using the indium mediated Barbier-reaction followed by the attachment of a thioglycoside, such as 2, using the thiol-ene coupling in aqueous media.

Part II: Synthesis of the Lewis b hexasaccharide

The purpose is to synthesis the Lewis b hexasaccharide 66, which is to be linked to a porphyrin skeleton and used for binding studies against a receptor at the surface of the bacteria Helicobacter pylori. Four building blocks are required for the synthesis of Lewis b; a thioglucosamine donor 76, a thiogalactoside donor 77 a thiofuranoside donor 78 and a disaccharide acceptor 79.
Preface

The research presented in this progress report was performed in the period between April 2010 and February 2011 at Department of Chemistry, Aarhus University, under the supervision of associate professor Henrik Helligsø Jensen, and from March 2011 to June 2011 at Centre for Synthesis and Chemical Biology, University College Dublin, under the supervision of Professor Stefan Oscarson. This progress report is submitted for the Midterm Review as part of the PhD programme at the Faculty of Science, Aarhus University.

In this report, two different projects will be described. The first project “Development of novel methods for attachment of carbohydrates to proteins” was performed at Department of Chemistry, Aarhus University, under the supervision of associate professor Henrik Helligsø Jensen. The second project “Synthesis of the Lewis b hexasaccharide” was performed at Centre for Synthesis and Chemical Biology, University College Dublin, under the supervision of Professor Stefan Oscarson.

Furthermore, a small project regarding the synthesis of dimeric triazole linked glucosamine compounds was done at Department of Chemistry, Aarhus University, under the supervision of associate professor Henrik Helligsø Jensen. In collaboration with Vladimir Krén and co-workers, these compounds were tested as ligands for nature killer cell activation receptors. Due to space limitations for this progress report, the synthesis is not described here. Recently this work resulted in a scientific publication in the journal Carbohydrate Research and is attached as Appendix B.

Due to technical problems with the MS and MALDI apparatus at Centre for Synthesis and Chemical Biology, University College Dublin, and technical problems with the MS apparatus at Department of Chemistry, Aarhus University some synthesised compounds is not fully characterised by MS or MALDI-TOF analysis.
ACKNOWLEDGEMENT

First of all, I wish to thank associate professor Henrik Helligsø Jensen for giving me the opportunity to work as a PhD student in his research group, for his trust in me and for his excellent and enthusiastic supervision. Also I would like to thank former and present members of group 103 for creating a very pleasant and inspiring working environment. Thanks to the members of the inSPIN laboratory, especially Erik H. T. Nielsen, Julie L. H. Madsen and Heidi K. Frahm for help and advice with the synthesis of peptides and MALDI analysis.

I would like to thank Professor Stefan Oscarson for allowing me to work in his group in Dublin for four months and for involving me in the interesting project of synthesising the Lewis b hexasaccharide. Thanks to the members of the Oscarson group for nice discussions about the project and helping me out in the laboratory, especially thanks to Post. Doc. Martin Hollinger and PhD student Rebecca Ulc. Also thanks to Professor Martina Lahmann and her group at Bangor University for fruitful discussions about the synthesis of the Lewis b structure.

Post. Doc. Mikkel D. Petersen, PhD student Julie L.H. Madsen and PhD student Tina S. Rasmussen are gratefully acknowledged for proofreading this progress report.

I am grateful to the Faculty of Science, Aarhus University for funding my PhD, as well as to the Hakon Lund Foundation, Aarhus University Research Foundation and to the Oticon Foundation for financial support during my stay at University College Dublin.

I would like to thank associate professor Eoin M. Scanlan for nice discussion about the Lewis b project, for taking good care of me in Dublin, letting my friends and family stay in his apartment during visits and for a never ending supply of chocolate cookies and emergency beers.

Finally, I would like to thank my family, friends and my fiancé Bo, for their love and support.

Helle Christensen
**LIST OF ABBREVIATIONS**

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Standard units and one- and three letter abbreviations for proteinogenic amino acids are not included.
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PART I
DEVELOPMENT OF NOVEL METHODS FOR ATTACHMENT OF CARBOHYDRATES TO PROTEINS

1.1 Introduction
Glycoproteins are involved in numerous vital biological events, however despite of their importance, progress towards understanding their structure and function has been very slow due to the lack of pure and structurally well-defined samples of glycoproteins. Glycoproteins are often found as heterogeneous mixtures in low concentrations in nature, complicating their isolation and characterization. In some cases, well-defined glycoproteins can only be obtained by chemically or enzymatic synthesis and these compounds are increasingly being used to address important problems in glycobiology research and glycomedicine. ¹

1.2 Natural glycoproteins
Protein glycosylation comprises a large family of posttranslational events in eukaryotic organisms. More than 50% of proteins in human cells are glycosylated.² The attached carbohydrate residues affect protein folding and stability,³ cell signalling and regulation,⁴ immunogenicity as well as other biological properties and activities such as fertilization, inflammation, pathogen recognition and the development of autoimmune diseases and some types of cancer.¹

In glycoproteins two main classes of glycosidic linkages is found; the oligosaccharide is either covalently bound to the oxygen atom of the side chain of serine or threonine, termed O-linked, or the amide nitrogen atom in the side chain of asparagine, termed N-linked. To be glycosylated, an asparagine residue must form part of the tripeptide sequence Asn-X-Ser, where X is any amino acid apart from proline. N-glycosylation is a co-translational modification that occurs simultaneously with translation of mRNA into protein, and begins in the endoplasmic reticulum and continues in the Golgi complex, while O-glycosylation is a post-translational modification and takes place entirely in the Golgi complex.⁵ Other natural linkages are also found in nature, such as the S-glycosidic linkage.⁶

Since protein glycosylation is not under direct genetic control, a heterogeneous mixture of glycoforms, which possess the same peptide backbone, but differ in the nature and site of glycosylation is formed.¹ It is therefore difficult to identify the specific oligosaccharide structures that are responsible for an individual biological process or protein function.

A major problem in glycobiology and glycoscience is the lack of pure and structurally well-defined glycoproteins.⁷ Methodologies for the synthesis of homological glycoproteins are therefore of high interest.

1.3 Formation of synthetic glycoproteins

Since most proteins contain multiple copies of each amino acid, an important challenge in the synthesis of glycoproteins is the development of strategies, which can selectively modify a protein at a specific site. In addition, site-specific modifications of a protein in two or more distinct locations are of high interest and utilize chemical modifications, which are orthogonal to each other. Ideally, the protein functionalisation should proceed rapidly with high yield without perturbation of the tertiary structure. As a consequence, orthogonal methodologies for adding a glycosyl-unit to a unique site on a protein under mild aqueous conditions are valuable.¹

The commonly most employed synthesis of glycopeptides uses preformed glycosyl amino acids, which are combined in a stepwise assembly via solid-phase peptide synthesis (SPPS). This requires proper selection of protecting groups, which is necessary to block the functional groups on both the glycan and the peptide part. A limitation of this strategy is that SPPS cannot be used for the synthesis of long peptide chains. This problem can be overcome using native chemical ligation,⁸ which involves the reaction of an N-terminal cysteine residue with a C-terminal thioester. The reaction proceeds via transthioesterification followed by a spontaneous and irreversible S-N acyl shift to give the native peptide bond, illustrated in Scheme 1.⁹

![Scheme 1](image1.png)

Scheme 1 Schematic illustration of native chemical ligation.

Another method for the preparation of well-defined glycoproteins uses a combination of site-directed mutagenesis and chemical attachment of synthetic oligosaccharides. This dual step strategy provides a flexible method, which allows both regio- and glycan-specific glycosylations of proteins. As cysteine is one of the rarest amino acids, it is the most commonly targeted residue when site-selective modifications are required, resulting in S-linked glycoproteins.¹ By introduction of a cysteine, a chemoselective thiol tag on a preselected position in the protein is set up. The free cysteine thiol can then be modified with electrophilic thiol-specific carbohydrate reagents, such as glycosyl methanethiosulfonates¹⁰ or glycosyl selenylsulfides¹¹ resulting in disulfide linked glycoproteins, illustrated in Scheme 2.

![Scheme 2](image2.png)

Scheme 2 Site-directed mutagenesis in combination with chemoselective ligation allows for site-selective protein modification.¹

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¹ Dawson, P.E; Muir, T.W; Clark-Lewis, I; Kent, S.B.H; Science, 1994, 5186, 776-779.
An alternative approach for preparing well-defined glycoproteins is to introduce an unnatural amino acid, such as dehydroalanine (Dha), azidohomoalanine (Aha) and homopropargylglycin (Hpg), into the protein and use these for attachment of the saccharide. Aha, Hpg and Dha are shown in Scheme 3.

![Scheme 3 Structure of Dha, Hpg and Aha.](image)

A procedure to directly convert cysteine in a protein to Dha by oxidative elimination using O-mesitylenesulfonylhydroxylamine has been reported by Davis and co-workers. Site-specific glycosylation of Dha can then be achieved by conjugate addition of a glycosyl thiol nucleophile illustrated in Scheme 4.

![Scheme 4 Synthesis of a thioether linked glycopeptides using the formation of dehydroalanine.](image)

The amino acids Hpg and Aha can be incorporated into proteins by using a modified aminoacyl-tRNA synthetases to charge the non-natural amino acid to a corresponding tRNA, which then incorporates the amino acid into a polypeptide or protein during recombinant synthesis. The copper(I)-catalysed azide-alkyne cycloaddition (CuAAC) has been used for the attachment of a glycan to Hpg and Aha residues as shown in Scheme 5.

![Scheme 5 Formation of triazole linked glycopeptides using CuAAC. A: Reaction between Hpg and an azidoglycoside. B: Reaction between Aha and an alkynyl-C-glycoside.](image)

One example of an orthogonal protein modification has been published by Davis and co-workers and makes use of the CuAAC between Hpg and an azidoglycoside as well as the reaction between cysteine and a methane-thiosulfonate functionalised glycoside.

Other techniques for the synthesis of glycoproteins is described in the literature but will not be further mentioned here. Even though several techniques exist for glycoprotein synthesis, availability of other ligation protocols, preferably in mild aqueous solution and orthogonal to already existing methods would extend the scope of protein glycosylations.

1.4 Project idea

The aim of this project is to establish a novel chemical approach to link a carbohydrate to a protein under mild conditions to preserve protein activity. By installing an aldehyde-tag in a protein, we envision that this can be used as a chemical handle for conjugation through the indium mediated Barbier-type allylation. This reaction is known to be compatible with aqueous media, but has until this point exclusively been used for synthetic and not for bioconjugative purposes.\textsuperscript{15}

Bertozzi and co-workers\textsuperscript{16} have reported a method for the site-specific introduction of an aldehyde group into a recombinant protein. It uses a short amino acid recognition motif, which \textit{in vivo} directs the oxidation of a specific cysteine to formylglycine (FGly) using the formylglycine generating enzyme (FGE). This is illustrated in Scheme 6A. Other approaches for the introduction of an aldehyde functionality into a protein is by the periodate oxidation of an N-terminal serine or threonine residue or by using the enzymatic co-factor pyridoxal-5'-phosphate (PLP) for the modification of an N-terminal glycine, which is illustrated in Scheme 6B.

By performing the indium mediated Barbier-type allylation between the aldehyde functionality and a carbohydrate moiety containing an allylbromide functionality, such as 1, a glycopeptide can be formed as illustrated in Scheme 7.

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The product of the Barbier-type allylation is a homoallylic alcohol, which can be useful for further conjugation. By using the photoinduced thiol-ene coupling, we envision that a thioglycoside 2 can be attached to the alkene functionality, shown in Scheme 7. In this way, the same aldehyde tag can function as a double attachment site for conjugation.

Both the indium mediated Barbier-type allylation and the photoinduced thiol-ene coupling is compatible with an aqueous environment takes place at room temperature, which is ideal for protein functionalisation. Furthermore, the indium mediated Barbier-type allylation is orthogonal to the glycoprotein synthesis using disulphide and triazole formation mentioned in section 1.3, and can therefore be considered to be a significant expansion of current methodologies for glycoprotein synthesis. This will allow for the preparation of the first homogeneous protein containing three well-defined and different modifications with complete control of site of attachment.

To develop the methodology for attachment of a glycan to a protein using the Barbier reaction followed by the thiol-ene coupling, a simplified test system will be needed for optimization of the reaction conditions. An artificial amino acid building block containing an aldehyde tag can be incorporated into a peptide through SPPS. Also a carbohydrate containing an allylbromide functionality, 1, and a thioglycoside 2 will to be synthesised.

1.5 Indium-mediated Barbier-type allylation

As mentioned in the project idea, aldehydes can undergo the Barbier-type allylation reaction. This is a one-pot reaction, where the carbonyl group reacts with the metal activated allyl halide to form a homoallylic alcohol. The product, containing both a double bond and a hydroxyl functionality can be further derivatised (Scheme 8).

\[
\text{Scheme 8 Indium-mediated allylation of an aldehyde with allylbromide.}
\]

Indium is an attractive metal in the Barbier reaction due to its low first ionization potential, which makes it more reactive than for instance Mg, Zn and Sn, as well as its inertness towards water and its relatively low toxicity. Also indium has a low tendency to form oxides in the presence of oxygen, which makes pre-activation of the metal unnecessary. The use of indium metal as a reducing agent in the Barbier-type allylation reaction was first reported by Araki et al. in anhydrous DMF. Li et al. have later reported the use of aqueous media for this reaction.

The regioselectivity for the allylation reaction with γ-substituted allyl halides is mostly determined by steric effects. The reaction gives the γ-adduct exclusively except when the γ-substituent is too bulky. The chosen solvent can affect the regioselectivity as well. In anhydrous solvents or in aqueous solution, the γ-product is observed exclusively. However, the regioselectivity can be changed from γ-selective to

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almost completely α-selectivity by using an organic solvent containing exact 6 eq water relative to the amount of aldehyde.\(^{22}\)

![Scheme 9](image)

_Scheme 9_ Possible regioisomers for the allylation reaction with a γ-substituted allyl halide.

Schmidt et al.\(^{23}\) were the first to use the Barbier-type allylation reaction with tin for unprotected carbohydrates. Later Kim et al.\(^{24}\) reported the allylation of unprotected carbohydrates using indium.

### 1.6 Thiol-ene coupling

The homoallylic alcohol, formed in the Barbier-type allylation, can be modified by adding a thiol to the alkene using an alkene hydrothiolation reaction, the so-called thiol-ene coupling (TEC).\(^{25}\) TEC is a thermally or photoinduced reaction which proceeds by a radical mechanism in an anti-Markovnikov regioselective fashion, shown in Scheme 10.\(^{26}\)

![Scheme 10](image)

_Scheme 10_ Mechanism for the thiol-ene coupling.

The advantages of the TCE reaction are that it is compatible with oxygen and water, can be carried out at room temperature, is totally atom economical, tolerant to a wide range of functional groups and mostly high yielding. Another advantage is the absence of a toxic transition-metal catalyst. The main observed side-product arises from the thyl radical homocoupling leading to disulfide formation. A known drawback of the TEC is the reversibility of the thyl radical addition to the alkene double bond, however this vary substantially depending on the structure of the reagents, the reaction temperature and the concentration of the thiol.\(^{27}\) A point of concern is the low tolerance of biomolecules to high-energy UV irradiation, however, in the presence of a suitable photoinitiator, such as 2,2-dimethoxy-2-phenylacetophenone (DPAP), the TEC reaction can be performed with low-energy light at 365 nm.

![Scheme 11](image)

_Scheme 11_ Use of DPAP as photoinitiator.

---

Since the reaction can proceed in aqueous solutions, it is well suited for reactions with proteins and oligosaccharides.\textsuperscript{27}

### 1.7 Formation of an aldehyde functionality

#### 1.7.1 Introduction of formylglycine

As previously described, Bertozzi and co-workers\textsuperscript{16} have reported a method for the site-specific introduction of an aldehyde group into a recombinant protein. It uses a short amino acid recognition motif, which \textit{in vivo} directs the oxidation of a specific cysteine to formylglycine (FGly) using the formylglycine generating enzyme (FGE). This is illustrated in Scheme 12. In Nature this unusual FGly amino acid is the essential residue found in type I sulfatase enzymes. The modification is thought to occur co-translationally and is critical for the sulfatases catalytic function.

![Scheme 12](image_url)

Scheme 12 Formylglycine-generating enzyme oxidises Cys to FGly if it is found in the consensus sequence.

Bertozzi and co-workers incorporated the FGly aldehyde tag-sequence into recombinant proteins using a bacterial expression system by incorporating the DNA sequence encoding the recognition motif into the cDNA of the target proteins using standard molecular biology techniques. The protein was then co-expressed with bacterial FGE, giving the FGly modified protein.\textsuperscript{16}

FGE recognises its substrate solely based on a conserved 13 amino acid consensus sequence around the target cysteine, that is highly conserved in all type I sulfatases across all species.\textsuperscript{28} Bertozzi and co-workers have shown that a minimised 6-residue sequence LCxPxR (Scheme 12), derived from the most highly conserved portion of the FGE recognition site, also efficiently directs the conversion of Cys to FGly.\textsuperscript{16} The recognition motif can be placed both at the C- or N-terminus of recombinant proteins with high conversion of the Cys to FGly using FGE.\textsuperscript{29} This can be used for site-specific modification by replacing a natural glycosylation site with the consensus sequence.

In order to be able to incorporate a FGly into a short peptide using SPPS, Bertozzi and co-workers\textsuperscript{17} have reported an efficient synthesis of a Fmoc-protected FGly building block in six steps in >70% overall yield with the aldehyde functionality protected as a diethylacetal (Fmoc-FGly(OEt)\textsubscript{2}OH) \textsuperscript{3}.

![Scheme 13](image_url)

Scheme 13 Incorporation of FmocFGly(OEt)\textsubscript{2}OH \textsuperscript{3} into a tetradecapeptide, reported by Bertozzi and co-workers.\textsuperscript{27}


The building block was incorporation into a synthetic peptide containing the consensus sequence recognised by FGE (Scheme 13). The diethylacetal could be removed alongside the other side chain protecting groups during the acidic cleavage step of Fmoc-based SPPS.

This procedure affords an approach to synthesise a peptide containing the FGly amino acid for testing and optimising the Indium-mediated Barbier reaction and the TEC reaction, before exploring the reactions on proteins.

1.7.2 Synthesis of N-terminal aldehydes

Another approach for introducing aldehydes into peptides is by the periodate oxidation of N-terminal serine and threonine residues (Scheme 14).\(^{30}\)

![Scheme 14](image)

Scheme 14 Periodate oxidation of an N-terminal serine yielding an N-terminal aldehyde functionality.

Geoghegan et al.\(^ {30}\) have reported a site-selective oxidation of a 2-amino alcohol group with NaI\(_2\) in sodium phosphate buffer at pH 7 at room temperature. A disadvantage of this reaction is that sodium periodate may not always be compatible with proteins containing a large number of cysteine or methionine residues.\(^ {31}\)

The enzymatic co-factor pyridoxal-5’-phosphate (PLP) can also be used for modifying proteins and peptides containing an N-terminal glycine to an aldehyde without modifying lysine side chain amines.\(^ {32}\) This technique has been shown to tolerate free cysteine residues\(^ {31}\) and can be performed in aqueous buffer at pH 6.5 at 37°C.\(^ {32}\)

The PLP-mediated transamination most likely begins with the formation of a Schiff’s base. The imine formed at the N-terminus has an α proton with a much lower pK\(_a\) value than lysine side chain amines, which allows tautomerisation to occur only at this site. Subsequent hydrolysis affords the aldehyde functionality as shown in Scheme 15.\(^ {32}\)

![Scheme 15](image)

Scheme 15 General mechanism for PLP mediated oxidation of N-terminal glycine to an aldehyde.

The development of the reaction for proteins was done using horse heart myoglobin, which has an N-terminal glycine residue.\(^ {32}\) Alam et al.\(^ {33}\) have recently used this PLP-mediated formation of N-terminal aldehyde to functionalise the N-terminus of horse heart myoglobin by the Mukaiyama aldol reaction.

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1.8 Results and discussion

1.8.1 Test of solvents systems for the indium mediated Barbier-type allylation

Initially a series of simple Barbier allylations was performed to find the most favourable solvent system. The indium mediated Barbier-type allylation has been reported to proceed in organic solvents, in aqueous solvents and in mixtures of these. Since the goal is to use the Barbier reaction for protein functionalisation an aqueous media is desired.

A qualitative screenings experiment at room temperature was set up using allylbromide 4, benzaldehyde 5 and indium in a mixture of organic solvent and either water or phosphate buffer (pH 7, 50 mM) to examine which solvent system, who gave the fastest reaction time. Some of the typical used solvents; ethanol, dioxane, tetrahydrofuran (THF), N,N-dimethylformamide (DMF) and acetonitrile were used. The results are shown in Scheme 16.

![Scheme 16 Qualitative screening for the optimal solvent system for the indium mediated Barbier-type allylation. Time for complete conversion of benzaldehyde 5 is shown. Benzaldehyde 5 1 eq, allylbromide 4 2 eq and indium 2 eq. Phosphate buffer pH 7.0 (50mM). 1:1 mixture of organic and aqueous solvent.](image)

<table>
<thead>
<tr>
<th>Organic solvent</th>
<th>Aq. solvent</th>
<th>Reaction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeCN</td>
<td>H₂O Buffer</td>
<td>90 min</td>
</tr>
<tr>
<td>EtOH</td>
<td>H₂O Buffer</td>
<td>10 min</td>
</tr>
<tr>
<td>Dioxane</td>
<td>H₂O Buffer</td>
<td>10 min</td>
</tr>
<tr>
<td>DMF</td>
<td>H₂O Buffer</td>
<td>60 min</td>
</tr>
<tr>
<td>THF</td>
<td>H₂O Buffer</td>
<td>20 min</td>
</tr>
<tr>
<td>-</td>
<td>H₂O Buffer</td>
<td>ON</td>
</tr>
</tbody>
</table>

The reactions were continuously monitored by TLC analysis to follow the conversion of the reactions. No attempt was done to quantify the amount of product, since this experiment only served to see which solvent systems were favourable to use. The formation of a milky white colour in the reaction mixture was a good reaction that the reaction was started. All reactions went to completion, as analysed by TLC, but major differences was obtained in the reaction times.

As can be notices from Scheme 16, performing the reactions in H₂O or buffer without an organic co-solvent, as well as using THF as the organic co-solvent resulted in very long reaction times. The reaction in acetonitrile and dioxane were considerably faster with H₂O compared to buffer even though dioxane was reasonable fast together with both. The choice of H₂O or buffer did not make any difference in the reaction time for reactions in DMF or EtOH. From these experiments it could be concluded that EtOH and dioxane resulted in the fastest reactions for this reaction in H₂O while EtOH was the best solvent to use together with phosphate buffer.

