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Versatile Approaches to Sugar Amino Acid Building Blocks as Precursors of Glycopeptides

Christelle Jablonski-Lorin*[a], Matthias Nold[a], Arthur Bodenmüller[b], and Ernst Hungerbühler*[a]

Abstract: The synthesis of monosaccharide units functionalised with different amino acids is described herein. Sugar amino acid templates linked at position 6 of the sugar were prepared by various nucleophilic substitutions introducing a spacer of variable length or by traceless Staudinger ligation. Sugar amino acid templates attached at the anomeric position and representing a class of precursors of glycopeptides were synthesized in three steps *via* addition of a solution of trimethylsilylnitrate-trimethylsilylazide on a glycal, followed by subsequent reduction of the sugar azide and coupling with diverse amino acids.

Keywords: Glycopeptides · Staudinger ligation · Sugar amino acid templates · Sugar azide

The growing recognition of the roles of carbohydrates in fundamental biological processes and their potential application as new therapeutics has emerged in recent years. Several groups have reported the effects in the activity, stability and metabolism of glycosylated peptide drugs.[1,2] Positive effects in the bioactivity, pharmacokinetics and immunogenicity of proteins were observed while the activity of certain protein-linked glycans were being studied and compared to the activity of the isolated protein.[2] The hydrophilic character of the glycopeptides tested emerged as a determinant factor that improved dramatically the water solubility of the biomolecule. The role of glycoproteins in molecular recognition was also proved.[1]

In collaboration with our spin-off company SynphaBase, we defined simplified glycopeptide targets suitable for further molecular recognition study and for the preparation of bulk pharmaceutical rather than the synthetically complex, branched oligosaccharides typical of eukaryotic proteins. We focused our efforts on the synthesis of monosaccharide units functionalised with different amino acids. These sugar amino acid templates represent interesting precursors of glycopeptides and are required in large amounts and great variety for the understanding of the biological roles of protein-linked glycans. To our knowledge, only a restricted number of such sugar amino acid scaffolds are commercially available in mg amount and then only at a high price. The chemical synthesis of such targets is difficult as carbohydrates are highly functionalized with hydroxyl groups of similar reactivity.

Our first interest was related to the elaboration of sugar amino acid templates linked at the C-6 position of the monosaccharide (Fig.: type I). We attempted the synthesis first *via* a classical approach involving a nucleophilic substitution for the introduction of a spacer, cleavage of the protecting group and coupling with different amino acids. This approach is very versatile since it resulted in a high degree of freedom in the choice of the protected sugar unit, the length and functionality of the spacer and the protecting groups of the amino acids. Scheme 1 illustrates the multistep synthetic route for the preparation of the functionalised derivate of 1,2,3,4-tetra-O-benzyl glucopyranoside (**3**).

A possible extension of this part of the project is the introduction of the spacer into a secondary alcohol functionality of the sugar unit. As a first example, students at the FHNW are currently optimising the synthesis of **7a**, **7b**, **7c** (Scheme 2). The starting material **4** was produced on a kilogram scale, intermediate products **5** and **6** were prepared on a 100 g scale. All the final compounds described herein were characterised by LCMS-MS (Agilent system 1100), ^{1}H- and ^{13}C-NMR experiments (Bruker Avance 400 MHz), ^{1}H-^{1}H COSY and NOESY experiments are required to confirm the structures.

*Correspondence: Prof. Dr E. Hungerbühler[a]
Tel.:+41 61 467 43 88
Fax: +41 61 467 44 57
E-Mail: ernst.hungerbuehler@fhnw.ch,
christelle.jablonski@fhnw.ch
[a]Fachhochschule Nordwest Schweiz (FHNW)
Hochschule für Life Sciences
Institut für Chemie und Bioanalytik
Gründenstrasse 40
CH-4132 Muttenz
[b]SynphaBase AG
Gründenstrasse 40
CH-4132 Muttenz