1.8.2 Test of buffer systems for the indium mediated Barbier-type allylation

Besides phosphate buffer, a wide range of other buffers for biological systems are available. Having established the utility of EtOH as a favourable organic solvent in the indium mediated Barbier-type allylation, five buffers; TEAA (triethylammonium acetate), HEPES (4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid), MES (2-(N-morpholino)ethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid) and phosphate buffer (NaH₂PO₄/Na₂HPO₄) were examined together with EtOH as solvent in the Barbier reaction using allylbromide 4, benzaldehyde 5 and indium.
(as shown in Scheme 16). The reactions were performed in a 1:1 mixture of EtOH and buffer at room temperature. Different pH values were examined for determining the optimal reaction conditions. Other common buffers such as Tris (tris(hydroxymethyl)aminomethane) were avoided since the aldehyde functionality is incompatible with a primary amine functionality. Again the reactions were continuously analysed by TLC and the product 6 to aldehyde 5 ratio was determined by analysing the TLC. The results are shown in Table 1.

**Table 1** Qualitative screening of buffer systems at room temperature for the indium mediated Barbier-type alkylation using allylbromide 4, benzaldehyde 5 and indium. Time for conversion of benzaldehyde is shown. Conditions: Benzaldehyde 5 1 eq, allylbromide 4 2 eq and indium 2 eq. Solvent is a 1:1 mixture of EtOH and aqueous buffer (50 mM).

<table>
<thead>
<tr>
<th>Buffer PH</th>
<th>TEAA</th>
<th>HEPES</th>
<th>MES</th>
<th>MOPS</th>
<th>NaH₂PO₄/Na₂HPO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>FC</td>
<td>-</td>
<td>FC</td>
<td>40 min</td>
<td>-</td>
</tr>
<tr>
<td>6.0</td>
<td>FC</td>
<td>-</td>
<td>2:1 P/A 18 h</td>
<td>-</td>
<td>FC 15 min</td>
</tr>
<tr>
<td>6.5</td>
<td>FC</td>
<td>-</td>
<td>1:1 P/A 18 h</td>
<td>FC 25 min</td>
<td>2:1 P/A 18 h</td>
</tr>
<tr>
<td>7.0</td>
<td>FC</td>
<td>NC</td>
<td>2:1 P/A 18 h</td>
<td>FC 25 min</td>
<td>FC 10 min</td>
</tr>
<tr>
<td>7.5</td>
<td>-</td>
<td>NC</td>
<td>-</td>
<td>2:1 P/A 18 h</td>
<td>FC 10 min</td>
</tr>
<tr>
<td>8.0</td>
<td>1:1 P/A 18 h</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

FC: Full conversion of benzaldehyde. NC: No conversion of benzaldehyde after 18 h. P/A: Product 6/Aldehyde 5.

All mixtures with TEAA turned instantly white after the addition of Indium. Full conversion of the aldehyde to the product was observed using pH 6.0, 6.5 and 7.0 after 10 min, while the reaction at pH 5.5 was slow and reached full conversion over night. At pH 8.0 only around half of the starting material was converted after 60 min and no further conversion was observed after 18 h. The employment of HEPES as buffer was not encouraging since only the reaction pH 6.5 gave any conversion of the starting material, but the reaction did not go to completion even after 18 h. No conversion was observed using pH 7.0, 7.5 and 8.0.

Using the MES and MOPS buffer, the reaction times were quite different across the pH values. Using the MES buffer product formation was observed in all four samples after 5 min. After 25 min full conversion of benzaldehyde was observed in pH 6.5, while the reaction in pH 5.5 completed after 40 min. pH 6.0 and 7.0 did not go to full conversion even after 18 h. In the reactions with MOPS as buffer conversion of aldehyde to product was observed in three samples, pH 6.5, 7.0 and 7.5 after 5 min. Full conversion was observed at pH 7.0 after 25 min, while pH 6.5 and 7.5 did not go to full conversion. No reaction was observed in the reaction mixture at pH 8.0.

Using phosphate buffer, full consumption of the aldehyde was observed after 10 min in pH 6.5, 7.0 and 7.5 while the conversion in pH 6.0 was slightly slower.

Overall from Scheme 16 and Table 1 it can be concluded that EtOH in combination with phosphate buffer at pH 6.5-7.5 or TEAA buffer at pH 6.0-7.0 offers the fastest conversion of benzaldehyde 5 to 1-phenylbut-3-en-1-ol (6) using indium as the mediating metal. In the following phosphate buffer at pH 7.0 in combination with EtOH are employed.

**1.8.3 Synthesis of allylic GlcNAc bromide 8**

A carbohydrate structure containing an allylbromide functionality needs to be synthesised for use in the indium mediated Barbier reaction. Here the synthesis of GlcNAc containing an allylbromide functionality 7 is described.
Peracetylated β-GlcNAc 8 was synthesised, as shown in Scheme 17. Following the procedure by Myszka et al., D-glucosamine hydrochloride 9 was transformed into the imine 10 using p-anisaldehyde. This was then converted into the tetraacetate 11 followed by hydrolysis of the imine to the hydrochloride salt 12. The amine 12 was acetylated to afford the desired GlcNAc donor 8. All intermediates were purified by re-crystallisations.

**Scheme 17** Synthesis of the peracetylated GlcNAc donor 8.

Ethyl bromocrotonate 13 was reduced to trans-4-bromo-2-buten-1-ol 14 using DIBAL. By using a methodology previously published by our group, the acceptor 14 was glycosylated with the GlcNAc donor 8 using Yb(OTf)₂ as promoter, resulting in 7 in 69% yield under microwave conditions.

**Scheme 18** Synthesis of allylbromide functionalized GlcNAc 7.

**1.8.4 Barbier reaction with the allylic GlcNAc bromide 7**

The optimised Barbier-conditions were examined using the allylic GlcNAc bromide 7 and benzaldehyde 5 as shown in Scheme 19. This reaction has previously been shown to afford the γ-product 15. According to TLC analysis, full conversion of the starting material 7 was archived. Unfortunately, besides the product 15, the by-product 16 was also formed due to the presence of EtOH. 16 was difficult to separate from the desired product 15 using column chromatography; therefore, the yield was not determined.

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34 Myszka, H; Bednarczyk, D; Najdar, M; Kaca, W; Carbohydrate Res., 2003, 338, 133-141.
36 This synthesis has previously been done by Ph.D.-student Marie Jensen.
37 This was done by Ph.D.-student Marie Jensen and project student Mathilde Vind Teilmann using different organic solvents and water as co-solvent.
Scheme 19 Test of Barbier conditions using allylic GlcNAc bromide 7 and benzaldehyde 5.

For use in later synthesis, a part of the allylic GlcNAc bromide 7 was deprotected. First classical Zemplén deprotection, catalytic NaOMe in MeOH, was used, however, besides cleavage of the acetyl groups, complete conversion of the bromide to the methoxide was achieved. Instead potassium carbonate in EtOH was useful, since in only a minor part of the deprotected product the bromide was substituted with ethoxide 17 (Scheme 20). However, storage of 1 was not tolerated due to further substitution of the bromide, so the deprotected allylic GlcNAc bromide 1 was synthesised just prior to use and subsequently used without any workup or characterisation.

Scheme 20 Deprotection of 7.

1.8.5 Synthesis of thioglycosides 18 and 19
Thioglycosides were required for use in the TEC reaction. It was decided to synthesise two 1-glycosyl thiols; peracetylated 2-acetamido-2-deoxy-1-thio-β-D-glucose 18 and peracetylated 1-thio-β-D-glucose 19 following the procedure reported by Floyd et al. N-acetyl-D-glucosamine 20 and pentaacetyl-β-D-glucopyranose 21 was converted respectively to the α-glucosyl chloride 22 or bromide 23 (Scheme 21 A and B). The reactions were α-selective due to the anomeric effect.

Scheme 21 Synthesis of glycosyl halides 22 and 23.

Glycosyl halides 22 and 23 were then converted into the corresponding glycosyl-β-thiols 18 and 19 through treatment with thiourea to afford the isothiouronium salts 24 and 25 followed by mild hydrolysis with sodium metabisulfite, as shown in and Scheme 22.

These two compounds could be used in the TEC reaction as the acetyl protected compounds or could be deprotected using classical Zemplén conditions.

### 1.8.6 Combined Barbier-reaction and thiol-ene coupling

A test reaction was performed by coupling the peracetylated β-GlcSH 19 to the previously synthesised homo allylic alcohol 15 (Scheme 19) using the photoinduced TEC reaction, as shown in Scheme 23. The reaction was accomplish using 2,2-dimethoxy-2-phenylacetophenone (DPAP) as photoinitiator and irradiating the reaction mixture with a handhold UVA lamp (λ_{max} 365 nm, 12W) at room temperature. Dichloromethane was used as solvent and the reaction was continuously monitored by LR-MS spectroscopy. After 1h and 15 min, the reaction had reached completion. LR-MS analysis showed that the product formed has a mass, which is 2 units lower than the expected mass 26. This indicates that the hydroxyl functionality has been oxidised to a ketone 27.

The reaction was repeated using cyclohexanecarbaldehyde 28, to avoid the oxidation. This time, the reaction was done in a one-pot manner, shown in Scheme 24 and followed by LR-MS.

Scheme 22 Synthesis of thioglycosides 18 and 19.

**Scheme 23** Photoinduced TEC reaction using DPAP as the photoinitiator, CH₂Cl₂ as solvent and by irradiating the mixture with a handhold UVA lamp (λ_{max} 365 nm). Expected mass 894 (26+Na), observed mass: 892 (27+Na).

The reaction was repeated using cyclohexanecarbaldehyde 28, to avoid the oxidation. This time, the reaction was done in a one-pot manner, shown in Scheme 24 and followed by LR-MS.

Scheme 24 One-pot synthesis of 30 using cyclohexanealdehyde 28, the allylic GLcNAc bromide 7 and the thioglucoside 19.
The homo allylic alcohol 29 was synthesised in a 1:1 mixture of EtOH and aqueous buffer. The product 29 was formed as well as the bi-product 16 (Scheme 19) resulting from substitution of bromide with ethoxide. The thioglycoside 19 and DPAP was added, and the resulting mixture was stirred under a UVA lamp (λmax 365 nm, 12W) at room temperature. The reaction was continuously monitored by LR-MS spectroscopy. After 2 h, full conversion of the homoallylic alcohol 29 was archived, and a mass corresponding to the disaccharide 30 was observed. The product was not further analysed or quantified. The conclusion is that the TEC reaction is compatible with the EtOH/NaHPO₄ buffer system, and the Indium-mediated Barbier reaction and the TEC reaction can be carried out using a one-pot strategy.

1.8.7 Synthesis of a peptide containing formylglycine

As described in section 1.7.1 (page 7) Bertozzi and co-workers¹⁶ have established a procedure for incorporation of FGly into a recombinant protein using the conserved consensus sequence recognised by FGE. The amino acid FGly containing the aldehyde functionality protected as a diethylacetal, Fmoc-FGly(OEt)₂OH 1, has previously been synthesised in the group by Ph.D.-student Marie Jensen and project student Mathilde Vind Teilmann by the procedure reported by Bertozzi and co-workers¹⁷.

It was decided to synthesise a small pentapeptide containing the sequence made after the oxidation of the cysteine to formylglycine with FGE, which is H₂N-Leu-FGly-Thr-Pro-Ser-OH (Scheme 12, page 7). A substantial amount of the pentapeptide was desired, so it was decided to synthesise the pentapeptide by solution phase synthesis using the Fmoc strategy.

Peptides are synthesised from the C- to the N-terminal, consequently the C-terminal of the serine Fmoc-Ser(tBu)-OH 31 needed to be protected before the amino acid can be elongated. The first attempted approach was to protect the acid as a primary amide. An approach inspired by the work reported by Pozdnev⁴¹ was carried out, as shown in Scheme 25. First a mixed anhydride is formed by reaction with Boc anhydride following by reaction with ammonia to afford the amide 32.

![Scheme 25 Synthesis of Fmoc-Ser(tBu)-OH](image)

Two different conditions were examined for the coupling of 32 with the Fmoc-Pro-OH. Using 20% piperidine in DMF for the Fmoc-deprotection, subsequently concentration of the crude mixture, and then coupling with Fmoc-Pro-OH using EDC, HOBt and DIPEA resulted in deprotection of the Fmoc group of 33. Using Et₂NH in CH₂Cl₂ for the deprotection of 32 followed by the same condition for the Fmoc-Pro-OH coupling did also not afford the desired product 33, Scheme 24. This approach was therefore abandoned.

Instead the C-terminal carboxylic acid was protected as a benzyl amide. Fmoc-Ser(tBu)-OH 31 was coupled to BnNH₂ using DCC and HOBt as coupling reagents. After workup, the resulting Fmoc-Ser(tBu)-

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NHBn amide (34) was purified by silica column chromatography and isolated in 90% yield. The product was then carried through repeated Fmoc deprotection using DBU, amino acid coupling using HBTU and HOBt and workup steps followed by purification and isolation of the intermediates. The synthetic route is shown in Scheme 26 and afforded the protected pentapeptide 38 in good yield.

Several attempts to deprotect the pentapeptide 38 were carried out. The first attempt used a mixture of 85:5:5:5 of TFA/thioanisole/anisole/H$_2$O as reported by Bertozzi and co-workers.$^{16,17}$ The reaction was followed by MALDI-TOF spectroscopy. After 2h full conversion of the protected peptide 38 was observed, and a compound was formed with a mass at 817 and 833 (Table 2). This did not correspond to the expected mass for 39 (Table 2), but match with deprotection of the peptide and the formation of dehydroalanine at the serine position 40 (Scheme 27). Using a 95:5 mixture of TFA/H$_2$O the masses 817 and 833 was again observed.

To confirm the presence of dehydroalanine in 40, a test for the alken functionality was carried out using EtSH in phosphate buffer (pH 8.5, 37°C) by a procedure by Bernandes et al.$^{12}$ (Scheme 27). However, no conversion of the starting material 40 to 41 was observed by MALDI-TOF spectroscopy.

To confirm the presence of formylglycine in 40, a hydroxylamine was added in order to form the corresponding oxime, but no conversion of 40 to 42 was observed using MALDI-TOF spectroscopy using the three conditions shown below (Scheme 27).

- methoxamine (KOAc/HOAc buffer, pH 4.6, 37°C).
- o-benzylhydroxylamine (phosphate buffer, pH 6.5, room temperature).
- o-benzylhydroxylamine (MES buffer pH 5.5, 37°C).$^{16}$

Table 2 Calculated mass of 39 and 40 shown in scheme Scheme 26 and Scheme 27.

<table>
<thead>
<tr>
<th>Product</th>
<th>+Na</th>
<th>+K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product 39</td>
<td>835</td>
<td>851</td>
</tr>
<tr>
<td>Product with Dha 40</td>
<td>817</td>
<td>833</td>
</tr>
</tbody>
</table>
Before the removal of the acid labile protecting groups, it was decided to remove the Fmoc group in **38** using diethyl amine in acetonitrile. The Fmoc group in **38** was cleaved in a clean reaction as analysed by LR-MS. This was followed by deprotection of the acid labile groups with a mixture of TFA/thioanisole/anisole/H$_2$O (85:5:5:5). The reaction was continuously followed by MALDI-TOF analysis. After 10 min, the intermediate was consumed, and MALDI-TOF analysis indicated formation of **43**. However, after precipitation from Et$_2$O, addition of H$_2$O and freeze-drying, no product could be detected by MALDI-TOF analysis. This procedure was carried out several times without success.

**Scheme 28** Deprotection of **38** using Et$_3$NH in MeCN followed by TFA/thioanisole/anisole/H$_2$O. After workup, no product could be detected by MALDI-TOF analysis.

### 1.8.8 Optimisation of the peptide deprotection

Since the acidic deprotection of the pentapeptide **38** proved to be difficult, it was decided to switch to a smaller system to see, if the diethylacetal could be cleaved with success. The C-terminal of the Fmoc-FGly(OEt)$_2$-OH **3** was first protected as a benzyl amine **44**, as shown in Scheme 29.

**Scheme 29** Synthesis of Fmoc-FGly(OEt)$_2$-NHBn **44** and attempt to deprotect the diethylacetal to afford **45**. Williams and co-workers$^{42}$ have in their synthesis of the peptide Capreomicin IB previously reported that this diethylacetal group could be cleaved in a 1:1 mixture of acetone and 2M HCl under reflux. TLC analysis indicated complete conversion of the starting material **44** after 2 h, but NMR analysis on the crude material after workup was difficult to interpret and did not clearly reveal the outcome of the

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reaction. To ease the NMR interpretation, it was decided to replace the Fmoc protecting group in 44 with an acetyl. Scheme 30 shows the synthesis of Ac-FGly(OEt)₂-NHBn 46.

Scheme 30 Conversion of Fmoc-FGly(OEt)₂-NHBn 44 to Ac-FGly(OEt)₂-NHBn 46 and attempt to deprotect the diethylacetal to afford 47.

Several conditions were screened for the cleavage of the diethylacetal 46, shown in Table 3. Using 1:1 mixtures of aqueous HCl and acetone or dioxane or using a 1:1 mixture of TFA and H₂O resulted in full consumption of the starting material 46. After work up, the crude mixtures was analysed by ¹H-NMR spectroscopy, but the NMR spectra were still difficult to interpret. As a final attempt to confirm the cleavage of the aldehyde functionality in 46, the reaction was carried out in a NMR tube using a 1:1 mixture of 0.5 M DCl in D₂O and acetonitrile d₃. After 5 min at room temperature, the reaction was analysed by NMR spectroscopy, showing no conversion. After heating the mixture to 80°C for 20 min, the signals from the diethylacetal had disappeared. No aldehyde signal was observed due to the presence of a deuterium instead of a hydrogen atom in 47. It was concluded that the diethylacetal was cleaved, but during work-up the aldehyde functionality is probably transformed back to the protected acetal or into a geminal diol.

Table 3 Conditions screened for cleavage of the diethylacetal 46 shown in Scheme 30. The reactions were first stirred at 0°C and then allowed to warm up to room temperature and stirred over night. The reactions were continuously monitored by TLC analysis.

<table>
<thead>
<tr>
<th>#</th>
<th>Conditions</th>
<th>TLC analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:1 2M HCl/Acetone</td>
<td>Full conversion of 46</td>
</tr>
<tr>
<td>3</td>
<td>1:1 2M HCl/dioxane</td>
<td>Full conversion of 46</td>
</tr>
<tr>
<td>4</td>
<td>cat.: 1:1TFA/THF/H₂O</td>
<td>No conversion of 46</td>
</tr>
<tr>
<td>5</td>
<td>1:1 TFA/H₂O</td>
<td>Full conversion of 46</td>
</tr>
<tr>
<td>6</td>
<td>cat.:1:1 pTsOH/Acetone/H₂O</td>
<td>No conversion of 46</td>
</tr>
</tbody>
</table>

As a final attempt to confirm the cleavage of the aldehyde functionality in 46, the reaction was carried out in a NMR tube using a 1:1 mixture of 0.5 M DCl in D₂O and acetonitrile d₃. After 5 min at room temperature, the reaction was analysed by NMR spectroscopy, showing no conversion. After heating the mixture to 80°C for 20 min, the signals from the diethylacetal had disappeared. No aldehyde signal was observed due to the presence of a deuterium instead of a hydrogen atom in 47. It was concluded that the diethylacetal was cleaved, but during work-up the aldehyde functionality is probably transformed back to the protected acetal or into a geminal diol.

Scheme 31 One-pot cleavage of the diethylacetal 46 to afford 47 using a 1:1 mixture of HCl and MeCN/EtOH/dioxane or DMF, followed by C-C bond formation using the indium-mediated Barbier reaction between 47 and allylbromide 4.

A last idea was to do the acetal cleavage and the indium-mediated Barbier-reaction in a one pot reaction (Scheme 31). Four different solvent were tested for the reaction: MeCN, EtOH, dioxane and DMF. A 1:1 mixture of an aqueous solution of HCl and the solvent of choice containing 46 was heated to 80°C and monitored by TLC analysis. After 1 h Na₃PO₄ was added (to pH 7) together with indium and allyl bromide.

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43 MS analysis was not possible at this time due to technical problems.
4. The mixture was stirred at room temperature over night. Disappointingly TLC analysis showed no conversion of 47 to 48.43

This FGly strategy was abandoned and methods for preparing peptides having the aldehyde functionality at the N-terminal were instead investigated.

1.8.9 **Synthesis of an N-terminal aldehyde functionality**

In order to synthesise an N-terminal aldehyde functionality, a dipeptide containing a N-terminal serine residue 49 was synthesised. This was then oxidised with NaIO₄ to the corresponding aldehyde.

The small dipeptide H₂N-Ser-Phe-NHBn 49 was synthesised using the Boc strategy shown in Scheme 32. The free carboxylic acid in Boc-Phe-OH was protected as a benzyl amine 50. Acidic cleavage of the Boc group followed by coupling to Boc-Ser-OH afforded the dipeptide 51. After cleavage of the Boc group the free N-terminal amine 50 was obtained. NaIO₄ oxidation of the N-terminal amine in sodium phosphate buffer provided the aldehyde 52.

1.8.10 **Indium-mediated Barbier reaction on dipeptide 52**

Aldehyde 52 was used in the indium-mediated Barbier reaction with allylbromide 4 in a 1:1 mixture of phosphate buffer (pH 7.0) and an organic solvent: MeCN, EtOH, dioxane, DMF or THF, shown in Scheme 33A. The reaction mixture was stirred at room temperature over night. The reaction was analysed using LR-MS.

Scheme 32 Synthesis of dipeptide 49 with a N-terminal aldehyde functionality and oxidation of 49 to 52.

Scheme 33 A) Test of the indium-mediated Barbier reaction using the N-terminal aldehyde 52. Allylbromide 4 was used as allylating agent in screenings reaction with different 1:1 mixtures of organic solvents and phosphate buffer. Product formation was analysed by LR-MS. Only reactions with EtOH and dioxane afforded the product 53. B) Failed coupling of the allylic GlcNAc bromide 1 to the N-terminal aldehyde 52.
The organic solvents MeCN, DMF and THF did not result in product formation, while EtOH gave full conversion of the aldehyde 52 to the allylic alcohol 53. Dioxane gave only partly conversion of 52 to 53. Next was the N-terminal aldehyde 52 coupled to the deprotected allylic GlcNAc bromide 1 (Scheme 33 B) using EtOH and phosphate buffer, but LR-MS showed formation of a unidentified product with a mass 12 units lower than the expected product 54 (+Na). This reaction has currently only been performed once.