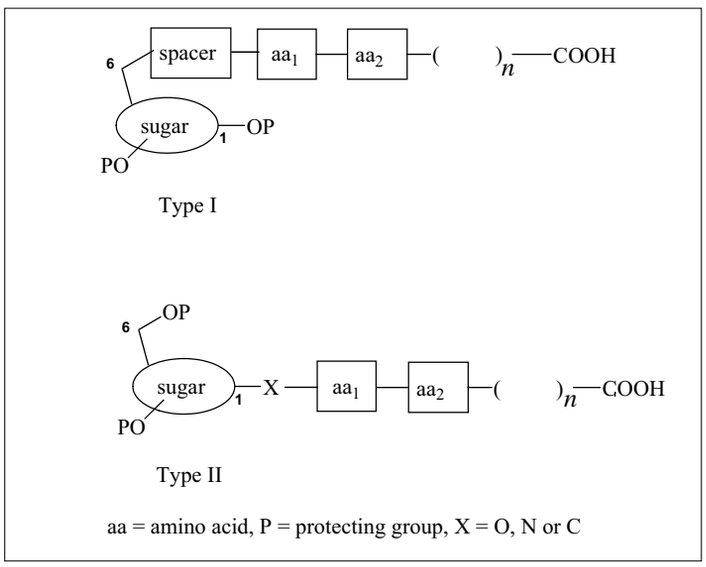
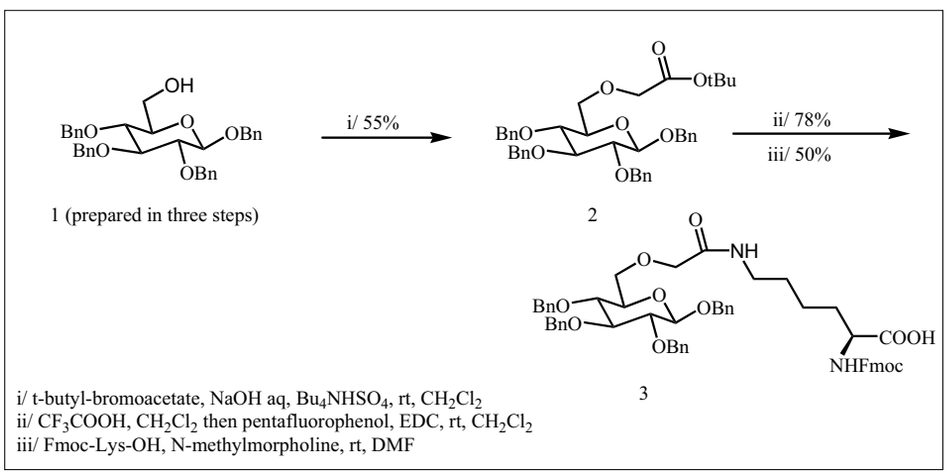
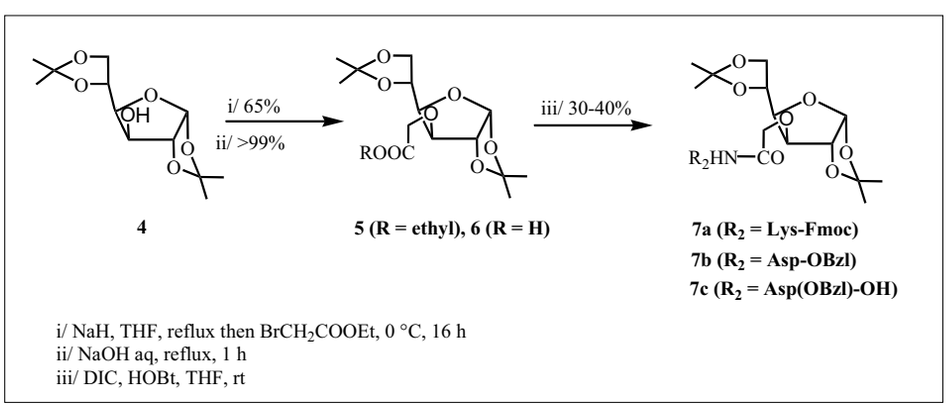


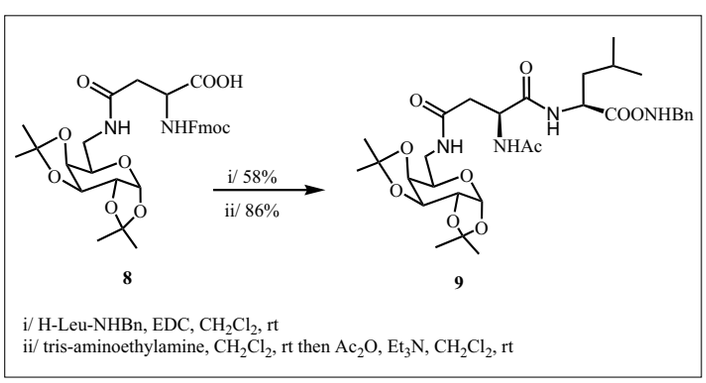
Fig. General structure of the glycopeptide targets



Scheme 1. Example of the synthesis of glycopeptide of type I



Scheme 2. Glycopeptide templates derivatives from 1,2:4,6-di-O-isopropylidene glucofuranose



Scheme 3. Example of glycopeptide of type I bearing two amino acids

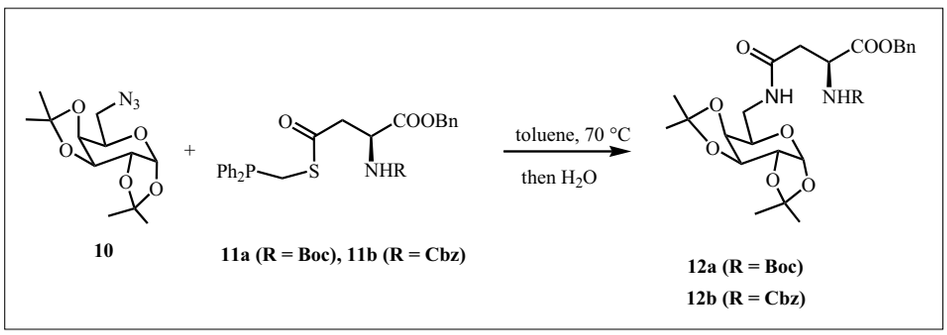
More elaborate templates of type I were also synthesized: Scheme 3 displays the further linkage of a second amino acid to the functionalised derivate of 1,2:3,4-di-O-isopropylidene galactopyranoside (**8**), as well as the final cleavage of protecting group leading to the protected hydrophilic glycopeptide **9**. Whereas the coupling of this second amino was performed manually, we envisaged exploring the great advantages of an automated synthesis of short peptides in solution phase. We are mostly taking advantage of the Fmoc strategy for the terminal NH of the amino acid allowing the protection of the sugar with acid labile groups and the further protection of the amino acid with benzyl protecting groups.

We have also investigated a more direct approach to our sugar amino acid templates of type I *via* traceless Staudinger ligation.[3,4] This novel reaction – compatible with diverse functional groups – for the formation of amide bonds involved an azide function as in **10** and a well-engineered phosphine partner such as **11a** or **11b** (Scheme 4). The Staudinger ligation consists of the nucleophilic attack of a phosphine on an azide to give a phosphazide which is spontaneously transformed into a reactive azaylide. The subsequent hydrolysis results in amide-linked products. The preparation of **11a** and **11b** required six steps including the purification of each intermediate by chromatography and was optimised to yield the functionalized phosphine in 57% yield compared to 43% described in the literature.

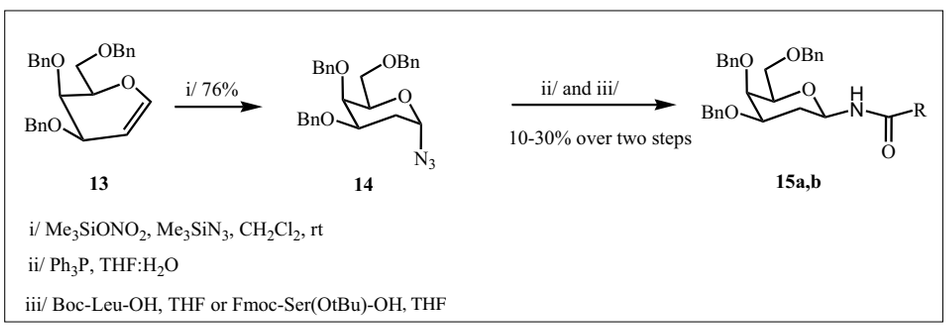
The synthesis of glycopeptides **12a** and **12b** was accomplished in a straightforward fashion: the azido sugar **10**[5] reacted with the phosphino derivative **11a** or **11b** (Scheme 4). The coupling was achieved in moderate to medium yields (22–47%), however the high selectivity generally obtained in this type of coupling encouraged us to evaluate the Staudinger ligation for future synthesis of glycopeptides of type I and II.

Natural protein-linked glycans exist only as glycoconjugates in which the sugar and amino acid moieties are directly linked at the anomeric carbon of the sugar. The lack of stability of O- and N-glycosides during *in vivo* enzymatic processes compared to C-glycosides can explain the great current interest in the preparation of C-glycopeptides mimetics.

Because of the natural pre-eminence of glycopeptides linked at C-1, we also investigated the synthesis of sugar-amino acid templates of type II (Fig.) with the anomeric centre as point of attachment. In this case, 2-azido-2-deoxy azido galactopyranose (**14**) was readily accessible from the commer-



Scheme 4. Traceless Staudinger ligation for the synthesis of glycopeptides **12a** and **12b**



Scheme 5. Formation of the sugar azide **14** and coupling reaction to generate glycopeptides of type II **15a** and **15b**

cially available 3,4,6-tri-O-benzyl galactal using the method described by Reddy *et al*.[6] (Scheme 5). A one-pot procedure for the reduction of the azide to amino group and subsequent coupling reaction with a choice of simple protected amino acids was successfully accomplished to generate our first sugar-amino acid templates of type II, **15a** and **15b**, with excellent selectivity (only the β anomer was obtained) and in moderate yield (20–40% over two steps).

We intend to pursue the project by developing the synthesis of more glycopeptides by the well-established methods described in this article, since the strategies developed afford the choice of a large variety of sugar, spacer and protected amino acids. The investigation of a three-component Staudinger ligation also appears very promising.[7]

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