1.8.11 Failed synthesis of N-terminal of horse heart myoglobin 55

Horse heart myoglobin has a N-terminal glycine residue and can be oxidised using PLP as reported by Francis and co-workers. It was decided to synthesise the first ten amino acids of the N-terminus, H2N-GLSDGEWQQV-CH2-OH 55, to use the decapeptide as a model substrate for the indium-mediated Barbier reaction. The decapeptide was synthesised using SPPS using a Fmoc-L-Val-wang resin. Each coupling step was accomplished using HBTU and DIPEA in DMF. Piperazine instead of piperidine was used for the deprotection, since the D-G sequence is prone to scrambling, forming the β-amino acid. The peptide was cleaved off the resin, precipitated and freeze-dried. Unfortunately it was difficult to find a suitable solvent for the purification using reverse phase HPLC. Only HFIPA (1,1,1,3,3,3-hexafluoro-2-propanol) could dissolve the peptide, which is known to be a problematically solvent to use in HPLC purification due to formation of very broad peaks. The HPLC purification was therefore not successful.

1.8.12 Oxidation of N-terminal Serine using NaIO4

A decapeptide 56 with an N-terminal serine was kindly provided by Erik Nielsen from the INSPIN laboratory (Scheme 34). Using NaIO4 in phosphate buffer, the N-terminal serine 56 was oxidised to form the aldehyde functionality 57 (analysed with MALDI-TOF spectroscopy). This mixture was divided into two and EtOH was added. Allylbromide 4 and indium was added to one part and the deprotected allylic GlcNAc bromide 1 and indium was added to the other part. The resulting mixtures were stirred at room temperature over night.

MALDI-TOF analysis on the reaction with allylbromide 4 revealed partly conversion till the desired product 58. Prolonged stirring did not lead to full conversion. MALDI-TOF analysis also indicated that the Barbier reaction had worked using the deprotected allylic GlcNAc bromide 1, however the observed mass was 2 units lower than the expected product (+Na) indicating the homoallylic alcohol has been oxidised to a ketone 59 (See APPENDIX A for MALDI-TOF analysis). Only partly conversion of the aldehyde 57 was observed.

Scheme 34 Attachment of allylbromide 4 and the allylic GlcNAc bromide 1 to the N-terminal aldehyde 57. None of the reactions did go to completion.
These reactions prove that the indium mediated Barbier-reaction is a durable way for the preparation of glycopeptides, even though further optimisation is necessary to obtain full conversion of the aldehyde.

1.9 Conclusion
The aim of this project was to establish a novel method for the site-selective attachment of a carbohydrate to a peptide or protein using the indium-mediated Barbier reaction in aqueous solution. Initially, several experiments were carried out using a simplified test system using benzaldehyde and allylbromide to determine the optimal conditions for this reaction. The rate of the reaction was considerably faster using an organic co-solvent compared to conducting the reaction with either water or phosphate buffer as the only solvent. Ethanol turned out to be the best organic co-solvent, while both phosphate buffer and TEAA buffer were equally efficient in the screening experiments using a 1:1 mixture of ethanol and buffer.

An allylic GlcNAc bromide was synthesised and applied in further studies of the indium-mediated Barbier reaction. The bromide was easily displaced by ethoxide in the Barbier reaction, and the resulting by-product was observed in all Barbier reactions using ethanol as solvent.

A peptide containing an aldehyde functionality was synthesised using the amino acid building block Fmoc-FGly(OEt)₂-OH. However, the deprotection of the diethyacetal was problematic. Instead a peptide containing an N-terminal serine was oxidised to form the aldehyde functionality using NaIO₄ in phosphate buffer. The N-terminal aldehyde was successfully allylated using the Barbier-reaction using both allylbromide and the allylic GlcNAc bromide. However, when the allylic GlcNAc bromide was applied, MALDI-TOF spectroscopy indicated that the homoallylic alcohol was oxidised to form a ketone.

The homo-allylic alcohol could be further conjugated using the thiol-ene reaction. For this purpose two thio-glucosides were synthesised. In a one-pot proof of principle experiment, the allylic GlcNAc bromide was first reacted with cyclohexanecarbaldehyde by the indium-mediated Barbier reaction. Then a thioglucoside and the photoinitiater DPAP were added and the resulting mixture was stirred under UV irradiation. Analysing the reaction mixture with MS indicated the formation of the desired disaccharide.

The results obtained so far have shown that the indium-mediated Barbier reaction can be used to functionalise peptides containing an aldehyde functionality with a carbohydrate containing an allylbromide functionality using indium in a 1:1 mixture of EtOH and phosphate buffer. Initial experiments have indicated that the alkene functionality formed in the Barbier reaction can be used for further conjugation using the thiol-ene reaction in a one-pot procedure.

This work presented was carried out from April 2010 to February 2011 where the project temporarily was put on hold in favour of the synthesis of the Lewis b hexasaccharide at University College Dublin. In July 2011 Alam et al.¹⁵ published a paper in Chemical communications describing site-selective functionalisation of N-terminal aldehydes using the indium-mediated Barbier reaction. By the use of the periodate oxidation, a N-terminal serine in a di- and tetrapeptide is converted to a N-terminal aldehyde, which is allylated using allylbromide and indium in sodium phosphate buffer. Furthermore they form the aldehyde functionality at the N-terminus of horse heart myoglobin by oxidising the N-terminal glycine residue with PLP in sodium phosphate buffer. Also in this case they are able to form the allylation product using allylbromide and indium in a 4:6 mixture of tert-butanol/water.
In the light of this publication, there is no rationale to continue the methodology development of the indium-mediated Barbier reaction for the synthesis of glycoproteins, and the project is therefore abandoned.

1.10 Future perspectives

Further development of the one-pot indium-mediated Barbier allylation followed by the thiol-ene coupling will be carried out in order to synthesise novel trisaccharides. The Barbier reaction between an aldehyde functionalised glycoside 60 and an allylic glycoside bromide 1, both in unprotected form, will result in a disaccharide 61. This can subsequently be coupled to an unprotected thioglycoside 2 by forming a thioether linkage using the thiol-ene coupling, resulting in a trisaccharide 62. This will be carried out using a one-pot strategy in aqueous solution. The idea is illustrated in Scheme 35.

Scheme 35 Future perspectives; synthesis of trisaccharides in a one-pot reaction.

This method would complement the results, recently published by Crich and co-workers. They have reported the synthesis of novel disaccharides 63 by reacting a thioglucoside 64 with an allylic disulfide 65 by adding triphenylphosphine or silver nitrate. These reactions are carried out at room temperature in a protic solvent without the need for the use of protecting groups. This is illustrated in Scheme 36.

Scheme 36 Work by Crich and co-workers. Synthesis of disaccharides 63 using the desulfurative rearrangement between a thioglucoside 64 and an allylic disulfide 65.

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PART II
SYNTHESIS OF THE LEWIS B HEXASACCHARIDE

2.1 Helicobacter pylori

*Helicobacter pylori* are spiral shaped gram negative bacteria, which cause gastric and duodenal ulcers and are a major cause of gastric cancer. The bacterium selectively colonizes the gastric epithelium. Unlike other viruses and bacteria, *Helicobacter pylori* have evolved the ability to survive the highly acidic environment by metabolising urea to ammonia and carbon dioxide via urease; in this way, the bacterium surrounds itself with a layer of ammonia, which neutralises stomach acid in its immediate vicinity. Therefore, the bacteria can persist for decades in the harsh gastric environment due to inability of the host to eliminate the infection. The bacteria express at least five types of adhesins, which enables them to adhere to the stomach epithelium, and they produce several cytotoxins that destroy stomach epithelial cells, creating painful ulcers. The resulting chronic inflammation promotes cell proliferation and thus predisposes the individual to stomach cancer.\(^{45}\)

2.2 Blood group antigens

Included among cell surface glycoconjugates are the carbohydrate antigens of the Lewis family. These carbohydrates serve a variety of functions such as control of cell growth and differentiation, cell-cell adhesion and immune response in inflammatory processes.

Structurally, the blood group determinants fall into two basic categories known as type I and type II. Type I is characterised by a backbone comprised of a galactose 1-3β linked to N-acetylglucosamine, while type II contains a 1-4β linkage between the same building blocks. The position and extent of α-fucosylation of these backbone structures give rise to the four types of Lewis sugars. Monofucosylation at the C$_4$-hydroxyl of the N-acetylglucosamine (type I series) affords the Lewis a type, whereas fucosylation of the C$_3$-hydroxyl of this sugar (type II series) constitutes the Lewis x type. Additional fucosylation of Lewis a and Lewis x at the C$_2$-hydroxyl of the galactose unit affords, respectively, the Lewis b and Lewis y types.\(^{46}\) The structures are shown in Scheme 37.

![Scheme 37 Structure of Lewis a, Lewis b, Lewis x and Lewis y.](image)


2.3 Connection between Helicobacter pylori and Lewis b

One of the five adhesins expressed by Helicobacter pylori is the “blood group antigen binding adhesin” (BabA) and is an outer membrane protein. BabA binds to the Lewis b antigen on the surface of gastric epithelial cells.\(^{47}\) That means, Helicobacter pylori attaches to the surface of Lewis b expressing cells.

Analyses of binding specificities of Helicobacter pylori strains from across the world suggest that the BabA adhesin has evolved in response to host mucosal glycosylation patterns to permit Helicobacter pylori to adapt to its host, and to maintain persistent colonisation.\(^{45}\)

2.4 Project idea

The aim of this project is to synthesise the Lewis b hexasaccharide (66 shown in Scheme 38) for the use in two separate, but related projects.

\[ \text{Scheme 38 Structure of the spacer-equipped Lewis b hexasaccharide 66 and the porphyrin skeleton 67.} \]

2.4.1 Investigation of binding between BabA and Lewis b

The first project seeks to investigate the carbohydrate-protein bindings between the Lewis b hexasaccharide and BabA.

Since carbohydrate – protein interactions in general are relatively weak, it would be advantageous to have a defined cluster able to display more than one carbohydrate unit. By attaching the Lewis b carbohydrate to a porphyrin, it will be possible to synthesise different dendrimers. In collaboration with Associate Professor Eoin M. Scanlan and co-workers in Trinity College, Dublin, the Lewis b conjugate 66 will therefore by click chemistry be linked to the porphyrin skeleton 67, shown in Scheme 38, with different loadings (1-4) with conditions previously developed in Scanlan’s group\(^{48}\).

The Lewis b 66 structure shown in Scheme 38 has to be modified further in order to be used in the click reaction by coupling it with a linker containing a terminal alkyne. The purpose of this project is to investigate how the binding to BabA is affected by presenting a specific number of the Lewis b structure to the protein. The porphyrin is in this project used as a scaffold to attach the Lewis b hexasaccharide.


2.4.2 Investigation of the use of a Lewis b linked porphyrin as a drug delivery system

The second application for the Lewis b linked porphyrin will be to use it as a drug delivery system for treating infections with *Helicobacter pylori* by photodynamic therapy (PDT).\(^{49}\)

PDT is based on the use of photosensitisers, such as a porphyrin, which are preferentially taken up and/or retained by diseased tissues. Upon photo-activation with visible light at the appropriate wavelength, the generation of cytotoxic species, such as reactive singlet oxygen, leads to irreversible destruction of the treated tissue.\(^{50}\) The attachment of the Lewis b structure to the porphyrin should enable the selective accumulation of the porphyrin within the tissue infected with *Helicobacter pylori*. Since the expression of BabA on the surface of *Helicobacter Pylori* is down regulated after the infection, other surface adhesins may need to be targeted in order to promote bacterial uptake of the PDT reagent. Scanlan and co-workers\(^{48}\) have developed conditions making it possible to attach two different carbohydrates to the porphyrin skeleton. By varying the carbohydrate moiety, it may be possible to target the bacterium both pre and post infection and to prevent the bacteria from spreading to unaffected areas *in vivo*.

2.5 Previously reported synthesis of the Lewis b hexasaccharide

Several strategies for the synthesis of the Lewis b hexasaccharide have been reported. Danishefsky and co-workers\(^{51,46}\) have synthesised the Lewis b hexasaccharide using a polymer-based oligosaccharide construction method with glycals as glycosyl donors. First an iodosulfonamide tetrasaccharide donor 68 was constructed, which then was then glycosylated on to a lactal lactose acceptor 69, shown in Scheme 39.

![Scheme 39 Synthesis of Lewis b hexasaccharide using glycals as glycosyl donors reported by Danishefsky and co-workers.](image)

Chernyak et al.\(^{52}\) has also reported the synthesis of Lewis b the hexasaccharide using a convergent synthesis, first they constructed a thioglycosides tetrasaccharide donor 70 and a spacer-equipped 3’,4–dial lactose acceptor 71. The tetrasaccharide was selective linked to the 3’ position of the lactoside acceptor using 20 mol. eq. DMTST. The deprotection of the hexasaccharide was complicated due to problems with removal of the phthalimido group. The strategy is illustrated in Scheme 40.

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Furthermore Lahmann et al.\textsuperscript{53} has reported the synthesis of the Lewis b hexasaccharide, here forming a tetrasaccharide acceptor block containing a spacer \textsuperscript{72} by a 2+2 glycosylation between thiogluco-side donor \textsuperscript{73} and lactoside acceptor \textsuperscript{74}, which then was bisfucosylated using the fucosyl donor \textsuperscript{75}. The deprotection of the hexasaccharide was accomplished using Pd/C with HCl.

\textbf{2.6 Synthetic strategy for the synthesis of the Lewis b hexasaccharide}

This work was done in Professor Stefan Oscarsons laboratory at University college Dublin. The strategy used in this project is inspired by the work recently published by Fourniére et al.,\textsuperscript{54} who reported the linear synthesis of the Lewis b pentasaccharide, and is developed by former post.doc. in the Oscarson group, Lionel Tschopp.\textsuperscript{55}

Four different building blocks are required for the synthesis of Lewis b hexasaccharide \textsuperscript{66}, three monosaccharide donors: the thioglucosamine \textsuperscript{76}, the thiogalactoside \textsuperscript{77} and the thiofuranoside \textsuperscript{78} and a disaccharide acceptor, the spacer-equipped lactose acceptor \textsuperscript{79}, shown in Scheme 42. The thiogalactoside \textsuperscript{77} and the thiofuranoside \textsuperscript{78} were already available in the group of Oscarson.

The Lewis b hexasaccharide \textsuperscript{66} can be prepared from the diol tetrasaccharide acceptor \textsuperscript{72} (Scheme 42.), which can be bisfucosylated using the thioethyl fucosyl donor \textsuperscript{78} in a halide-assisted coupling, where the bromo fucoside \textsuperscript{75} is prepared \textit{in situ} by addition of bromine to the glycosylation mixture already containing the acceptor \textsuperscript{72}, the thioethyl fucoside \textsuperscript{78} and tetraethyl ammonium bromide (TEAB).

\textsuperscript{55} Unpublished results
Catalytic hydrogenation will then remove the benzyl ethers and reduce the azido group to an amine to afford 66.

The diol tetrasaccharide acceptor 72, can be synthesised by glycosylation of the trisaccharide acceptor 80 with the thioethyl galactoside donor 77 using DMTST as promoter, followed by regioselective opening of the benzylidene acetal with NaBH₃CN and HCl/Et₂O and subsequently Zemplén deprotection.

The trisaccharide 80 can be synthesised by NIS/AgOTf promoted glycosylation of the lactoside acceptor 79 with the thioethyl thioglucosamine donor 76. Cleavage of the phthaloyl protecting group with ethylenediamine, followed by acetylation of the amino group with acetic anhydride in pyridine, and last, cleavage of the 3-acetyl group by Zemplén deprotection affords the trisaccharide 80.

Scheme 42 Retrosynthesis of the spacer equipped Lewis b hexasaccharide 66.

To synthesize the Lewis b hexasaccharide, the spacer-equipped lactose acceptor 79 and the thioglucosamine donor 76 will be synthesised, and the four building blocks will then be linked together by glycosidic linkages to form the hexasaccharide 66.

2.7 Results and discussion

2.7.1 Synthesis of lactoside acceptor 79

The synthetic route to synthesise the lactoside acceptor 79 started from D-lactose 81, seen is Scheme 43. 81 was refluxed in acetic anhydride with sodium acetate to afford the peracetylated D-lactose 82 in 74% yield. Due to the neighbouring group participation at C-2 only the β-anomer was obtained.

Following the procedure by Chen and co-workers⁵⁶, the peracetylated β-D-lactose 82 was then glycosylated with 3-chloropropan-1-ol in the presence of boron trifluoride etherate as promotor to yield the spacer-equipped lactoside 83 in 56% yield. BF₃·Et₂O activates the anomeric acetyl group as leaving

group which leads to an oxonium ion intermediate. Due to the neighbouring acetyl group at C-2 the nucleophilic attack can only take place from above to give the β-configurated glycoside.

The chloro-spacer was converted into an azido-spacer applying S$_2$N$_2$ conditions. Using the procedure reported by Chen and co-workers$^{56}$, the desired lactoside 84 was afforded in 90% yield using tetrabutylammonium iodide (TBAI) and NaN$_3$ at 60°C for 2 h. The carbohydrate 84 was deprotected applying the classical Zemplén procedure. Treatment with a catalytic amount of freshly prepared sodium methoxide in dry methanol afforded the deprotected lactoside 85 in 96% yield.

The deprotected lactoside 85 was converted into the diol 74 following the procedure shown in Scheme 44. The 3′,4′-O-isopropylideneation was performed using 10 equivalents of 2,2-dimethoxypropane (DMP) in anhydrous acetonitrile in the presence of vanadyl triflate VO(OTf)$_2$ as a catalyst.$^{57,58}$ The reaction had to be carried out for at least two to three days to obtain the thermodynamically more stable 3′,4′-O-isopropylidene compared to the 4′,6′-O-isopropylidene. The isopropylidene protected lactoside 86 was not isolated, but used directly in the next step. Benzylation of 86 by treatment with sodium hydride and benzyl bromide in dry DMF afforded the benzyl protected lactoside 87. Subsequently this was treated with a mixture of TFA/H$_2$O (9:1) to remove the isopropylidene protecting group. The lactoside unit 74 was obtained in 46% yield over three steps.

Scheme 43 Synthesis of disaccharide 85.

Scheme 44 Synthesis of diol 74.

57 VO(OTf)$_2$ was prepared by Ph.D-student Rebecca Ulc and this procedure was developed by Rebecca Ulc as well.

By the procedure reported by Sundgren et al.,\(^{59}\) diol 74 was stereo-selectively transformed into the corresponding 3,4-endo-benzylidene derivative 88 using benzaldehyde dimethyl acetal and camphorsulfonic acid (CSA). Subsequent NaBH₃CN/HCl-mediated reductive opening of the acetal ring afforded the 3′-hydroxy derivative 79, as illustrated in Scheme 45. Formation of the 3,4-exo-benzylidene acetal was observed by TLC analysis, and this diastereo-isomer was hard to separate from the 3,4-endo-benzylidene 88 derivative by column chromatography. Several experiments were the reaction was careful monitored by TLC analysis did not improve the yield of the 4′-hydroxy derivative 79 to the reported 80%.

Scheme 45 Synthesis of the lactoside acceptor 79.

### 2.7.2 Synthesis of thioglucoside donor 76

The thioglucoside donor 76 was prepared following a procedure by Sun et al.\(^{60}\) from D-glucosamine hydrochloride 89, shown in Scheme 46.

Scheme 46 Synthesis of thioglucoside donor 76.

D-glucosamine hydrochloride 89 was converted to the peracetylated phthalimido protected glucoside 90 in only 27% yields. Glycosylation of 90 with ethane thiol in the presence of BF₃·OET₂ afforded the peracetylated thioglucoside 91, which then was deprotected using Zemplén conditions. Subsequent treatment of 91 with benzaldehyde dimethyl acetal and p-toluenesulfonic acid monohydrate afforded the 4,6-O-benzylidene derivative 93. The free 3-OH was protected using acetic anhydride and pyridine to afford the phthalimido protected thioglucoside donor 76.

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2.7.3 Synthesis of trisaccharide acceptor 80

All the building blocks for the synthesis of the Lewis b hexasaccharide were now available. The lactoside acceptor 79 and the phthalimido protected thioglucoside donor 76 had first to be put together by a glycosidic linkage to form a trisaccharide. An alternative strategy for the formation of the desired trisaccharide could be to use the peracetylated GlcNAc donor 8, previously synthesised (Scheme 17, page 11), as the glycosyl donor instead of the phthalimido protected thioglucoside donor 76. Both procedures were carried out.

2.6.3.1 Using phthalimido protected thioglucoside donor 76

Coupling of the lactoside acceptor 79 with the phthalimido protected thioglucoside donor 76 using NIS/AgOTf as promoter system in CH₂Cl₂ gave the trisaccharide 94 in good yield. The phthaloyl protecting group ensured complete β-selectivity during the reaction. To avoid problems with later removal of the phthaloyl group, experienced in the deprotection sequence of the hexasaccharide reported by Chernyak et al., the phthalidomido group was exchanged to the acetyl amido group at this stage. This was done using ethylenediamine (EDA) in refluxing ethanol, subsequent acetylation of the amine as well as the free 3''-OH to afford 95, followed by removal of the 3''-acetyl group with Zemplén conditions to afford the desired trisaccharide 80. This is illustrated in Scheme 47.

Scheme 47 Synthesis of the trisaccharide acceptor 80.

2.6.3.2 Using peracetylated GlcNAc donor 8

Inspired by a publication by Jensen and co-workers, another strategy for the synthesis of the trisaccharide acceptor 80 was carried out by using the peracetylated GlcNAc donor 8, illustrated in Scheme 48.

Three different conditions were examined for the glycosylation using a catalytic amount of bismuth triflate as promoter. Bismuth triflate catalyses the oxazolidine formation and its activation in situ.

61 Other metals such as Sc and Yb triflate can catalyse the formation of the oxazoline and its activation. Recently Martin Jæger Petersen, former master student in group 103, Århus, reported the use of Bi(OTf)₃.
Table 4 Examined reactions conditions for the synthesis of the trisaccharide 96 using the GlcNAc donor 8.

<table>
<thead>
<tr>
<th>#</th>
<th>Donor 8:Acceptor 79</th>
<th>Bi(OTf)₃</th>
<th>Temperature</th>
<th>Time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3:1</td>
<td>45%</td>
<td>Reflux</td>
<td>24 h</td>
<td>62%</td>
</tr>
<tr>
<td>2</td>
<td>3:1</td>
<td>45%</td>
<td>80 °C µW</td>
<td>4 h</td>
<td>54%</td>
</tr>
<tr>
<td>3</td>
<td>(2x1.5):1</td>
<td>2x22.5%</td>
<td>Reflux</td>
<td>48 h</td>
<td>72%</td>
</tr>
</tbody>
</table>

Using 1 eq. acceptor 79, 3 eq. of the donor 8 and 45% Bi(OTf)₃ (that is 15% with respect to donor) and refluxing the reaction mixture in CH₂Cl₂ for 24 h, partial consumption of the acceptor and full conversion of the donor was observed. 62% of 96 could be isolated (Table 4, entry 1). Carrying the reaction out at 80 °C at microwave conditions for 4 h, again only partial consumption of the acceptor and full conversion of the donor was observed, resulting in 54% yield (Table 4, entry 2). The best result was obtained by adding 1.5 eq. of the donor 8 with respect to the acceptor 79 and 22.5% Bi(OTf)₃ in CH₂Cl₂, and heating the reaction mixture to reflux for 24 h, and then add another 1.5 eq. of the donor 8 and 22.5% Bi(OTf)₃, followed by additional heating for 24 h. This afforded the product 96 in 72% yield (Table 4, entry 3).

Scheme 48 Synthesis of trisaccharide acceptor 80 using GlcNAc donor 8.

Deprotection of the acetal groups by sodium methoxide in methanol provided the triol 97, which subsequently was treated with benzaldehyde dimethyl acetal and p-toluenesulfonic acid monohydrate in the presence of the drying agent CaSO₄ to afford the acceptor 80.

The overall yield for the reaction shown in Scheme 47 is 59% (for four steps) and is similar to the overall yield for the reaction shown in Scheme 48, which is 58% (for three steps). Taken into account the easy preparation of the peracetylated GlcNAc donor 8, this second procedure is definitely an interesting approach to obtain the trisaccharide acceptor 80.

2.7.4 Synthesis of tetrasaccharide 98

After the formation of the trisaccharide acceptor 80, next step is to attach the thiogalactoside donor 77 to obtain the tetrasaccharide 98. The thiogalactoside donor 77 was available in the Oscarson group from another project. Unfortunately all attempts to form tetrasaccharide 98 failed. See Scheme 49.
Several promoter systems were tried using an acceptor/donor ratio of 1:1.4:

a) DMTST (3.5 eq), 4 Å MS, dry CH₂Cl₂, N₂-atmosphere, 0°C to room temperature, ON.

b) NIS (2 eq), AgOTf (0.3 eq), 4 Å MS, dry CH₂Cl₂, N₂-atmosphere, -78 °C to room temperature, ON.

c) NIS (1.6 eq), TMSOTf (cat.), 4 Å MS, dry CH₂Cl₂, N₂-atmosphere, 0°C to room temperature, ON.

d) First: Br₂, donor, 4 Å MS, dry CH₂Cl₂, N₂-atmosphere, room temperature, 30 min. Then acceptor, AgOTf, 2,6-ditertbutyl pyridine, -78°C to room temperature, ON.

This reaction has previously been done by Lionel Tschopp using conditions a. However, using conditions a, b or c, full consumption of the donor 77 was observed, while the acceptor 80 was not fully converted as analysed by TLC. Formation of several compounds was observed using TLC analysis, but separation with flash column chromatography, isolating the spot with highest intensity, did not result in product isolation, as only three anomic protons could be observed by NMR analysis.⁶² Using conditions d, where a bromo galactoside is prepared from thiogalactoside 77, did again not result in full conversion of the acceptor 80. Isolation of the spot with highest intensity on TLC, indicated the formation of a compound with five anomic protons, observed by NMR analysis.

Discussion the problems with the tetrasaccharide formation with professor Martina Lahmann and her co-workers at Bangor university revealed, that they had observed the same problems using the thiogalactoside donor 77 with an acetate in the C₂-position. However, by changing the C₂ acetate of the donor to a benzoate did in their case overcome this problem. Due to lack of time, this has not been tested.

2.8 Conclusion

In order to synthesise the Lewis b hexasaccharide, a spacer-equipped lactose acceptor 79 and a thioglucosamine donor 76 was synthesised. The synthesis of the 3'-hydroxy lactoside 79 acceptor was complicated due to problems obtaining the 3,4-endo-benzylidene derivative 88.

The thioglucosamine donor 76 was glycosylated on to the lactoside acceptor 79 in 75% yield. By using the peracetylated glucosamine donor 8 for this glycosylation, the corresponding trisaccharide could be obtained in 72% yield. By using the peracetylated glucosamine donor 8, problems with cleavage of the phthaloyl protecting group can be avoided.

Several attempts to synthesise the tetrasaccharide 98 was carried out by trying to glycosylate the thiogalactoside donor 77 on to the trisaccharide acceptor 80, but without success.

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⁶² MS analysis was not performed due to technical problems.
2.9 **Future perspectives**

The future plan is to complete the synthesis of the Lewis b structure following the synthetic strategy, outlined in Scheme 42. This will be done at Aarhus University. The thiogalactose donor 99 with a benzoate at the C2-position will be synthesised. This donor will be used in the tetrasaccharide synthesis, and will hopefully solve the problems encountered in this step.

Furthermore, the use of the peracetylated GlcNAc donor 8 in a regioselective glycosidation on the 3',4'-lactoside acceptor 74 will be examined, using the bismuth triflate promoter system. This is illustrated in Scheme 50. By using a non-basic benzylation methodology, it would be possible to convert the trisaccharide 100 to the previously synthesised 95, this way the challenging formation of the 3,4 endo-benzylidene derivative 88 can be avoided.

![Scheme 50](image-url)
EXPERIMENTAL SECTION

3.1 General methods

Department of chemistry, Aarhus University

All reagents except otherwise stated were used as purchased without further purification. Oven dried glassware (ca. 120 °C) was used for reactions carried out under nitrogen atmosphere. Solvents were dried according to standard procedures prior to use. Flash chromatography was performed with Merck silica 60 (230-400 mesh) as stationary phase and TLC was performed on silica-coated aluminium plates (Merck 60 F254). TLC plates were visualised with ceric sulfate/ammonium molybdate in 10% H2SO4 or with KMnO4 stain.

1H-NMR (400 MHz) and 13C-NMR (100MHz) were recorded on a Varian Mercury 400 spectrometer. Multiplicities are reported using the following abbreviations: s = singlet, br s = broad singlet, d = doublet, dd = double doublet, t = triplet, dt = double triplet, q = quartet, m = multiplet. The chemical shifts are reported in ppm relative to the solvent residual peak. Spectra were assigned based on gCOSY and gHMBC experiments.

ES-MS were recorded on a Micromass LC-TOF instrument using electrospray ionization (ESI). High resolution spectra were recorded with either of the following compounds as internal standard: Boc-L-alanine: C8H15NO4Na: 212.0899; BzGlyPheOMe: C19H20N2O4Na: 363.1321; BocSer(OBn)SerLeuOMe: C25H39N3O8Na: 532.2635; erythromycin: C37H67NO13Na: 756.4510. Masses of standards and analytes are calculated and reported in Daltons for uncharged species.

MALDI-TOF MS was recorded on a Bruker Daltonics mass spectrometer using an α-cyano-4-hydroxycinnamic acid based matrix. Melting points were measured on a Büchi B-540 instrument and are uncorrected. Optical rotation was measured on a PE-241 polarimeter and reported in units of deg cm2 g−1.

ES-MS was performed on a CEM Liberty microwave assisted peptide synthesiser. All resins, reagents and aminoacids were purchased from Iris Biotech, Germany.

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The 1H and 13C NMR spectra (δ in ppm, relative to Me4Si in CDCl3 or relative to acetone in D2O) were recorded with a Varian spectrometer 500/125 MHz at 25 °C. Assignments were aided by 1H–1H and 1H–13C correlation experiments. HRMS spectra were recorded on a micromass LC-TOF instrument from Waters. Optical rotations were measured on a Perkin Elmer polarimeter with a Na lamp (589 nm) and are not corrected. TLC was carried out on precoated 60 F254 silica gel alumina plates (Merck) using UV-light, H2SO4 (10% in ethanol), ninhydrin solution (ninhydrin–CH3COOH–ethanol [0.3:3:100 w/v/v] and/or AMC-solution (ammonium sulphate, cerium (IV) sulphate, 10% H2SO4 [5:0.1:100 w/v/v]). Column chromatography was performed on silica gel (Apollo scientific, pore size 60 Å, particle size 40–63 μm).

3.2 EXPERIMENTAL SECTION PART I

1-Phenylbut-3-en-1-ol (6)

Benzaldehyde 5 (10 µL, 0.098 mmol) and allylbromide 4 (17 µL, 0.197 mmol) was dissolved in the solvent of choice (250 µL) and H₂O or the buffer of choice (250 µL). Indium was added (23 mg, 0.200 mmol) and the reaction was stirred at rt and followed by TLC analysis. Rᶠ: 0.21(1:14 EtOAc/pentane). The reaction times are shown in Scheme 16 and Table 1. LRMS (ES): Calculated for C₁₀H₁₂ONa 171.0786. Found m/z 171.1.

2-Deoxy-2-[p-methoxybenzylidene(amo)]-D-glucopyranose (10)

To a solution of D-glucosamine hydrochloride 9 (12.5 g, 58.0 mmol) in a freshly prepared aqueous solution of 1 M NaOH (60.0 mL) under stirring was added p-anisaldehyde (8.5 mL, 70.0 mmol). After a short time crystallisation began. Then the mixture was refrigerated and after 2 days the precipitated product was filtered off and washed with cold water, followed by a mixture of 1:1 EtOH/Et₂O. The resulting product was dried, resulting in 12.6 g crude, which was used without any characterisation (42.6 mmol, 73 %).

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-[p-methoxybenzylidene(amo)]-β-D-glucopyranose (11)

Compound 10 (12.7, 42.6 mmol) was added sequentially to a cooled (ice-water) mixture of pyridine (67.5 mL) and Ac₂O (37.5 mL). The mixture was stirred in ice-bath for 1 h and at rt for 3 days. The yellow solution was poured into 250 mL of ice-water. The precipitated white product was filtered and washed with cold water. The crystals were added toluene, which was removed under vacuum, resulting in 17.1 g of 11 as a white powder (36.7 mmol, 86 %).
\[ ^1H-NMR \ (400 \ MHz, \ CDCl_3) \ \delta \ 8.14 \ (s, 1H, N CH), \ 7.64 \ (d, \ J = 8.4 \ Hz, 2H, Ar-H), \ 6.90 \ (d, \ J = 8.8 \ Hz, 2H, Ar-H), \ 5.93 \ (d, \ J_{H1-H2} = 8.0 \ Hz, 1H, H1), \ 5.41 \ (t, \ J = 9.6 \ Hz, 1H, H-3), \ 5.13 \ (t, \ J = 9.6 \ Hz, 1H, H-4), \ 4.36 \ (dd, \ J_{H6a-H5} = 4.4Hz, \ J_{H6a-H6b} = 12.4 \ Hz, 1H, H-6a), \ 3.96 \ (d, \ J = 9.6 Hz, 1H, H-3), \ 5.13 \ (t, \ J = 9.6 \ Hz, 1H, H-4), \ 4.36 \ (dd, \ J_{H6a-H5} = 4.4Hz, \ J_{H6a-H6b} = 12.4 \ Hz, 1H, H-6a) ppm. \]

13C-NMR (100 MHz, CDCl_3) \[ \delta \ 170.9, \ 170.1, \ 170.0, \ 169.0, \ 164.5, \ 162.5, \ 130.5 (2C), \ 128.5, \ 114.3 (2C), 93.4, \ 73.5, \ 73.2, \ 73.0, \ 68.3, \ 62.1, \ 55.6, \ 21.0 (2C), 20.9, \ 20.7 ppm. \]

The NMR data are in accordance with those previously reported.

1,3,4,6-Tetra-O-acetyl-\( \beta \)-D-glucosamine hydrochloride (12)

[Diagram]

Glucoside 11 (17.0 g, 36.5 mmol) was dissolved in warm acetone (150 mL) and then HCl (5 M, 7.5 mL) was added with immediate formation of a precipitate. The mixture was cooled in ice-bath, and after addition of Et_2O (150 mL) it was stirred for 2 h and refrigerated for 3 days. The precipitated product was filtered, washed with Et_2O and dried on vacuum to afford 12.9 g of a white solid (33.6 mmol, 92%).

\[ ^1H-NMR \ (400 \ MHz, \ CD_3OD) \ \delta \ 5.86 \ (d, \ J_{H1-H2} = 8.8 \ Hz, 1H, H-1), \ 5.35 \ (dd, \ J_{H13-H2} = 9.4 \ Hz, J_{H13-H1} = 10.4 \ Hz, 1H, H-3), \ 5.09 \ (dd, \ J_{H4-H5} = 9.2 \ Hz, J_{H4-H6} = 10.0 \ Hz, 1H, H-4), \ 4.30 \ (dd, \ J_{H6-H5} = 4.8 \ Hz, J_{H6-H6} = 12.8 \ Hz, 1H, H-6), \ 4.12 \ (dd, \ J_{H6-H5} = 2.0 \ Hz, J_{H6-H6} = 12.4 \ Hz, 1H, H-6b), \ 4.02 \ (dd, \ J_{H5-H6} = 4.8 \ Hz, J_{H5-H6} = 10.0 \ Hz, 1H, H-5), \ 3.64 \ (dd, \ J_{H2-H3} = 8.8Hz, J_{H2-H2} = 10.4Hz, 1H, H2), \ 2.18 \ (s, \ 3H, CH_3CO), \ 2.09 \ (s, \ 3H, CH_2CO), \ 2.04 \ (s, \ 6H, 2xCH_3CO) ppm. \]

13C-NMR (100 MHz, CDCl_3) \[ \delta \ 173.7, \ 173.2, \ 172.8, \ 171.4, \ 90.5, \ 72.3, \ 71.0, \ 68.3, \ 62.1, \ 55.6, \ 21.0 \ (2C), 20.9, \ 20.7 ppm. \]

The NMR data are in accordance with those previously reported.

2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-\( \beta \)-D-glucopyranose (8)

[Diagram]

Glucoside 12 (4.6 g, 11.9 mmol) was dissolved in dry CH_2Cl_2 (60 mL) and stirred at 0 °C under N_2 atmosphere. Et_3N (4.5 mL, 32.5 mmol) was added and the mixture was stirred for 15 min until the starting material was completely dissolved. AcCl (1.2 mL, 16.8 mL) was then added dropwise at 0 °C and the mixture was then allowed to slowly warm up to rt. After 1 h TLC analysis (EtOAc) showed full conversion of the starting material, and the mixture was diluted with CH_2Cl_2, washed twice with H_2O, brine, dried over MgSO_4, filtered and concentrated under reduced pressure. The product was recrystallized from CH_2Cl_2 and pentane to afford 4.33 g of the product 8 as white crystals (11.1 mmol, 93%). R_f: 0.35 (EtOAc).

$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ 5.69 (d, $J = 8.8$ Hz, 1H, H1), 5.62 (br d, $J = 9.5$ Hz, 1H, NH), 5.18 – 5.08 (m, 2H, H3, H4), 4.34 – 4.22 (m, 2H, H2, H6a), 4.13 (dd, $J = 12.5$, 2.2 Hz, 1H, H6b), 3.83 – 3.77 (m, 1H, H5), 2.11 (s, 3H, CH$_3$), 2.08 (s, 3H, CH$_3$), 2.04 (s, 2H, CH$_3$), 2.03 (s, 2H, CH$_3$), 1.92 (s, 2H, CH$_3$) ppm.

$^{13}$C-NMR (125 MHz, CDCl$_3$) $\delta$ 171.3, 170.8, 170.2, 169.7, 169.4 (5xC=O), 92.8 (C1), 73.1 (C5), 72.8 (C4), 68.0 (C3), 61.8 (C6), 53.2 (C2), 23.3 (C$_3$H$_7$), 21.0 (C$_3$H$_7$), 20.8 (C$_3$H$_7$), 20.7 (C$_3$H$_7$) ppm.

NMR data are in accordance with those previously reported. LRMS (ES): Calculated for C$_{16}$H$_{23}$NO$_{10}$Na m/z 412.1220. Found m/z 412.06.

Trans-4-bromo-2-buten-1-ol (14)

Ethyl bromocrotonate 12 (75%, 1.7 mL, 9.25 mmol) was dissolved in dry toluene (12 mL) and cooled on an ice bath. DIBAL (1 M in toluene, 22 mL, 22.0 mmol) was added drop wise over 30 min at 0 °C and the reaction mixture was stirred additional 2 hours at rt before quenching with 1 M HCl. The residue was diluted with CH$_2$Cl$_2$ and the organic phase was washed with H$_2$O, brine, dried over MgSO$_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc/pentane, 1:3) to afford 718 mg of 14 as clear oil (4.75 mmol, 51%). The product was unstable and was used immediately for further reaction. R$_f$: 0.29 (EtOAc/pentane, 1:3)

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 5.98 – 5.84 (m, 2H, H2, H3), 4.14 (d, $J = 3.5$ Hz, 2H, H1), 3.95 (d, $J = 6.3$ Hz, 2H, H4), 2.45 (s, 1H, OH) ppm. Spectral data were in accordance with previously published.

E-4-bromobut-2-enyloxy 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranoside (7)

Peracetylated GlcNAc donor (8) (1.43 g, 3.67 mmol), Yb(OTf)$_3$ (342 mg, 0.55 mmol) and trans-4-bromo-2-buten-1-ol (14) (708 mg, 4.69 mmol) were placed in a microwave vial and dissolved in dry CH$_2$Cl$_2$ (10 mL). The reaction flask was flushed with nitrogen. The reaction mixture was heated to 80°C for 30 min under microwave irradiation. The reaction mixture was diluted with CH$_2$Cl$_2$, washed with H$_2$O, followed by extraction of aqueous phase with CH$_2$Cl$_2$. The combined organic phases was dried over MgSO$_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (EtOAc/CH$_2$Cl$_2$, 1:2) to afford 1.21 g of 7 as white solid (2.52 mmol, 69%). R$_f$: 0.31 (EtOAc/CH$_2$Cl$_2$, 1:2).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.99 – 5.88 (m, 1H, H3’), 5.86 – 5.74 (m, 1H, H2’), 5.62 (d, $J = 8.8$ Hz, 1H, NH), 5.26 (dd, $J = 10.6, 9.3$ Hz, 1H, H3), 5.06 (t, $J = 9.6$ Hz, 1H, H4), 4.68 (d, $J = 8.3$ Hz, 1H, H1), 4.34 (dd, $J_{\text{gem}} = 13.2, J_{1'a,2'} = 4.0$ Hz, 1H, H1’a), 4.24 (dd, $J = 12.3, 4.7$ Hz, 1H, H6a), 4.19 – 4.00 (m, 2H, H6b, H1'b), 3.98 – 3.81 (m, 3H, H4’, H2), 3.69 (ddd, $J = 10.0, 4.7, 2.5$ Hz, 1H, H5), 2.08 (m, 3H, COCH$_3$), 2.02 (s, 3H, COCH$_3$), 2.01 (s, 3H, COCH$_3$), 1.96 (s, 3H, COCH$_3$) ppm.

$^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ 170.9, 170.8, 170.4, 169.5 (4x COCH$_3$), 130.5, 129.4 (C2’, C3’), 100.0 (C1), 72.4 (C3), 71.9 (C5), 68.7 (C4), 68.3 (C1’), 62.3 (C6), 54.7 (C2), 31.9 (C4’), 23.5, 20.9, 20.8, 20.7 (4xCOCH$_3$) ppm.

LR-MS (ES): Calculated for C$_{18}$H$_{26}$BrNO$_7$Na m/z 502.0689. Found m/z 502.1.

2-[Hydroxy(phenyl)methyl]but-3-en-1-yl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranoside (15)

$E$-4-bromobut-2-enyloxy 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranoside 7 (60 mg, 0.126 mmol) was dissolved in EtOH (0.5 mL) and phosphate buffer (0.5 mL, 50 mM, pH 7.0). Benzaldehyde 5 (6.4 µL, 63 µmol) and indium (20 mg, 0.174 mmol) were added and the mixture was stirred at room temperature for 17 h, resulting in a thick white mixture. The mixture was filtered through a cotton plug and concentrated under reduced pressure. Attempt to purify the product with flash column chromatography (1:1 EtOAc/CH$_2$Cl$_2$) failed. R$_f$: 0.30 (EtOAc/CH$_2$Cl$_2$, 1:1).

LR-MS (ES): Calculated for C$_{25}$H$_{33}$NO$_{10}$Na m/z 530.0202. Found m/z 529.9.

$E$-4-bromobut-2-enyloxy 2-acetamido-2-deoxy-β-D-gluco-pyranoside (1) and $E$-4-ethoxybut-2-enyloxy 2-acetamido-2-deoxy-β-D-gluco-pyranoside (17)

The peracetylated allylic bromo glucoside (7) (17.0 mg, 0.035 mmol) was dissolved in dry EtOH (1 mL) and dry K$_2$CO$_3$ (1.2 mg, 0.009 mmol) was added. The mixture was stirred at rt and followed by MS analysis. After 4 h there was full conversion of the starting material 7 to the expected product 1 and the by-product 17. The mixture was used immediately in the next step for the Barbier reaction (Scheme 20).

LRMS (ES) 1: Calculated for C$_{12}$H$_{20}$$^{79}$BrNO$_6$K m/z 392.0111. Found m/z 391.8.

LRMS (ES) 17: Calculated for C$_{14}$H$_{25}$NO$_7$K m/z 358.1268. Found m/z 357.9.
2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (23)

Pentaacetyl-β-D-glucopyranose 21 (2.52 g, 6.46 mmol) was dissolved in dry CH₂Cl₂ (20 mL). To this, hydrogen bromide (33 % in acetic acid, 15 mL) was added. The reaction mixture was stirred at rt under N₂ for 1.5 h. The reaction mixture was diluted with CH₂Cl₂ and H₂O, the aqueous layer was extracted three times with CH₂Cl₂, the combined organic phases was washed with a saturated aqueous solution of NaHCO₃, washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The product was crystallized from ethyl acetate/pentane to afford 2.38 g of the product 23 as a fluffy white powder (5.81 mmol, 90%). Rf 0.60 (1:1ethyl acetate/pentane).

¹H NMR (400 MHz, CDCl₃) δ 6.58 (d, J = 4.0 Hz, 1H, H1), 5.52 (t, J = 9.7 Hz, 1H, H3), 5.12 (t, J = 9.8 Hz, 1H, H4), 4.80 (dd, J = 10.0, 4.0 Hz, 1H, H2), 4.35 – 4.22 (m, 2H, H5, H6a), 4.12 – 4.05 (m, 1H, H6b), 2.06 (s, 3H, COC₃H₃), 2.06 (s, 3H, COC₃H₃), 2.01 (s, 3H, COC₃H₃), 2.00 (s, 3H, COC₃H₃) ppm. Spectral data are in accordance with previously published.

³¹C-NMR (100 MHz, CDCl₃) δc 170.6, 170.0, 169.9, 169.6 (4xCOCH₃), 86.8 (C1), 72.3 (C5), 70.7 (C2), 70.3 (C3), 67.3 (C4), 61.1 (C6), 20.8, 20.8, 20.7, 20.7 (4xCOCH₃) ppm. Spectral data are in accordance with previously published. LR-MS (ES): Calculated for C₁₄H₁₉BrO₇Na m/z 433.0110. Found m/z 432.7.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-1-isothiouronium bromide (25)

Thiourea (657 mg, 8.63 mmol, 1.5 eq) and the glucosyl bromide (23) (2.36 g, 5.76 mmol, 1 eq) were dissolved in acetone (HPLC-grade, 15 mL) under N₂ atmosphere. The reaction mixture was heated to 60 °C. After 2 hours, a white precipitate was observed. This was removed by filtration and the filtrate was again heated to reflux. This process was repeated until no more precipitate was formed. The precipitates were combined and re-crystallized from acetone/pentane to afford 1.74 g of the product 25 as a white powder (3.56 mmol, 62%).

¹H-NMR (400 MHz, DMSO) δ 9.26 (br s, 2H, NH₂), 9.10 (br s, 2H, NH₂), 5.72 (d, J = 10.0 Hz, 1H, H1), 5.32 (t, J = 9.4 Hz, 1H, H3), 5.11 (m, 2H, H2, H4), 4.24-4.15 (m, 2H, H5, H6a), 4.14 – 4.03 (m, 1H, H6b), 2.06 (s, 3H, COCH₃), 2.03 (s, 1H, COCH₃), 2.00 (s, 3H, COCH₃), 1.98 (s, 3H, COCH₃) ppm. Spectral data are in accordance with previously published.
2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranoside (19)

Sodium metabisulfite (Na₂S₂O₅, 933 mg, 4.91 mmol) and 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-1-isothiouronium bromide (25) (1.71 g, 3.50 mmol) was dissolved in a mixture of CH₂Cl₂ (15 mL) and H₂O (7.5 mL). The reaction mixture was heated to reflux under Ar. After 6 h, TLC-analysis (1:1 pentane/ethyl acetate) showed incomplete conversion of starting material. Additional Na₂S₂O₅ (300 mg, 1.58 mmol) was added, and the mixture was stirred for additional 1.5 h at reflux and at rt overnight. The phases were then separated, and the aqueous layer was re-extracted with twice with CH₂Cl₂, dried over MgSO₄, filtered and concentrated under reduced pressure to afford 1.21 g of the product 19 as a white solid (3.32 mmol, 95%).

¹H-NMR (400 MHz, CDCl₃) δH 5.15 (t, J = 9.4 Hz, 1H, H3), 5.06 (t, J = 9.7 Hz, 1H, H4), 4.93 (t, J = 9.5 Hz, 1H, H2), 4.52 (t, J = 9.9 Hz, 1H, H1), 4.21 (dd, dd = 12.5, 4.8 Hz, 1H, H2a), 4.08 (dd, J = 12.5, 2.2 Hz, 1H, H6b), 3.69 (ddd, J = 9.9, 4.7, 2.2 Hz, 1H, H5), 2.29 (d, J = 10.0 Hz, 1H, SH), 2.05 (s, 3H, COCH₃), 2.04 (s, 3H, COCH₃), 1.97 (s, 3H, COCH₃) ppm. ¹³C-NMR (100 MHz, CDCl₃) δC 170.8 (COCH₃), 170.2 (COCH₃), 169.8 (COCH₃), 169.5 (COCH₃), 78.8 (C1), 76.4 (C5), 73.7, 73.6 (C2, C3), 68.2 (C4), 62.1 (C6), 20.9 (COCH₃), 20.8 (COCH₃), 20.7 (COCH₃) ppm. Spectral data are in accordance with previously published.³⁸ LRMS (ES): Calculated for C₁₄H₂₀O₉Na m/z 387.0726. Found m/z 386.9.

2-Acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl chloride (22)

N-Acetyl D-glucosamine (20) (5.00 g, 22.6 mmol) was added to stirring acetyl chloride (15.0 mL) and the resulting suspension was stirred under nitrogen atmosphere for 2 days at rt. CH₂Cl₂ (50 mL) was then added and the resulting solution was poured into ice water (100 mL) under vigorous stirring. The organic layer was transferred to a beaker with aqueous NaHCO₃ (100 mL) and ice and stirred until no further CO₂(g) was developed. The organic layer was then separated and dried over MgSO₄. The work up did not take longer than 15 min. The organic layer was then filtered, and the residue washed thoroughly with CH₂Cl₂. The resulting solution was concentrated under vacuum. Diethyl ether (30 mL) was added and crystallisation began. The mixture was left at 4 °C for 24 h and filtered to afford 6.65 g of the product 22 as a white crystalline solid (18.2 mmol, 80 %). Rf 0.18 (EtOAc).

¹H-NMR (400 MHz, CDCl₃) δH 6.18 (d, 1H, J = 4.0 Hz, H-1), 5.83 (d, 1H, J = 8.8 Hz, N-H), 5.31 (dd, 1H, J = 9.6 Hz, J = 10.8 Hz, H-3), 5.20 (dd, 1H, J = 3.6 Hz, J = 12.0 Hz, H-4), 4.53 (ddd, 1H, J = 3.6 Hz, J = 8.8 Hz, J = 12.4 Hz, H-2), 4.30-4.10 (m, 3H, H-6a, H-6b, H-5), 2.09 (s, 3H, COCH₃), 2.04 (s, 6H, 2-COCH₃), 1.98 (s, 3H, COCH₃) ppm. ¹³C-NMR (100 MHz, CDCl₃) δC 171.7, 170.8, 170.3, 169.3, 93.9, 71.1, 70.4, 67.2, 61.4, 53.7, 23.3, 20.9, 20.8 ppm. NMR data matched previously reported data.³⁹ LRMS (ES): Calculated for C₁₄H₂₀ClNO₆Na m/z 388.1. Found m/z 387.9.
2-Acetamido-3,4,6-tri-O-acetyl-β-D-glucopyranosyl-1-isothiouronium chloride (24)

![Chemical Structure](image)

2-Acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl chloride (22) (2.20 g, 6.02 mmol, 1 eq) and thiourea (687 mg, 9.03 mmol, 1.5 eq) were dissolved in acetone (HPLC-grade, 15 mL) under Ar. The reaction mixture was heated to 60 °C. After 3 hours, a white solid precipitated. The precipitate was removed by filtration, the filtrate was returned to reflux and this process was repeated until the solid ceased to precipitate. The solids were combined and re-crystallized from acetone/pentane to afford 2.59 g of the product 24 as a white solid (4.56 mmol, 97 %).

$^1$H NMR (400 MHz, DMSO) δ $^H$ 9.42 (br s, 1H, NH$_2$), 9.22 (br s, 1H, NH$_2$), 8.42 (d, $J = 6.6$ Hz, 1H, NH), 5.69 (d, $J = 9.2$ Hz, 1H, H1), 5.14 (t, $J = 9.7$ Hz, 1H, H3), 4.95 (t, $J = 7.9$ Hz, 1H, H4), 4.33–4.13 (m, 2H, H5, H6a), 4.13–4.94 (m, 2H, H2, H6b), 2.02 (s, 3H, COC$_3$H$_3$), 1.99 (s, 3H, COC$_3$H$_3$), 1.95 (s, 3H, COC$_3$H$_3$), 1.82 (s, 3H, NHCOC$_3$H$_3$) ppm. Spectral data are in accordance with previously published.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-glucopyranose (18)

![Chemical Structure](image)

2-Acetamido-3,4,6-tri-O-acetyl-β-D-glucopyranosyl-1-isothiouronium chloride (24) (2.58 g, 5.83 mmol) and sodium metabisulfite (Na$_2$S$_2$O$_5$, 1.55 g, 8.17 mmol) were dissolved in a mixture of CH$_2$Cl$_2$ (15 mL) and H$_2$O (10 mL). The mixture was heated to reflux under N$_2$. After 1.5 h, the reaction mixture was cooled to rt and the phases separated. The aqueous layer was re-extracted twice with CH$_2$Cl$_2$, the combined organic layers were washed with brine, dried over MgSO$_4$, filtered and then concentrated under reduced pressure to afford 1.52 g of the crude thioglucoside as an 1:5 α:β mixture (NMR analysis). The resulting white solid was crystallized from ethyl acetate/pentane to afford 1.27 g of the β-anomer 18 (3.48 mmol, 60%).

$^1$H-NMR (400 MHz, CDCl$_3$) δ $^H$ 5.70 (d, $J = 9.4$ Hz, 1H, NH), 5.15–5.04 (m, 2H, H3, H4), 4.58 (t, $J = 9.7$ Hz, 1H, H1), 4.23 (dd, $J = 12.4$, 4.8 Hz, 1H, H6a), 4.16–4.07 (m, 2H, H2, H6b), 3.69 (ddd, $J = 9.6$, 4.7, 2.1 Hz, 1H, H5), 2.57 (d, $J = 9.4$ Hz, 1H, SH), 2.09 (s, 3H, COCH$_3$), 2.04 (s, 3H, COCH$_3$), 2.02 (s, 3H, COCH$_3$), 1.98 (s, 2H, NHCOC$_3$H$_3$) ppm. Spectral data are in accordance with previously published.$^{38}$ LRMS (ES): Calculated for C$_{14}$H$_{21}$NO$_8$SNa m/z 386.0886. Found m/z 385.8.
2-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranoside)-4-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside)-1-phenylbutan-1-one (27)

2-Hydroxy(phenyl)methyl]but-3-en-1-yl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranoside 15 (11 mg, 22 µmol) and 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (19) (10 mg, 26 µmol) was dissolved in CH2Cl2 in a 1 mL glass vial. The mixture was stirred at room temperature under a UVA lamp (λ 365 nm, 12 W) for 1 h and 15 min. The reaction was continuously analysed with LR-MS analysis.


2-(Cyclohexyl(hydroxy)methyl)but-3-en-1-yl-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (29)

E-4-bromobut-2-enyloxy 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranoside (7) (96 mg, 0.200 mmol) was dissolved in EtOH (0.5 mL) and sodium phosphate buffer (0.5 mL, 50 mM, pH 7.0). Cyclohexanecarbaldehyde 28 (24 µL, 0.200 mmol) and indium (46 mg, 0.401 mmol) was added, resulting in instant formation of a thick white mixture, and the reaction mixture was stirred at room temperature over night. TLC analysis and LR-MS showed full conversion of the product. The crude mixture was used directly in the next step without further purification.

LRMS (ES): Calculated for C25H39NO17Na m/z 536.2472. Found m/z 536.2471.

1-Cyclohexyl-2-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranoside)-4-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside)butan-1-ol (30)

To the crude product 2-(cyclohexyl(hydroxy)methyl)but-3-en-1-yl-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-gluco-pyranoside (29) (0.200 mmol) in a 1:1 mixture of EtOH/sodium phosphate buffer (1 mL, 25 mM, pH 7.0) was added 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (19) (87 mg, 0.239 mmol) and DPAP (5 mg, 19.5 µmol). The mixture was stirred at room temperature under a UVA lamp (λ 365 nm, 12 W) for 2 h. The reaction was continuously analysed with LR-MS analysis. After 1.5 h full conversion was observed. The mixture was concentrated. At this point the product was not purified.

LRMS (ES): Calculated for C39H59NO19SNa m/z 900.3300. Found m/z 899.7.
Fmoc-Ser(tBu)-NH₂ (32)

To a solution of Fmoc-Ser(tBu)-OH (804 mg, 2.10 mmol) in dry 1,4-dioxane (20 mL) was added dry pyridine (85 µL, 1.05 mmol) followed by Boc₂O (729 mg, 2.94 mmol). The resulting mixture was stirred at 40 °C under N₂ atmosphere for 21 h. NH₄HCO₃ (232 mg, 2.94 mmol) was added, and the mixture was stirred at 40 °C for 6 h. The reaction mixture was concentrated under vacuum and purified by flash column chromatography (gradient eluent system 1:4 EtOAc/pentane to EtOAc) to afford 663 mg of Fmoc-Ser(tBu)-NH₂ 32 as white crystals (1.73 mmol, 83 %). Rf: 0.24 (1:1 EtOAc/pentane).

¹H-NMR (400 MHz, CDCl₃) δH 7.76 (d, 2H, J 7.5 Hz, ArH), 7.62-7.59 (m, 2H, ArH), 7.40 (t, 2H, J 7.2 Hz, ArH), 7.31 (t, 2H, J 7.6 Hz, ArH), 6.62 (br s, 1H, NH), 6.09 (br s, 1H, NH), 5.86 (br s, 1H, NH), 4.43 (d, 2H, J 6.9 Hz, CH₂Fmoc), 4.26 (br s, 1H, αH), 4.22 (t, 1H, J 6.8 Hz, FmocCH), 3.81 (br s, 1H, CH₂Ser), 3.41 (t, 1H, J 8.4 Hz, CH₂Ser), 1.21 (s, 9H, C(CH₃)₃) ppm. ¹³C-NMR (100 MHz, CDCl₃) δC 173.8 (CONH), 156.2 (CONH), 143.9, 143.8, 141.4, 127.8, 127.2, 125.2, 125.1, 120.1 (Aromatic-C), 74.4 (C(CH₃)₃), 67.1 (CH₂Fmoc, 61.8 (CH₂Ser), 54.5 (Ca), 47.3 (CHFmoc), 27.5 ([C(CH₃)₃] ppm. HRMS (ES): Calculated for C₂₉H₂₆N₂O₅Na m/z 405.1790. Found m/z 405.1790. [α]₂⁰⁺K +37.8 (c = 1, CHCl₃). mp (uncorr): 94.6-96.7 °C

Fmoc-Ser(tBu)-NHBBn (34)

Fmoc-L-Ser(tBu)-OH (506 mg, 1.32 mmol), benzylamine (158 µL, 1.45 mmol), dicyclohexylcarbodiimide (328 mg, 1.59 mmol) and 1-hydroxybenzotriazole (293 mg, 1.91 mmol) were dissolved in anhydrous CH₂Cl₂ (20 mL) and stirred at rt under N₂-atmosphere for 20 h. The reaction mixture was filtered through Celite and washed with a saturated aqueous solution of NaHCO₃, then dried over MgSO₄, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (1:2 EtOAc/Pentane) to afford 561 mg of Fmoc-Ser(tBu)-NHBBn 34 as a white solid (1.19 mmol, 90 %). Rf: 0.59 (1:1 EtOAc/pentane).

¹H-NMR (400 MHz, CDCl₃) δH 7.76 (d, 2H, J 7.5 Hz, ArH), 7.59 (d, 2H, J 7.4 Hz, ArH), 7.42-7.38 (m, 2H, ArH), 7.33-7.27 (m, 7H, ArH), 6.89 (br s, 1H, NH), 5.77 (1H, bs, NH), 4.49 (s, 2H, NCH₂Ph), 4.42 (d, 2H, J 6.9 Hz, CH₂Fmoc), 4.26 (br s, 1H, αH), 4.22 (t, 1H, J 6.9 Hz, FmocCH), 3.85 (dd, 1H, J 8.3 Hz, J₃.₇ Hz, CH₂Ser), 3.40 (t, 1H, J 8.3 Hz, CHH₂Ser), 1.15 (s, 9H, C(CH₃)₃) ppm. ¹³C-NMR (100 MHz, CDCl₃) δC 170.4 (CONH), 141.6 (OCONH), 138.2, 128.9, 128.0, 127.8, 127.7, 127.3, 125.3, 120.3 (Aromatic-C), 74.4 (C(CH₃)₃), 67.0 (CH₂Fmoc), 62.1 (CH₂Ser), 54.4 (Ca), 47.4 (CHFmoc), 43.8 (NCH₂Ph), 27.6 (C(CH₃)₃) ppm. Spectroscopic data is in accordance with literature.¹⁶ LRMS (ES): Calculated for C₂₉H₂₆N₂O₅Na m/z 495.2260. Found m/z 495.0.

Fmoc-Pro-Ser(tBu)-NHBn (35)

Fmoc-Ser(tBu)-NHBn 34 (334 mg, 0.707 mmol) was dissolved in dry CH₂Cl₂ (6 mL) in a flame dried flask to which 1,8-diaza-bicyclo[5.4.0]undec-7-ene (DBU) (111 µL, 0.742 mmol) was added. The reaction was stirred at rt under N₂-atmosphere for 30 min until TLC analysis (2:1 ethyl acetate/pentane) indicated complete consumption of the starting material with the formation of a single product. N-Hydroxybenzotriazole (HOBt) (165 mg, 1.08 mmol) was added to the reaction which was allowed to stir for 5 mins. Diisopropylethylamine (Dipea) (246 µL, 1.41 mmol), O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (405 mg, 1.07 mmol) and Fmoc-L-ProOH (327 mg, 0.918 mmol) was added. The reaction mixture was allowed to stir at rt overnight. The reaction was diluted with water and CH₂Cl₂, the phases were separated and the aqueous phase was re-extracted twice with CH₂Cl₂. The combined organic phases were washed twice with hydrochloric acid (1M aqueous solution), a saturated aqueous solution of NaHCO₃, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (1:2 ethyl acetate/pentane) to afford 325 mg of Fmoc-Pro-Ser(tBu)-NHBn 35 (0.570 mmol, 81 %) as a white amorphous solid. Rₚ: 0.29 (2:1 EtOAc/pentane).

¹H-NMR (400 MHz, CDCl₃) δH 7.77 (d, J = 7.1 Hz, 2H, ArH), 7.51 – 7.44 (m, 2H, ArH), 7.44 – 7.36 (m, 2H, ArH), 7.35 – 7.27 (m, 3H, ArH), 7.21 (d, J = 7.5 Hz, 1H, ArH), 7.16-7.06 (m, 2H, ArH), 7.06 – 6.95 (m, 1H, ArH), 6.90 (br s, 1H, NH), 6.69 (br s, 1H, NH), 4.66 (dd, 1H, J 7.0 Hz, J 14.9 Hz, CH₂Ph), 4.53-4.46 (m, 1H, αHSer), 4.33-4.26 (m, 2H, CH₂Fmoc, αHPro), 4.24-4.17 (m, 1H, CH₂Ph), 4.13-4.10 (m, 1H, CHFmoc), 3.98-3.91 (m, 2H, CH₂Pro), 3.61-3.49 (m, 2H, CH₂Ser), 3.42 (dd, 1H, J 5.2 Hz, J 8.1 Hz, CH₂Ph), 2.80-2.69 (2H, m, CH₂Pro), 2.00-1.90 (2H, m, CH₂Pro), 1.08 (s, 9H, C(CH₃)₃) ppm. ¹³C-NMR (100 MHz, CDCl₃) δC 171.4, 170.1, 156.2 (3xCO), 143.9, 143.6, 141.4, 138.5 (5xArC), 128.3, 127.9, 127.8, 127.3, 127.1, 127.0, 125.1, 125.0, 120.1 (13xArCH), 73.6 (C(CH₃)₃), 68.1 (CH₂Fmoc), 61.6 (αCPro), 61.5 (CH₂Ser), 53.2 (αCSer), 47.3 (CH₂Pro), 47.0 (CHFmoc), 43.2 (CH₂Ph), 29.7 (CH₂Pro), 27.4 (3C, C(CH₃)₃), 24.7 (CH₂Pro) ppm. Spectroscopic data is in accordance with literature. LRMS (ES): Calculated for C₃₄H₃₉N₃O₅Na m/z 592.2787. Found m/z 592.2787.

Fmoc-Thr(tBu)-Pro-Ser(tBu)-NHBn (36)

Fmoc-Pro-Ser(tBu)-NHBn 35 (309 mg, 0.543 mmol) was dissolved in dry CH₂Cl₂ (5 mL) in a flame dried flask to which DBU (85 µL, 0.570 mmol) was added. The reaction was stirred at rt under N₂-atmosphere for 30 min until TLC analysis (EtOAc) indicated complete consumption of the starting material with the formation of a single product. HOBt (127 mg, 0.830 mmol) was added to the reaction which was allowed to stir for 5 min.
Dipea (190 µL, 1.09 mmol), HBTU (307 mg, 0.809 mmol) and Fmoc-L-Thr(tBu)OH (280 mg, 0.705 mmol) was added. The reaction mixture was allowed to stir at rt overnight. The reaction was diluted with water and CH₂Cl₂, the phases were separated and the aqueous phase was re-extracted twice with CH₂Cl₂. The combined organic phases were washed twice with hydrochloric acid (1M aqueous solution), a saturated aqueous solution of NaHCO₃, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (gradient eluent system 1:1 EtOAc/pentane) to afford 343 mg of Fmoc-Thr(tBu)-Pro-Ser(tBu)-NHBn 36 (0.472 mmol, 87 %) as a colourless foam. Rf: 0.24 (2:1 EtOAc/pentane).

\(^1\)H-NMR (400 MHz, CDCl₃) δH 7.77 (d, 2H, J 7.6 Hz, ArH), 7.64-7.57 (m, 2H, ArH), 7.43-7.10 (m, 9H, ArH), 6.88 (d, 1H, J 8.0 Hz, NH), 5.54 (d, 1H, J 7.6 Hz, NH), 4.63-4.51 (m, 1H, CH₂Ph), 4.51-4.39 (m, 4H, αCHThr, αCHSer, CHFmoc, αCHPro), 4.39-4.10 (m, 2H, CH₂Ph, CHFmoc), 4.02-3.85 (m, 2H, CH₂Pro), 3.88-3.78 (m, 1H, CHFmoc), 3.74-3.62 (m, 1H, CH₂CH₃), 3.52 (dd, J = 8.9, 5.0 Hz, 1H, CHHSer), 2.14-1.82 (m, 4H, 2xCH₂Pro), 1.23 (s, 9H, C(CH₃)₃), 1.17 (s, 9H, C(CH₃)₃), 0.86 (d, 3H, J 6.3 Hz, CHCH₃) ppm. \(^13\)C-NMR (100 MHz, CDCl₃) δC 170.9, 170.5, 169.5 (3xCO), 155.4 (OCNHNH), 143.7, 143.5, 141.2, 138.0 (5xArC), 128.2, 127.9, 127.6, 127.1, 126.9, 124.9, 124.8, 119.9 (13xArcH), 74.5 (C(CH₃)₃), 73.6 (C(CH₃)₃), 68.8 (CH₃Fmoc), 66.6 (CH₂CH₃), 61.6 (αCPro), 61.2 (CH₂Ser), 57.1 (αCThr), 53.7 (αCSer), 48.8 (CH₂Pro), 47.1 (CHFmoc), 43.5 (CH₂Ph), 29.1 (CH₂Pro), 27.9 (C(CH₃)₃), 27.3 (C(CH₃)₃), 24.9 (CH₂Pro), 18.2 (CHCH₃) ppm. HRMS (ES): Calculated for C₆₃H₅₄N₉O₇Na m/z 749.3882. Found m/z 749.3882.

Fmoc-FGly(OEt)₂-Thr(tBu)-Pro-Ser(tBu)-NHBn (37)

Fmoc-Thr(tBu)-Pro-Ser(tBu)-NHBn 36 (291 mg, 0.401 mmol) was dissolved in dry CH₂Cl₂ (6 mL) in a flame dried flask to which DBU (63 µL, 0.421 mmol) was added. The reaction was stirred at rt under N₂-atmosphere for 2 h until TLC analysis (EtOAc) indicated complete consumption of the starting material with the formation of a single product. HOBT (93.7 mg, 0.612 mmol) was added to the reaction which was allowed to stir for 5 min. Dipea (140 µL, 0.802 mmol), HBTU (228 mg, 0.601 mmol) and FmocFmoc(OEt)₂OH (3) (225 mg, 0.563 mmol) was added. The reaction mixture was allowed to stir at rt overnight. The reaction was diluted with water and CH₂Cl₂, the phases were separated and the aqueous phase was re-extracted twice with CH₂Cl₂. The combined organic phases were washed twice with HCl (1M aqueous solution), a saturated aqueous solution of NaHCO₃, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (gradient eluent system 1:1 EtOAc/pentane to EtOAc) to afford 241 mg of Fmoc-FGly(OEt)₂-Thr(tBu)-Pro-Ser(tBu)-NHBn 37 (0.272 mmol, 68 %) as a colourless foam. Rf: 0.19 (2:1 EtOAc).
Fmoc-Leu-FGly(OEt)_{2}-Thr(tBu)-Pro-Ser(tBu)-NHBn (38)

Fmoc-FGly(OEt)_{2}-Thr(tBu)-Pro-Ser(tBu)-NHBn (37) (235 mg, 0.265 mmol) was dissolved in dry CH_{2}Cl_{2} (6 mL) in a flame dried flask to which DBU (42 µL, 0.278 mmol) was added. The reaction was stirred at rt under N\textsubscript{2}-atmosphere for 2 h until TLC analysis (EtOAc) indicated complete consumption of the starting material with the formation of a single product. HOBt (62 mg, 0.405 mmol) was added to the reaction which was allowed to stir for 5 min. Dipea (92 µL, 0.530 mmol), HBTU (151 mg, 0.398 mmol) and FmocLeuOH (122 mg, 0.345 mmol) was added. The reaction mixture was allowed to stir at rt overnight. The reaction was diluted with water and CH_{2}Cl_{2}, the phases were separated and the aqueous phase was re-extracted twice with CH_{2}Cl_{2}. The combined organic phases were washed twice with HCl (1M aqueous solution), a saturated aqueous solution of NaHCO\textsubscript{3}, brine, dried over MgSO\textsubscript{4}, filtered and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (gradient eluent system 2:1 EtOAc/pentane to EtOAc) to afford 211 mg of 38 (0.211 mmol, 80 %) as a bright yellow solid. R\textsubscript{f}: 0.40 (EtOAc).

\textsuperscript{13}C-NMR (400 MHz, CDCl\textsubscript{3}) \delta_{c} 172.5, 171.2, 170.4, 169.8, 167.2 (4xCO), 156.1 (OCONH), 143.9, 143.8, 141.3, 138.5 (5xArC), 128.4, 127.8, 127.6, 127.1, 127.0, 125.2, 125.1, 120.0 (13xArCH), 101.3 (CH(OEt)) \textsubscript{2}, 75.0 (C(CH\textsubscript{3}))\textsubscript{2}, 73.8 (C(CH\textsubscript{3}))\textsubscript{2}, 68.3 (CH_{2}Fmoc), 67.1 (CHCH\textsubscript{2}), 65.4 (OCH\textsubscript{2}CH\textsubscript{2}), 64.0 (OCH_{2}CH\textsubscript{2}CH\textsubscript{2}), 61.8 (αCPro), 61.4 (CH\textsubscript{2}Ser), 56.4 (αCThr), 55.7 (αCFGly), 53.9 (αCSer), 53.6 (αCVal), 48.9 (CH\textsubscript{2}Pro), 47.2 (CHFmoc), 43.3 (CH\textsubscript{2}Ph), 41.6 (CH\textsubscript{2}-CH(CH\textsubscript{2})\textsubscript{2}), 29.2 (CH\textsubscript{2}Pro), 28.1 (C(CH\textsubscript{3})\textsubscript{2}), 27.5 (C(CH\textsubscript{3})\textsubscript{2}), 25.1 (CH\textsubscript{2}Pro), 24.8 (CH\textsubscript{2}-CH(CH\textsubscript{2})\textsubscript{2}), 23.1 (CH\textsubscript{2}-CH(CH\textsubscript{2})\textsubscript{2}), 22.0 (CH\textsubscript{2}-CH(CH\textsubscript{2})\textsubscript{2}), 18.6 (CHCH\textsubscript{2}), 15.3 (OCH\textsubscript{2}CH\textsubscript{2}), 15.2 (OCH_{2}CH\textsubscript{2}) ppm. HRMS (ES): Calculated for C\textsubscript{66}H\textsubscript{60}N\textsubscript{10}O\textsubscript{11}Na \textsuperscript{m/z} 908.4783. Found m/z 908.4783.

[α]_{D}^{294\text{C}} = -7.5 (c=1, CH\textsubscript{2}Cl).
Failed synthesis of Fmoc-Leu-FGly-Thr-Pro-Ser-NHBn (39)

Fmoc-Leu-FGly(OEt)₂-Thr(tBu)-Pro-Ser(tBu)-NHBn (38) (15 mg, 15 µmol) was dissolved in a mixture of TFA/thioanisole/anisole/H₂O (85/5/5/5, total 1 mL). The mixture was placed on a shaking machine, and left at room temperature until MALDI-TOF analysis indicated full consumption of the starting material. N₂-air was bubbled through the mixture until almost dryness, cold MTBE was added, the mixture was centrifuged at 0°C (10 min) and the supernatant was removed. This was repeated three times in total. H₂O and MeCN (1:1, total 2 mL) was added, the mixture was cooled to -78°C and then lyophilized.

MALDI-TOF MS Expected mass+Na m/z 835.364. Observed mass: 817.047. Indicates full deprotection of the peptide and formation of dehydroalanine instead of serine (40). See APPENDIX A for MALDI-TOF analysis.

General procedure for test of aldehyde functionality in 40

A fraction of the crude 40 from the acidic deprotection of 38, described above, (0.5 mg, 0.612 µmol) was dissolved in buffer (0.4 mL, MES buffer pH 5.5/Sodium phosphate buffer pH 6.5 /KOAc,HOAc buffer pH 4.6). The hydroxyl amine of choice (methoxyamine or o-benzylhydroxylamine, 1.83 µmol) was added and the mixture was stirred at either rt or 37 °C. The reaction was analysed by MALDI-TOF spectroscopy. No product formation was observed. See APPENDIX A for MALDI-TOF analysis.

Test of dehydroalanine functionality in 40

A fraction of the crude 39 from the acidic deprotection of 38, described above, (0.5 mg, 0.612 µmol) was dissolved in sodium phosphate buffer (0.8 mL, pH 8.5, 50 mM). Ethane thiol (23 µL, 3.06 µmol) was added and the mixture was stirred at 37 °C over night. The reaction was analysed by MALDI-TOF spectroscopy. No product formation was observed. See APPENDIX A for MALDI-TOF analysis.

Failed synthesis of H₂N-Leu-FGly-Thr-Pro-Ser-NHBn (43)

Fmoc-Leu-FGly(OEt)₂-Thr(tBu)-Pro-Ser(tBu)-NHBn (38) (11 mg, 11 µmol) was dissolved in MeCN (200 µL). Et₂NH (1.3 µL, 12 µmol) was added, and the mixture was stirred at rt for 1.5 h. LR-MS showed full conversion of the start material to H₂N-Leu-FGly(OEt)₂-Thr(tBu)-Pro-Ser(tBu)-NHBn. The mixture was concentrated under reduced pressure, and was then dissolved in a mixture of TFA/thioanisole/anisole/H₂O (85/5/5/5, total 1 mL) and stirred at rt. MALDI-TOF analysis showed full conversion of the intermediate and formation of the desired product 43 after 10 min. N₂-air was bubbled through the mixture until almost dryness, cold MTBE was added, the mixture was centrifuged at 0°C (10 min) and the supernatant was removed. This was repeated three times in total. H₂O and MeCN (1:1, total 2 mL) was added, the mixture was cooled to -78°C and then lyophilized. After work up, no product 43 could be observed.
Fmoc-Gly(OEt)_2-NHBn (44)

Fmoc-Gly(OEt)_2-OH 3 (222 mg, 0.556 mmol) was dissolved in dry CH₂Cl₂ (8 mL). Benzylamine (67 µL, 0.611 mmol), HOBT (120 mg, 0.778 mmol) and DCC (138 mg, 0.667 mmol) were added and the mixture was stirred at rt under N₂. The mixture was filtered through celite, diluted with CH₂Cl₂, washed with a saturated aqueous solution of NaHCO₃, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (gradient eluent system 1:2 EtOAc/pentane to EtOAc) to afford 232 mg of 44 as a white powder (86%, 0.475 mmol). Rf: 0.39 (1:2 EtOAc/pentane).

1H-NMR (400 MHz, CDCl₃) δ 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.60 (d, J = 7.3 Hz, 2H, ArH), 7.44 – 7.22 (m, 9H, Ar-H), 6.88 (br s, 1H, NH), 5.84 (br s, 1H, NH), 4.85 (br s, 1H, CH(OEt)_2), 4.60 – 4.32 (m, 5H, αCH-Fmoc, NCH₂Ph, CH₂Fmoc), 4.24 (t, J = 6.9 Hz, 1H, CHFmoc), 3.85 – 3.43 (m, 4H, 2xOCH₂CH₃), 1.23 – 115 (m, 6H, 2xOCH₂CH₃) ppm. 13C-NMR (100 MHz, CDCl₃) δC 168.2 (CONH), 156.5 (CONH), 143.9, 141.4 (Ar-C), 128.7, 127.9, 127.5, 125.3, 120.1 (Ar-CH), 102.3 (CH(OEt)_2), 67.8 (CH₃Fmoc), 65.2 (OCH₂CH₃), 64.6 (OCH₂CH₃), 59.5 (αCFGly), 47.3 (CHFmoc), 43.6 (CH₃Ph), 15.4 (OCH₂CH₃), 15.3 (OCH₂CH₃) ppm. HRMS (ES): Calculated for C₂₉H₂₇N₂O₃Na m/z 511.2209. Found m/z 511.2205.

Ac-Gly(OEt)_2-NHBn (46)

Fmoc-Gly(OEt)_2-NHBn 44 (237 mg, 0.485 mmol) was dissolved in dry CH₂Cl₂ (5 mL). DBU (80 µL, 0.534 mmol) was added and the mixture was stirred at rt under N₂-atmosphere for 3h. Ac₂O (100 µL, 1.07 mmol), Et₃N (39 µL, 0.534 mmol) and a catalytic amount of DMAP was added, and the resulting mixture was stirred at rt under N₂-atmosphere for 2h. Work up was accomplished by adding H₂O and diluting the mixture with CH₂Cl₂. The organic phase was washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (gradient eluent system 1:1 EtOAc/pentane to EtOAc) to afford 73 mg of 46 as a colourless oil (49%, 0.237 mmol). Rf: 0.39 (EtOAc).

1H-NMR (400 MHz, CDCl₃) δ 7.37 – 7.21 (m, 5H, ArH), 6.92 (s, 1H, NH), 6.56 (d, J = 6.4 Hz, 1H, NH), 4.83 (d, J = 2.7 Hz, 1H, CH(OEt)_2), 4.61 – 4.53 (m, 2H, αCH-Gly, CHHPh), 4.43 (dd, J = 15.2, 5.6 Hz, 1H, CHHPh), 3.85 – 3.46 (m, 4H, 2xOCH₂CH₃), 2.06 (s, 3H, CH₃), 1.24 – 1.15 (m, 6H, OCH₂CH₃) ppm. 13C-NMR (100 MHz, CDCl₃) δC 170.5, 168.2 (CONH), 138.2 (Ar-C), 128.7, 127.4 (5 Ar-CH), 102.0 (CH(OEt)_2), 65.3 (OCH₂CH₃), 64.5 (OCH₂CH₃), 56.0 (αCFGly), 43.6 (CH₃Ph), 23.4 (COCH₃), 15.4 (OCH₂CH₃), 15.3 (OCH₂CH₃) ppm. HRMS (ES): Calculated for C₁₀H₁₂N₂O₃Na m/z 331.1634. Found m/z 331.1630. [α]D²⁰⁷K +36.0 (c=1, CH₂Cl₂).
Conditions for deprotection of diethylacetal in 46 to afford 47

Ac-FGly(OEt)_2-NHBn 46 (10 mg, 32 µmol) was dissolved in 1 mL of the solvent of choice, shown in Table 3. The mixture was stirred at 0°C and allowed to warm up to rt over night. The reactions were followed by TLC analysis (EtOAc). Work up was accomplished by diluting the mixture with CH_2Cl_2 and adding H_2O. The organic phase was dried over MgSO_4, filtered, concentrated under reduced pressure and analysed with NMR spectroscopy. No aldehyde signal could be observed in ^1H-NMR.

Boc-Phe-NHBn (50)

Boc-L-Phe-OH (929 mg, 3.50 mmol), DCC (867 mg, 4.20 mmol) and HOBT (750 mg, 4.90 mmol) were dissolved in dry CH_2Cl_2 (50 mL). Benzyl amine (420 µL, 3.85 mmol) was added and the resulting mixture was stirred at rt under N_2 atmosphere ON. The mixture was filtered through celite, diluted with CH_2Cl_2, washed with an aqueous solution of HCl (0.25 M), twice with a saturated aqueous solution of NaHCO_3, washed with brine, dried over MgSO_4, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (1:4 EtOAc/pentane) to afford 1.17 g of 50 as a white powder (3.29 mmol, 94%). R_f: 0.23 (1:4 EtOAc/pentane).

^1H-NMR (400 MHz, CDCl_3) δ_H 7.31 – 7.22 (m, 6H, ArH), 7.21-7.16 (m, 2H, ArH), 7.13-7.05 (m, 2H, ArH), 6.14 (br s, 1H, NH), 5.09 (br s, 1H, NH), 4.44-4.27 (m, 3H, αCHPhe, NHCHAr), 3.19 – 2.97 (m, 2H, CHAr), 1.39 (s, 9H, C(CH_3)_3) ppm. Spectral data were in accordance with previously published. \(^{69}\) LRMS (ES): Calculated for C_{21}H_{26}N_2O_3Na m/z 377.1841. Found m/z 377.0.

Boc-Ser-Phe-NHBn (51)

Boc-PheNHbN 50 (1.15 g, 3.24 mmol) was dissolved in CH_2Cl_2 (40 mL). An aqueous solution of HCl (6M, 20 mL) was added and the mixture was stirred ON at rt. TLC analysis showed the incomplete conversion of the starting material, so the residue was concentrated under reduced pressure and re-dissolved in CH_2Cl_2 (10 mL) and TFA (1 mL) and heated to 30 °C for 1h. The mixture was concentrated and left at vacuum ON.

The deprotected amino acid was dissolved in dry CH$_2$Cl$_2$ (30 mL). N-methylmorpholine (0.72 mL, 6.48 mmol), BocSerOH (732 mg, 3.57 mmol), HOBt (744 mg, 4.86 mmol) and EDC (931 mg, 4.86 mmol) were added and the resulting mixture was stirred for at rt. After 40 h, the mixture was diluted with CH$_2$Cl$_2$ and washed with H$_2$O, the aqueous phase was re-extracted with CH$_2$Cl$_2$, the combined organic phases were dried over MgSO$_4$ filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (gradient eluent system 2:1 EtOAc/pentane to EtOAc) to afford 874 mg of 51 as a white powder (1.98 mmol, 61%).

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 7.33 – 7.23 (m, 6H, ArH), 7.23 – 7.16 (m, 2H, ArH), 7.13-7.05 (m, 3H, ArH), NH), 6.76 (br s, 1H, NH), 5.50 (d, $J = 6.7$ Hz, 1H, NH), 4.77 (dd, $J = 15.0, 7.0$ Hz, 1H, $\alpha$HPhe), 4.42 – 4.26 (m, 2H, $\text{NHC}_2\text{Ar}$), 3.30 – 3.02 (m, 3H, C$_2$H$_2$Ar, OH), 1.39 (s, 9H, C(C$_3$H$_3$)$_3$) ppm.

13C-NMR (100 MHz, CDCl$_3$) $\delta$ 171.0 (CONH), 170.6 (CONH), 165.8 (OCONH) 137.7, 136.4 (2xAromatic-C), 129.4, 128.9, 128.7, 127.9, 127.6, 127.3 (10xAromatic C-H), 63.0 (CH$_2$OH), 55.9, 54.7 ($\alpha$C-Ser, $\alpha$C-Phe), 43.8 (2xC$_2$H$_2$Ar), 28.4 (C(C$_3$H$_3$)$_3$) ppm.

LRMS (ES): Calculated for C$_{24}$H$_{31}$N$_3$O$_5$Na m/z 464.2161. Found m/z 463.9.

H-Ser-Phe-NHBn (49)

Boc-Ser-Phe-NHBn 51 (831 mg, 1.88 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL) and TFA (1 mL). The mixture was stirred at rt ON. TLC analysis (EtOAc) showed no conversion of the starting material, so additional TFA (1 mL) was added and the mixture was heated to 30 °C for 4 h. Still no change was observed by TLC analysis, so the mixture was concentrated under reduced pressure, and the crude was dissolved in neat TFA (2 mL) and heated to 65 °C ON. The mixture was then concentrated under reduced pressure, cooled on an ice bath before adding Et$_2$O, resulting in precipitation of a white solid, which was filtered off. The crude solid was then re-dissolved in an aqueous solution of HCl (0.1M). The aqueous phase was extracted twice with Et$_2$O, the pH of the aqueous phase was then raised to pH 14 by adding NaOH (1 M), resulting in formation of a white precipitate, which was filtered off and dried under reduced pressure affording 476 mg of the product 49 (74%, 1.39 mmol).

$^1$H NMR (400 MHz, CD$_2$OD) $\delta$ 7.23 – 7.11 (m, 8H, ArH), 7.11 – 7.06 (m, 2H, ArH), 4.59 (dd, $J = 8.3, 6.3$ Hz, 1H, $\alpha$CHPhe), 4.33 – 4.16 (m, 2H, NHCH$_2$Ar), 3.31 (t, $J = 5.9$ Hz, 1H, $\alpha$CHSer), 3.26 – 3.22 (m, 2H, CH$_2$OH), 3.09 (dd, $J = 13.8, 6.3$ Hz, 1H, CHHR), 2.89 (dd, $J = 13.8, 8.3$ Hz, 1H, CHHR) ppm. HRMS (ES): Calculated for C$_{19}$H$_{23}$N$_3$O$_3$Na m/z 364.1637. Found m/z 364.1640. [\(\alpha\)]$_D^{297\text{K}}$ -10.4 (c=1, MeOH).
HCOCO-Phe-NHBn (52)

H-Ser-Phe-NHBn 49 (78 mg, 0.206 mmol) was dissolved in sodium phosphate buffer (25 mM, pH=7.0, 1 mL) and NaIO₄ (88 mg, 0.412 mmol) was added. The resulting mixture was stirred at rt under the exclusion of light until TLC analysis showed full conversion of starting material (3h). Na₂SO₃ (26 mg, 0.206 mmol) was added to neutralize the excess of periodate. CH₂Cl₂ was added to the mixture, and the aqueous phase was extracted three times with CH₂Cl₂, the combined organic phases was washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure resulting in 49 mg of a bright yellow solid (crude yield 77%). NMR analysis of the crude showed an aldehyde functionality. The crude 52 was used as a test system in the next step without further purification. HRMS (ES): Calculated for C₁₈H₁₈N₂O₃Na m/z 333.1215. Found m/z 333.1208.

N-(1-(benzylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxypent-4-enamide (53)

HCOCO-Phe-NHBn 52 (crude, 10 mg, 32 µmol) was dissolved in sodium phosphate buffer (50mM, pH=7.0, 0.2 mL) and the solvent of choice (0.2 mL, MeCN/EtOH/dioxane/DMF/THF). Allylbromide 4 (5.6 µL, 64 µmol) and indium (7.4 mg, 64 µmol) was added, and the reaction mixture was stirred at rt ON. The reaction mixtures was analysed with LR-MS. Formation of 53 was observed using EtOH and dioxane as solvent. LRMS (ES): Calculated for C₂₁H₂₄N₂O₃Na m/z 375.1685. Found m/z 375.0.

N-terminus of horse heart myoglobin (55)

H₂N-GLSDGEWQV-OH 55 was synthesised using SPPS and performed on a CEM Liberty microwave assisted peptide synthesiser. The resin Fmoc-L-Val-Wang (370 mg, 100 mmol) was dissolved in DMF (5 mL) and CH₂Cl₂ (5 mL). For each coupling step 2M DIPEA and 0.5 MHBTU in DMF were used and the deprotection was accomplished using 5wt% piperazin and 0.1M HOBt in DMF. The L-amino acids Fmoc-Asp(tBu)-OH (5 eq), Fmoc-Gln(Trt)-OH (8 eq), Fmoc-Glu(tBu)-OH (5 eq), Fmoc-Gly-OH (6 eq), Fmoc-Ser(tBu)-OH (5 eq), Fmoc-Leu-OH (5 eq) and Fmoc-Trp(Boc)-OH (5 eq) were used. After complete synthesis, the resin was washed with DMF (4x), CH₂Cl₂ (4x), Et₂O (4x) and dried at vacuum. The cleavage of the resin was accomplished by adding a mixture of TFA/DODT/TIPS/H₂O (90:5:2.5:2.5) and heating the mixture to 40°C using the microwave. The mixture was filtered, the filtrate was concentrated and MBTE was added. The mixture was centrifuged at 0°C (10 min) and the supernatant was removed. This was repeated three times in total. H₂O and MeCN (1:1, total 2 mL) was added, the mixture was cooled to ~78°C and then lyophilized. The crude (72 mg) was dissolved in HFIPA. The purification by preparative reverse phase HPLC failed.
Decapeptide H$_2$SNNFGAILSS-NH$_2$ (56) (2.2 mg, 2.2 µmol) was dissolved in sodium phosphate buffer (25 mM, pH 7.0, 500 µL) and NaIO$_4$ (1.2 mg, 5.6 µmol) was added. The resulting mixture was stirred at rt ON in the dark. MALDI-TOF analysis showed full conversion of the starting material to the product 57. This solution was used directly in the next step for the Barbier reaction. See APPENDIX A for MALDI-TOF spectra.

MALDI-TOF MS: Calculated for C$_{42}$H$_{64}$N$_{12}$O$_{15}$Na m/z 999.4512. Found m/z 999.216

(2-hydroxypent-4-en-1-on)-NNFGAILSS-NH$_2$ (58)

MALDI-TOF MS: Calculated for C$_{45}$H$_{70}$N$_{12}$O$_{15}$Na m/z 1041.4981. Found m/z 1041.369.

Neoglycopeptide (59)

MALDI-TOF MS: Calculated for C$_{45}$H$_{85}$N$_{13}$O$_{21}$K m/z 1290.5620. Found m/z 1288.427.
3.3 EXPERIMENTAL SECTION PART II

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-O-acetyl-β-D-glucopyranoside (82)

Anhydrous sodium acetate (4.80 g, 58.5 mmol) was dissolved in acetic anhydride (70 mL) in a 250 mL round bottomed flask. The mixture was heated to reflux and α-D-lactose monohydrate (81) (10.0 g, 27.8 mmol) was added in portions, so that the mixture continued to reflux. After complete addition, the reaction mixture was stirred at reflux for 30 min and turned into a clear mixture. The mixture was then poured into 300 mL of crushed ice and stirred for 2 h. The precipitated product was filtered off and washed with cold water, dried in vacuum and recrystallised from EtOH (50 mL). This resulted in 14.0 g of a white solid of 82 (20.6 mmol, 74 %). Rf: 0.43 (1:1 EtOAc/toluene).

1H-NMR (500 MHz, CDCl3) δ: 5.66 (d, 1H, J = 8.3 Hz, H1), 5.34 (dd, 1H, J = 3.0 Hz, J = 1.2 Hz, H4'), 5.23 (t, 1H, J = 9.2 Hz, H3), 5.11–5.00 (m, 2H, H2, H3'), 4.94 (dd, 1H, J = 10.4 Hz, J = 3.4 Hz, H2'), 4.47 (d, 1H, J = 7.9 Hz, H1'), 4.44 (dd, 1H, J = 12.1 Hz, J = 1.8 Hz, H6'a), 4.15 – 4.04 (m, 2H, H5', H6b), 3.88 – 3.81 (m, 2H, H4, H6b), 3.77 – 3.72 (m, 1H, H5), 2.14 (s, 3H, CH3), 2.10 (s, 3H, CH3), 2.05 (s, 3H, CH3), 2.04 (s, 3H, CH3), 2.03 (s, 3H, CH3), 2.01 (s, 3H, CH3), 1.95 (s, 3H, CH3) ppm.

13C-NMR (125 MHz, CDCl3) δ: 170.5, 170.4, 170.2, 170.1, 169.7, 169.6, 169.1, 168.9 (8C, COCH3), 101.0 (1C, C1'), 91.7 (1C, C1), 75.8, 73.7, 72.8, 71.1, 70.9, 70.7, 69.2, 66.8 (8C), 61.9 (1C, C6), 61.0 (1C, C6'), 20.93, 20.90, 20.86, 20.73 (2C), 20.71, 20.69, 20.60 (8C, COCH3) ppm. LRMS (ES): Calculated for C28H38O19Na m/z 701.1905. Found m/z 701.26.

3-Chloropropyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (83)

This procedure was done using the Schlenk technique. The peracetylated β-D-lactose (82) (12.00 g, 17.68 mmol) was dried under high vacuum over night. It was then dissolved in dry CH2Cl2 (50 mL) under N2 atmosphere and cooled to 0°C (icebath). 3-Chloropropanol (3.7 mL, 44.21 mmol) was added followed by dropwise addition of BF3·Et2O (10.9 mL, 88.42 mmol). The resulting mixture was then stirred for 30 min at 0°C and then allowed to warm up to rt. The reaction was monitored by TLC analysis (1:1 EtOAc/toluene). After 19 h the reaction mixture was poured into crushed ice and stirred for 10 min. The layers was separated and the organic phase was washed with an saturated aqueous solution of NaHCO3 followed by brine, dried over MgSO4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (gradient eluent system EtOAc/toluene 1:9 → 1:2) to afford 7.01 g of the product 83 as a white solid (9.83 mmol, 56 %). Rf: 0.48 (1:1 EtOAc/toluene).
\[^1\text{H-NMR (500 MHz, CDCl}_3\text{)}\] $\delta$ 5.34 (d, 1H, $J$ 2.7 Hz, H4’), 5.20 (t, 1H, $J$ 9.3 Hz, H3), 5.10 (dd, 1H, $J$ 10.4 Hz, $J$ 7.9 Hz, H2’), 4.95 (dd, 1H, $J$ 10.4 Hz, $J$ 3.4 Hz, H3’), 4.88 (dd, 1H, $J$ 9.5 Hz, $J$ 8.0 Hz, H2), 4.52 – 4.44 (m, 3H, H1’, H6a’, H1), 4.16 – 4.05 (m, 3H, H5’, H6a, H6b’), 3.96-3.92 (m, 1H, -OCH$_2$-), 3.88-3.85 (m, 1H, H6a), 3.82 – 3.75 (m, 1H, H4), 3.70 – 3.64 (m, 1H, H5, -CH$_2$Cl), 2.15 (s, 3H, CH$_3$), 2.12 (s, 3H, CH$_3$), 2.06 (s, 3H, CH$_3$), 2.05 (s, 3H, CH$_3$), 1.96 (s, 3H, CH$_3$), ppm. $^{13}\text{C-NMR (125 MHz, CDCl}_3\text{)}\] $\delta$ 170.5, 170.4, 170.2, 170.1, 169.8, 169.8, 169.2 (7C, C=O), 101.2 (C1’), 100.9 (C1), 76.4 (C4), 72.9 (C3), 72.8 (C5), 71.8 (C2), 71.1 (C5’), 70.9 (C3’), 69.3 (C2’), 66.8 (C4’), 66.5 (-OCH$_2$-), 62.1 (C6’), 61.0 (C6), 41.4 (-CH$_2$N$_3$), 29.2 (-CH$_2$-), 21.0, 20.9, 20.8, 20.7 (3C), 20.6 (7C, CH$_3$) ppm.

LRMS (ES): Calculated for C$_{29}$H$_{41}$ClO$_1$Na m/z 735.19. Found m/z 735.26. The NMR data are in accordance with those previously reported.

3-Azidopropyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (84)

The lactoside 83 (1.62 g, 2.27 mmol) was dissolved in dry DMF (25 mL). NaN$_3$ (813 mg, 12.5 mmol) and TBAI (84 mg, 0.227 mmol) was added and the reaction mixture was stirred under N$_2$ atmosphere at 60 °C for 2h. The reaction mixture was diluted with EtOAc, washed with H$_2$O and brine, dried over MgSO$_4$, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (1:1 EtOAc/pentane) affording 1.47 g of the product 84 as a white foam (2.05 mmol, 90%). $R_f$: 0.46 (1:1 EtOAc/toluene).

$^1\text{H-NMR (500 MHz, CDCl}_3\text{)}\] $\delta$ 5.34 (d, 1H, $J$ 2.7 Hz, H4’), 5.19 (t, 1H, $J$ 9.3 Hz, H3), 5.10 (dd, 1H, $J$ 10.4 Hz, $J$ 7.9 Hz, H2’), 4.95 (dd, 1H, $J$ 10.4 Hz, $J$ 3.5 Hz, H3’), 4.87 (dd, 1H, $J$ 7.6 Hz, $J$ 6.4 Hz, H2), 4.51 – 4.44 (m, 3H, H1’, H6a’, H1), 4.16 – 4.04 (m, 3H, H5’, H6a, H6b’), 3.93 – 3.83 (m, 2H, H6b, -OCH$_2$-), 3.79 (t, 1H, $J$ 9.3 Hz, H4), 3.62 – 3.57 (m, 2H, H5, -OCH$_2$-), 3.36 – 3.32 (m, 2H, -CH$_2$N$_3$), 2.14 (s, 3H, CH$_3$), 2.11 (s, 3H, CH$_3$), 2.05 (s, 9H, CH$_3$), 2.04 (s, 3H, CH$_3$), 1.96 (s, 3H, CH$_3$), 1.90 – 1.75 (m, 2H, -CH$_2$-) ppm. $^{13}\text{C-NMR (125 MHz, CDCl}_3\text{)}\] $\delta$ 170.5, 170.3, 170.2, 169.9, 169.8, 169.2, 169.1 (7C, C=O), 101.2 (C1’), 100.8 (C1), 76.4 (C4), 73.0 (C-3), 72.9 (C-5), 71.9 (C-2), 71.2 (C-5’), 70.9 (C-3’), 69.3 (C-2’), 66.8 (C-4’), 66.6 (-OCH$_2$-), 62.1 (C-6’), 61.0 (C-6), 48.1 (-CH$_2$N$_3$), 29.2 (-CH$_2$-), 21.0, 20.9, 20.8, 20.7, 20.6 (7C, CH$_3$) ppm. LRMS (ES): Calculated for C$_{29}$H$_{41}$N$_3$O$_{18}$Na m/z 742.23. Found m/z 742.19. The NMR data are in accordance with those previously reported.

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Lactoside 84 (2.77 g, 3.84 mmol) was dissolved in dry MeOH (20 mL) followed by addition of a catalytic amount of freshly prepared NaOMe. The mixture was stirred under N₂ atmosphere at rt for 1 h. The reaction was monitored by TLC analysis (1:3 MeOH/CH₂Cl₂). The mixture was neutralized by adding a small amount of Dowex 50WX-8-100 ion-exchange resin and stirring the mixture for 15 min, the resin was then filtered off and washed with MeOH, the filtrate was concentrated under reduced pressure to afford 1.58 g of the deacetylated lactoside 85 as a white solid (3.70 mmol, 96%). Rfv: 0.23 (1:4 MeOH/CH₂Cl₂).

**1H-NMR (500 MHz, D₂O)** δ 4.55 (d, J = 8.0 Hz, 1H, H1), 4.52 (d, J = 7.8 Hz, 1H, H1'), 4.09-4.02 (m, 2H), 4.00 (d, J = 3.2 Hz, 1H), 3.91-3.82 (m, 4H), 3.82 – 3.76 (m, 2H), 3.76 – 3.69 (m, 3H), 3.67 (d, J = 4.4 Hz, 1H), 3.64 – 3.58 (m, 1H), 3.54 (t, J = 6.7 Hz, 2H), 3.39 (t, J = 8.1 Hz, 1H), 1.99 (qv, J = 6.5 Hz, 2H) ppm. **13C-NMR (125 MHz, D₂O)** δ 102.9 (C-1'), 102.1 (C-1), 78.4, 75.3, 74.7, 74.4, 72.8, 72.5, 70.9, 68.5, 67.4 (-OCH₂-), 61.0, 60.1, 47.9 (-OCH₃), 28.2 (-CH₂-) ppm. The NMR data are in accordance with those previously reported.

A 10 mL flask was charged with the vanadyl triflate catalyst (60 mg, 0.16 mmol). Dry acetonitrile (2.5 mL) was added to dissolve the catalyst resulting in a faint blue solution. 2,2-Dimethoxypropane (4.0 mL, 32.8 mmol) was added and the resulting mixture was stirred at rt for 10 min, during which the reaction mixture changed colour from faint blue to yellow to brown. The lactoside 85 was added (1.40 g, 3.28 mmol) and the mixture was then stirred at rt for 2 days. The reaction was observed using TLC analysis (MeOH/EtOAc 1:9). The reaction mixture was then concentrated under reduced pressure to obtain a yellow-brown substance. The crude product was used directly in the next step without further purification or characterisation. Rf: 0.47 (9:1 EtOAc/MeOH).

The crude lactoside was dissolved in dry DMF (7 mL) and stirred at 0 °C under N₂ atmosphere. NaH (1.3 g, 60%, 32.5 mmol) was added in portions and the mixture was stirred for 5 min. Benzyl bromide (3.9 mL, 32.8 mmol) was then added slowly, and the reaction mixture was then allowed to warm up to rt and was stirred over night. The progress of the reaction was monitored by TLC (1:3 EtOAc/pentane). The reaction mixture was then cooled in an ice-bath and quenched by drop wise adding CH₃OH followed by some drops of H₂O. The mixture was then extracted twice with EtOAc, the combined organic phases was washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (gradient eluent system EtOAc/pentane 1:9→1:4) to yield a yellow oil, which was used in the next step without further purification or characterisation.
The crude material was dissolved in CH₂Cl₂ (15 mL). TFA/H₂O (10:1, 2.0 mL:0.2 mL) was added and the mixture was stirred at rt for 25 min. The mixture was co-evaporated with toluene and concentrated to dryness. The product was purified by flash column chromatography (gradient eluent system 1:9 →1:3 EtOAc/toluene) affording 1.33 g of the product 74 as an orange oil (1.51 mmol, 46% over 3 steps, average 77 % per step). Rₛ: 0.52 (2:3 EtOAc/pentane).

¹H-NMR (500 MHz, CDCl₃) δ_H 7.40-7.14 (m, 25H, ArH), 4.99 (dd, J = 10.9 Hz, J = 7.2 Hz, 1H, -CH₂Ph), 4.84 (d, J = 10.9 Hz, 1H, -CH₂Ph), 4.79 (d, J = 9.1 Hz, 1H, -CH₂Ph), 4.76 (d, J = 6.7 Hz, 1H, -CH₂Ph), 4.72 (d, J = 8.6 Hz, 1H, -CH₂Ph), 4.68 (d, J = 11.6 Hz, 1H, -CH₂Ph), 4.59 (dd, J = 12.0, 4.7 Hz, 1H, -CH₂Ph), 4.47-4.42 (m, 3H, -CH₂Ph, H1'), 4.39-4.35 (m, 2H, -CH₂Ph), 4.03-3.93 (m, 3H, H4, H4', OCH₂), 3.82 (dd, J = 10.9, 4.1 Hz, 1H, H6a), 3.79-3.72 (m, 1H, OCH₂), 3.66-3.56 (m, 3H, H3, H6b, H6a'), 3.54-3.48 (m, 1H, -OCH₂), 3.51-3.46 (m, 7H, H2, H2', H3', H5, H5', CH₂N₃), 2.46 (d, J = 3.6 Hz, 1H, OH), 2.39 (d, J = 4.7 Hz, 1H, OH), 1.95-1.83 (m, 2H, -CH₂-) ppm.

¹³C-NMR (125 MHz, CDCl₃) δ_C 139.3, 138.8, 138.6, 138.4, 138.2 (5xArC), 128.9, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 127.9, 127.8, 127.5 (25 Ar-CH), 103.8 (C1), 102.8 (C1'), 83.1, 82.0, 81.3, 79.3, 76.7, 76.3, 75.5, 75.3, 73.8, 73.5, 73.2 (2xCH₂Ph, C2', C5'), 69.0, 68.9, 68.5 (C4', C6, C6'), 66.7 (-OCH₂-), 48.6 (-CH₂N₃), 29.5 (-CH₂-) ppm. HRMS (ES): Calculated for C₅₀H₅₇N₃O₁₁Na m/z 898.3891. Found m/z 898.3861. The NMR data are in accordance with those previously reported.

3-Azidopropyl (2,6-di-O-benzyl-3,4-endobenzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (88)

The diol 74 (5.05 g, 5.76 mmol) was co-evaporated with toluene and kept at high vacuum ON before use. It was then dissolved in dry THF (100 mL) and pTsOH monohydrate (335 mg, 1.76 mmol) was added followed by benzaldehyde dimethyl acetal (4.3 mL, 28.8 mmol). The reaction mixture was stirred at rt under N₂ atmosphere for 3 h and then quenched with triethylamine (3.5 mL). The reaction mixture was concentrated and co-evaporated with toluene. The crude mixture was used directly in the next step. Rₛ (endo): 0.52 (1:4 EtOAc/toluene).

Endo-product:

¹³C-NMR (125 MHz, CDCl₃) δ_C 139.2, 138.8, 138.7, 138.6, 138.5, 138.0 (6xArC), 129.4, 129.3, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 126.9, 125.5 (Ar-CH), 104.6 (C1), 103.8 (C1'), 102.2 (CH-Ph), 83.1, 82.0, 81.3, 79.3, 76.7, 76.3, 75.5, 75.3, 73.8, 73.5, 73.4, 72.1, 69.1, 68.4, 66.7 (-OCH₂-), 48.6 (-CH₂N₃), 29.5 (-CH₂-) ppm. HRMS (ES): Calculated for C₅₇H₆ₙN₃O₁₁Na m/z 988.4360. Found m/z 988.4334.
3-Azidopropyl (2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (79)

To a solution of crude 88 (5.76 mmol) in dry THF (70 mL) was added 3Å molecular sieves (500 mg) and NaCNBH₄ (2.9 g, 46.1 mmol). The solution was then stirred for 10 min under N₂ atm. HCl/Et₂O (2 M) was added drop wise, until no more starting material could be detected by TLC analysis (total 50 mL). The mixture was diluted with toluene, filtered through celite, washed with water and stirred ON with an aqueous solution of HCl (0.1 M). The organic layer was separated, washed with water, a saturated aqueous solution of NaHCO₃, dried over MgSO₄, filtered, concentrated under reduced pressure and purified by flash column chromatography (1:19 EtOAc/toluene) to afford 2.41 g of the product 79 as a yellow oil (2.51 mmol, 44%). Rₚ: 0.31 (1:9 EtOAc/toluene).

¹H-NMR (500 MHz, CDCl₃) δH 7.42-7.08 (m, 30H, Ar-H), 5.04 (m, 1H, 1xC₄H₄Ph), 4.86-4.63 (m, 6H, 6xC₄H₄Ph), 4.62-4.51 (m, 2H, 2xC₄H₄Ph, H1, H1’), 4.25 (d, J = 11.9 Hz, 1H, 1xC₄H₄Ph), 3.88 – 3.72 (m, 3H, H3, H6a, H6b'), 3.61 – 3.34 (m, 6H, H2, H4, H5, H2', H5', H6b), 3.52-3.34 (m, 6H, H2, H4, H5, H2', H5', H6b'), 3.32 (t, J = 6.8 Hz, 2H, -OCH₂-), 1.90-1.75 (m, 2H, -CH₂-) ppm. ¹³C-NMR (125 MHz, CDCl₃) δC 139.3, 138.9, 138.8, 138.7, 138.4, 138.3 (6xArc), 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.3 (Ar-CH), 103.7 (C1), 102.8 (C1'), 83.1, 81.9, 80.8, 76.8, 76.2, 75.5, 75.4, 75.3, 75.2, 75.1, 74.3, 73.6, 73.5, 73.4, 68.4, 68.2, 66.7 (-OCH₂-), 48.5 (-CH₂N₃), 29.5 (-CH₂-) ppm. HRMS (ES): Calculated for C₅₇H₆₃N₃O₁₁Na m/z 988.4360. Found m/z 988.4334. The NMR data are in accordance with those previously reported.⁵⁹,⁷¹

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-α,β-D-glucopyranoside (90)

D-Glucosamine hydrochloride 89 (25.0 g, 11.6 mmol) was dissolved in H₂O (150 mL) and stirred at rt. Sodium hydroxide (6.0 g, 14.5 mmol) was added followed by phthalic anhydride (18.9 g, 12.8 mmol). The mixture was stirred at rt over night. The reaction mixture was extracted with Et₂O three times; the aqueous phase was then co-evaporated with toluene and left at vacuum over night resulting in a yellow solid. The crude product was dissolved in pyridine (200 mL) at 0°C followed by careful addition of acetic anhydride (100 mL). The mixture was stirred at rt over night. The mixture was then diluted with CH₂Cl₂ and extracted three times with an aqueous solution of HCl (1M), then the three times with a saturated aqueous solution of NaHCO₃, washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (gradient eluent system EtOAc/toluene 1:4→1:2) to afford 15.0 g of the product 90 as an off-white powder as a 3:2 α/β mixture (31.4 mmol, 27 %). NMR data are in accordance with literature.⁷²

Ethyl-3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (91)

To a mixture of glucoside (90) (14.9 g, 31.2 mmol) in dry dichloroethane (150 mL) stirred at 0 °C under N₂ atmosphere was added EtSH (3.9 mL, 53.1 mmol) followed by drop wise addition of BF₃·Et₂O (9.6 mL, 78.0 mmol). The resulting mixture was stirred for 1 h at 0 °C and then at rt over night. Work up was accomplished by adding a saturated aqueous solution of NaHCO₃. The organic layer was separated and dried over MgSO₄, filtered and concentrated under reduced pressure. The product was purified by column chromatography (gradient eluent system EtOAc/pentane 1:3→1:2) to afford 10.9 g of the product 91 as colourless crystals (22.8 mmol, 73%). Rf: 0.45 (2:3 EtOAc/pentane).

¹H-NMR (500 MHz, CDCl₃) δ 7.87-7.84 (m, 2H, Phth), 7.75-7.72 (m, 2H, Phth), 5.82 (dd, Jₘ,ₙ = 10.1, Jₙ,ₐ = 9.3 Hz, 1H, H-3), 5.48 (dd, Jₐ,ₕ = 10.6 Hz, 1H, H-1), 5.17 (dd, Jₙ,ₕ = 10.0, Jₕ,ₖ = 9.4 Hz, 1H, H-4), 4.39 (t, Jₚ,ₙ = 10.4 Hz, 1H, H-2), 4.30 (dd, Jₖ,ₖ₉₉ = 12.3, Jₖ,ₖ₉₆ = 5.0 Hz, 1H, H-6), 4.18 (dd, Jₖ₉₆,ₕ = 12.3, Jₖ₉₆,₂ = 2.2 Hz, 1H, H-6b), 3.89 (dd, Jₖ₉₆,ₙ = 10.2, Jₖ₉₆,ₕ = 4.9), 3.85 (m, 2H, Hₗ₉₆), 2.75 – 2.59 (m, 2H, SCH₂CH₃), 2.10, 2.06, 1.86 (3s, 3CH₃CO) 1.21 (t, J = 7.4 Hz, 3H, SCH₂CH₃) ppm. ¹³C-NMR (125 MHz, CDCl₃) δ 170.8, 170.2, 169.6 (3C, CH₂C-O), 168.1, 167.1 (2C, NCO), 134.5 (CH, Phth), 134.2 (C, Phth), 123.9 (CH, Phth), 81.4 (C-1), 76.1 (C-5), 71.7 (C-3), 69.1 (C-4), 62.5 (C-6), 53.9 (C-2), 24.5 (SCH₂CH₃), 20.9, 20.8, 20.6 (3C, CH₂C-O), 15.0 (SCH₂CH₃) ppm. The NMR data are in accordance with those previously reported.⁷³

Ethyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (92)

Thioglucoside 91 (10.8 g, 22.5 mmol) was dissolved in dry MeOH (60 mL) followed by addition of MeONa (3 mL, 0.5 M). The mixture was stirred under N₂ atmosphere at rt for 4.5 h. The reaction was monitored by TLC analysis (1:1 EtOAc/pentane). The mixture was neutralized by adding a small amount of Dowex 50WX8-100 ion-exchange resin and stirring the mixture for 15 min, the resin was then filtered off and washed with MeOH, the filtrate was concentrated under reduced pressure to afford 7.68 g of the deprotected thioglucoside 92 as bright orange crystals (21.7 mmol, 96%). Rf: 0.57 (EtOAc).

¹H-NMR (500 MHz, CDCl₃) δ 7.81 – 7.77 (m, 2H, Phth), 7.71 – 7.67 (m, 2H, Phth), 5.31 (d, Jₘ,ₙ = 10.4 Hz, 1H, H-1), 4.31 (t, Jₙ,ₕ = 10.2, 1H, H-3), 4.13 (t, Jₜ,ₙ = 10.2 Hz, 1H, H-2), 3.90-3.81 (m, 2H, Hₖ₉₆,ₙ), 3.71-3.61 (m, 1H, H-4), 3.48 – 3.43 (m, 1H, H-5), 2.71 – 2.54 (m, 2H, SCH₂CH₃), 1.15 (t, J = 7.4 Hz, 3H, SCH₂CH₃) ppm. ¹³C-NMR (125 MHz, CDCl₃) δ 168.4 (2C, NCO), 134.4 (4XCH, Phth), 131.8 (2XCH, Phth), 81.6 (C-1), 79.9 (C-5), 72.9 (C-3), 71.5(C-4), 62.2 (C-6), 56.1 (C-2), 24.5 (SCH₂CH₃), 15.1 (SCH₂CH₃) ppm. The NMR data are in accordance with those previously reported.⁷³

Ethyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (93)

Thioglucoside 92 (7.65 g, 21.7 mmol) was dissolved in dry MeCN (100 mL). Activated molecular sieves (3 Å, 6 g) was added followed by benzaldehyde dimethylacetel (5.0 mL, 33.3 mmol) and the mixture was stirred at rt under N₂ atmosphere for 30 min. pTsOH H₂O (1.17 g, 6.15 mmol) was added and the mixture was stirred over night. The mixture was then poured into crushed ice (200 mL) and stirred for 10 min. The organic phase was separated, the aqueous phase was extracted with CH₂Cl₂ three times, the combined organic phases was dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (1:2 EtOAc/pentane) to afford 6.50 g of the product 93 as white crystals (14.7 mmol, 68%). Rₜ: 0.35 (1:2 EtOAc/pentane).

¹H-NMR (500 MHz, CDCl₃) δ_H 7.91 – 7.82 (m, 2H, Phth), 7.76 – 7.71 (m, 2H, Phth), 7.52 – 7.47 (m, 2H, Ph), 7.40 – 7.36 (m, 3H, Ph), 5.57 (s, 1H, PhCH), 5.42 (d, J₁,₂ = 10.5 Hz, 1H, H-1), 4.67 (t, J₂,₃ = J₂,₄ = 9.5 Hz, 1H, H-3), 4.41 (dd, J₆a,₆b = 10.4 Hz, J₆b,₂ = 4.7 Hz, 1H, H₂b), 4.34 (t, J₁,₂ = 10.3 Hz, 1H, H-2), 3.81 (t, J₅,₆a = J₆a,₆b = 10.2 Hz, 1H, H₆a), 3.71 (dt, J₄,₅ = J₅,₆a = 9.9 Hz, J₅,₆b = 4.9 Hz, 1H, H-5), 3.62 (t, J₃,₄ = J₄,₅ = 9.1 Hz, 1H, H-4), 2.76 – 2.62 (m, 2H, SCH₂CH₃), 2.50 (s, 1H, OH), 1.20 (t, J = 7.4 Hz, 3H, SCH₂CH₃) ppm. The NMR data are in accordance with those previously reported.⁷³

Ethyl 3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (76)

A solution of thioglucoside 93 (4.06 g, 9.20 mmol) and DMAP (20 mg) in acetic anhydride (40 mL) and pyridine (20 mL) was stirred at rt under N₂ atmosphere for 1 h. The mixture was then evaporated and co-evaporated with toluene and dried under vacuum to afford 4.37 g of the product 76 as a white powder (9.04 mmol, 98%). Rₜ: 0.60 (1:9 EtOAc/toluene).

¹H-NMR (500 MHz, CDCl₃) δ_H 7.91 – 7.83 (m, 2H, Phth), 7.77 – 7.70 (m, 2H, Phth), 7.49 – 7.42 (m, 2H, Ph), 7.39 – 7.32 (m, 3H, Ph), 5.92 (t, J = 9.5 Hz, 1H, H-3), 5.58 (d, J = 10.6 Hz, 1H, H-1), 5.55 (s, 1H, PhCH), 4.44 – 4.34 (m, 2H, H-4, H-5), 3.89 – 3.70 (m, 3H, H-2, H-6a, H₆b), 2.81 – 2.58 (m, 2H, SCH₂CH₃), 1.89 (s, 3H, CH₃CO), 1.21 (t, J = 7.4 Hz, 3H, SCH₂CH₃) ppm. ¹³C-NMR (125 MHz, CDCl₃) δ_C 170.3 (CH₃CO), 168.0, 167.6 (2×NCO), 137.2, 134.7, 133.4, 132.0, 131.5, 129.4, 128.5, 126.5, 123.9 (9 Ar-C), 101.9 (PhCH), 82.0 (C-1), 79.5 (C-4), 70.8, 70.7 (C-3, C-5), 68.9(C-6), 54.6 (C-2), 24.7 (SCH₂CH₃), 20.8 (CH₃CO), 15.2 (SCH₂CH₃) ppm. The NMR data are in accordance with those previously reported.⁶⁰

Synthesis of DMTST

A roundbottom flask with dry CH₂Cl₂ (8 mL) was cooled to 0 °C and dimethyl disulfide (0.9 mL, 10.0 mmol) was added followed by MeOTf (1.4 mL, 12.0 mmol). The reaction mixture was stirred for 2 h under N₂ atm at 0 °C. The clear solution started to precipitate (white) after 1.5 h. The flask was the kept in the refrigerator ON with N₂ balloon. The mixture was then diluted with dry Et₂O (100 mL), the crystals were filtered off, washed with dry Et₂O (2×50 mL), dried under suction for 10 min and at high vacuum ON to afford 2.444 g of a snow white powder (9.46 mmol, 95%).
3-Azidopropyl (3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (94)

Thioglucoside donor 76 (1.11 g, 3.30 mmol), acceptor 79 (2.21 g, 2.29 mmol) and 4Å powdered molecular sieves (1.06 g) was dried at high vacuum for 1 h. Dry CH₂Cl₂ (50 mL) was added and the mixture was cooled to -40°C and stirred under N₂ atmosphere. NIS (1.47 g, 6.55 mmol) was added followed by AgOTf (176 mg, 0.686 mmol) and the mixture was allowed to slowly warm up to -25°C. After 2 h TLC analysis (1:9 EtOAc/toluene) showed full consumption of donor but still presence of acceptor in the reaction mixture. Additional thiogluoside donor 76 (463 mg, 1.37 mmol) was added. After total 3 h, the reaction was quenched by the addition of Et₂N (200 µL), the mixture was filtered through celite, diluted with CH₂Cl₂ and washed with an aqueous solution of Na₂S₂O₃, sat. aq. NaHCO₃, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (gradient eluent system 1:50 to 1:20 EtOAc/toluene) to afford 2.42 g of the product as a white solid (1.74 mmol, 76%). R₁: 0.40 (1:9 EtOAc/toluene).

¹H-NMR (500 MHz, CDCl₃) δₙ; 7.51 – 7.03 (m, 37H, ArH), 6.93 – 6.85 (m, 2H, ArH), 5.99-5.89 (m, 1H, H3’’), 5.68 (d, J = 8.3 Hz, 1H, H1’’), 5.54 (s, 1H, CPh), 5.00-4.84 (m, 2H, C₂H₃Ph), 4.80-4.65 (m, 2H, C₂H₃Ph), 4.59-4.51 (m, 2H, CH₃Ph), 4.51-4.42 (m, 2H, CH₂Ph), 4.40 – 4.22 (m, 5H, CH₂Ph, H1’’), H-4’, H-5’’), 4.18 (d, J = 7.6 Hz, 1H, H1), 4.06 (d, J = 12.1 Hz, 1H, H6a), 3.92 – 3.72 (m, 5H, CH₂, H3, H6b, H4’, H6a’), 3.61 – 3.44 (m, 4H, H2, H3’, H6a’’, H6b’’), 3.42-3.24 (m, 7H, H4, H5, H2’, H5’, H6b’, -CH₂N₃), 1.85 (d, J = 6.2 Hz, 3H, COCH₃), 1.84 – 1.75 (m, 2H, -CH₂-) ppm. ¹³C-NMR (125 MHz, CDCl₃) δₖ; 170.2, 168.5, 167.5 (C=O), 139.5, 139.2, 138.9, 138.7, 138.6, 138.5, 138.1, 137.1, 129.4, 129.3, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.3, 127.1, 126.5, 125.5 (aromatic C), 103.7 (C1), 102.6 (C1’), 102.0 (CPh), 100.2 (C1’’), 83.2, 82.1, 81.9, 79.6, 79.1, 76.8, 75.9, 75.6, 75.3, 75.2, 75.0, 74.2, 73.6, 73.3, 73.2, 69.8, 69.0, 68.4, 67.9, 66.6, 66.2, 56.0 (C2-C6, C2’-C6’, C2’’-C6’’), 48.5 (-CH₃N₃), 29.5 (-CH₂-), 20.8 (COCH₃) ppm. The NMR data are in accordance with those previously reported.⁵⁹

3-Azidopropyl (2-acetamido-3-O-acetyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (95)

Trisaccharide 94 (2.42 g, 1.74 mmol) was dissolved in absolute EtOH (17 mL). Hydrazine monohydrate (3.4 mL, 69.7 mmol) was added. The clear mixture was stirred at reflux under N₂ atmosphere over night. TLC analysis (1:9 EtOAc/toluene, first using Ninhydrine, then H₂SO₄/MeOH) showed full conversion of the starting material. R₁ (starting material) 0.40 (1:9 EtOAc/toluene). R₁ (product) 0.17 (1:9 EtOAc/toluene). The reaction mixture was concentrated and co-evaporated with toluene and dried under high vacuum.
The crude compound was dissolved in acetic anhydride (5 mL) and pyridine (20 mL). A catalytic amount of DMAP was added, and the mixture was stirred at rt for 1.5 h (TLC 1:3 EtOAc/toluene). The reaction mixture was concentrated under high pressure and purified using flash column chromatography (gradient eluent system 1:6 to 1:3 EtOAc/toluene) to afford the 1.99 g of the desired product 95 as white crystals (1.53 mmol, 88%). \( R_f 0.23 \) (1:3 EtOAc/toluene).

\[ ^{13}C \text{ NMR (125 MHz, CDCl}_3 \text{)} \delta 171.2 (\text{NHCOCH}_3), 169.9 (\text{COCH}_3), 139.4, 139.3, 139.2, 138.8, 138.6, 138.4, 137.1, 129.4-126.3 (\text{Aromatic-C}), 103.8, 102.8, 101.7 (C1, C1', C1'', CHPh), 83.1, 81.9, 81.1, 80.7, 78.9, 76.6, 76.4, 75.6, 75.3, 75.2, 75.1, 74.7, 73.7, 73.6, 73.5, 72.4, 68.9, 68.5, 68.4, 66.8, 66.7, 54.8 (C2-C6, C2''-C6', C2''-C6'', 6xCH2Ph, -OCH2-), 48.5 (-CH2N3), 29.5 (-CH2-), ppm. The NMR data are in accordance with those previously reported. ^{59} \text{HRMS (ES): Calculated for } C_{74}H_{82}N_4O_{17}\text{Na m/z } 1321.5573. \text{Found m/z 1321.7640.}

3-Azidopropyl (2-acetamido-4,6-O-benzylidene-2-deoxy-\beta-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-benzyl-\beta-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-\beta-D-glucopyranoside (80)

**Method A**
Trisaccharide 95 (1.98 g, 1.52 mmol) was dissolved in dry CH2Cl2 (16 mL) and dry MeOH (8 mL). A catalytic amount of freshly prepared NaOMe was added, and the mixture was stirred at rt at N2 atmosphere over night. The mixture was concentrated without neutralisation to afford 1.97 g the product 80 as a bright yellow powder (1.56 mmol, quantitative yield). \( R_f 0.18 \) (1:3 EtOAc/toluene).

**Method B**
Trisaccharide 97 (120 mg, 0.103 mmol) was dissolved in dry DMF (700 µL). Benzaldehyde dimethyl acetal (62 µL, 0.410 mmol) was added followed by pTsOH·H2O (2 mg, 0.010 mmol) and CaSO4 (130 mg, 0.955 mmol). The mixture was stirred at rt under N2 atmosphere for 21 h. The mixture was neutralised by addition of Et3N (10 µL), diluted by CH2Cl2, washed with H2O, brine, dried over MgSO4, filtered and concentrated under reduced pressure. The product was purified using flash column chromatography (gradient eluent system 1:3 to 1:2 EtOAc/toluene) to afford 105 mg of the product 80 (0.084 mmol, 82%). \( R_f 0.25 \) (1:1 EtOAc/toluene).

\[ ^{13}C \text{ NMR (125 MHz, CDCl}_3 \text{)} \delta_c 172.4 (\text{NHCOCH}_3), 139.2, 139.1, 138.8, 138.7, 138.4, 138.3, 138.0, 137.3, 129.2-125.5 (\text{Aromatic-C}), 103.7, 102.7, 102.6, 102.0 (C1, C1', C1'', CHPh), 82.9, 81.8, 81.8, 81.5, 80.1, 76.5, 76.3, 75.5, 75.2, 75.1, 74.4, 73.6, 73.5, 73.4, 72.7, 68.7, 68.3, 66.6, 66.5, 58.9 (C2-C6, C2''-C6', C2''-C6'', 6xCH2Ph, -OCH2-), 48.4 (-CH2N3), 29.4 (-CH2-), 22.9 (NHCOCH3). \text{HRMS (ES-): Calculated for } C_{72}H_{79}N_4O_{16}\text{ m/z 1255.5491. Found m/z 1255.5502.}
3-Azidopropyl (2-acetamido-3,4,6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (96)

Donor 8 (84 mg, 0.216 mmol) and acceptor 79 (139 mg, 0.144 mmol) was dried under vacuum for 1 h, and was then dissolved in dry CH₂Cl₂ (1.4 mL). Bi(OTf)₃ (21 mg, 0.032 mmol) was added and the reaction mixture was stirred at reflux under N₂ atmosphere for 19 h. TLC analysis (1:1 EtOAc/toluene) showed no more donor but still acceptor in the reaction mixture. Additional donor 8 (84 mg, 0.216 mmol) and Bi(OTf)₃ (21 mg, 0.032 mmol) was then added and the reaction mixture was stirred at reflux under N₂ atmosphere for additional 24 h. TLC analysis showed full conversion of the acceptor. The reaction mixture was concentrated under reduced pressure and purified using flash column chromatograph (gradient eluent system 1:4 to 1:2 EtOAc/toluene) to afford 135 mg of the product 96 as a white solid (0.104 mmol, 72%). Rᵣ: 0.47 (1:1 EtOAc/toluene)

¹H-NMR (500 MHz, CDCl₃) δ H 7.43 – 7.10 (m, 30H, Ar-H), 5.08 – 4.92 (m, 4H), 4.91 – 4.65 (m, 6H), 4.59 (d, J = 12.2 Hz, 2H), 4.50 (d, J = 11.5 Hz, 1H), 4.44 (d, J = 7.7 Hz, 1H), 4.41 – 4.29 (m, 3H), 4.28 – 4.13 (m, 3H), 4.03 (dd, J = 18.4, 9.3 Hz, 1H), 3.99 – 3.88 (m, 3H), 3.78 – 3.61 (m, 4H), 3.54 – 3.46 (m, 2H), 3.46 – 3.33 (m, 4H), 2.04 (s, 3H, CH₃CO), 1.96 (s, 9H, 3xCH₃CO), 1.90 – 1.80 (m, 2H).¹³C-NMR (125 MHz, CDCl₃) δ C 171.0, 170.8, 169.9, 169.5 (4xCH₃CO), 139.4, 139.2, 139.2, 138.8, 138.6, 138.4 (Aromatic-C), 128.9-127.5, 126.4 (Aromatic-CH), 103.7, 102.7, 102.4 (C₁, C₁’, C₁’’), 83.1, 82.3, 81.9, 80.2, 76.6, 76.3, 75.6, 75.3, 75.1, 74.6, 73.6, 73.5, 73.1, 72.0, 68.9, 68.4, 68.4, 66.7, 65.7, 62.5, 54.7 (C₂-C₆, C₂’-C₆’, C₂”-C₆”’), 48.5 (-CH₂N₃), 29.5 (-CH₂-), 22.9 (NHCOCH₃), 20.8, 20.8, 20.7 (3xCH₂CO). HRMS (ES): Calculated for C₇₁H₈₂N₄O₁₉Na m/z 1317.5471. Found m/z 1317.5491. [α]₀²⁹⁰K = -6.8 (c=1, CHCl₃)

3-Azidopropyl (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (97)

Trisaccharide 96 (132 mg, 0.102 mmol) was dissolved in dry MeOH (2 mL) and a catalytic amount of freshly prepared NaOMe was added to the solution, which was then stirred at rt under N₂ atmosphere for 25 h. TLC analysis showed full conversion of the starting material and the mixture was concentrated without neutralisation to afford 120 mg of the product 97 as a bright yellow foam (0.103 mmol, quantitative yield). Rᵣ: 0.20 (1:1 EtOAc/toluene).
$^1$H NMR (500 MHz, CD$_3$OD) $\delta_H$ 7.34 – 6.87 (m, 30H, ArH), 4.98 (d, $J = 11.4$ Hz, 1H), 4.92 – 4.83 (m, 1H), 4.74 – 4.50 (m, 5H), 4.49 – 4.35 (m, 3H), 4.34 – 4.24 (m, 2H), 4.25 – 4.16 (m, 2H), 4.07 (d, $J = 11.9$ Hz, 1H), 3.95 (d, $J = 2.5$ Hz, 1H), 3.87 (dd, $J = 11.8$, 2.2 Hz, 1H), 3.82 – 3.57 (m, 5H), 3.56 – 3.45 (m, 2H), 3.45 – 3.35 (m, 4H), 3.35 – 3.21 (m, 2H), 1.76 – 1.66 (m, 2H), 1.61 (s, 3H, NHCOCH$_3$) ppm. $^{13}$C-NMR (125 MHz, CD$_3$OD) $\delta_C$ 174.9 (NHCOCH$_3$), 142.3, 141.9, 141.7, 141.4, 141.1, 140.9 (Aromatic C), 131.1-129.6 (Aromatic-CH), 106.0, 105.7, 104.9 (C1, C1', C1''), 85.4, 84.9, 84.3, 82.1, 79.9, 79.5, 78.4, 77.8, 77.4, 77.3, 77.2, 76.4, 76.0, 75.6, 74.2, 71.7, 70.5, 69.0, 64.7, 59.5 (C2-C6, C2'-C6', C2''-C6'', 5xCH$_2$Ph, -OCH$_2$-), 31.7 (-CH$_2$-), 24.6 (NHCOCH$_3$) ppm. HRMS (ES): Calculated for C$_{65}$H$_{76}$N$_4$O$_{16}$Na m/z 1191.5154. Found m/z 1191.63. [\alpha]$_{D}^{296}$K -8.8 (c=1, CHCl$_3$).
APPENDIX A: MALDI-TOF SPECTRA

Global deprotection

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<td>1021.563</td>
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Test of aldehyde functionality

Start material **40**

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<td><strong>40</strong></td>
<td>794.364</td>
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<tr>
<td>Product MeONH$_2$ <strong>42A</strong></td>
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Test of dehydroalanine functionality

![Chemical structures and mass spectrometry data](image)

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<tr>
<td>Product 41</td>
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Synthesis of \( H_2N\text{-Leu-FGly-Thr-Pro-Ser-NHBn} \) (43)

Before workup

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<td>Intermediate</td>
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<td>Product 43</td>
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<td>613,2962</td>
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Oxidation of N-terminal Serine using NaIO₄

\[ \text{H}_2\text{N-S-N-N-F-G-A-I-L-S-S-NH}_2 \overset{\text{NaIO}_4}{\longrightarrow} \text{H-N-N-F-G-A-I-L-S-S-NH}_2 \]

\text{phosphate buffer, pH=7, rt, dark, 2.5 h}

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<tr>
<td>Product 57</td>
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**Indium-mediated Barbier reaction using allyl bromide and allylic GlcNAc bromide**

![Chemical structures and diagrams showing the reaction process involving indium-mediated Barbier reaction using allyl bromide and allylic GlcNAc bromide.]

<table>
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<th>Exact mass+Na</th>
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<td>999.451</td>
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APPENDIX B: PUBLICATION

**Enzymatic synthesis of dimeric glycomimetic ligands of NK cell activation receptors**

Anna Drozdová, Pavla Bojarová, Karel Krênek, Lenka Weignerová, Birgit Henßen, Lothar Elling, Helle Christensen, Henrik H. Jensen, Helena Pelantová, Marek Kuzma, Karel Bezouška, Monika Krupová, David Adámek, Kristýna Slámová, Vladimír Krˇen

*Carbohydrate Research, 2011, 346, 1599–1609*