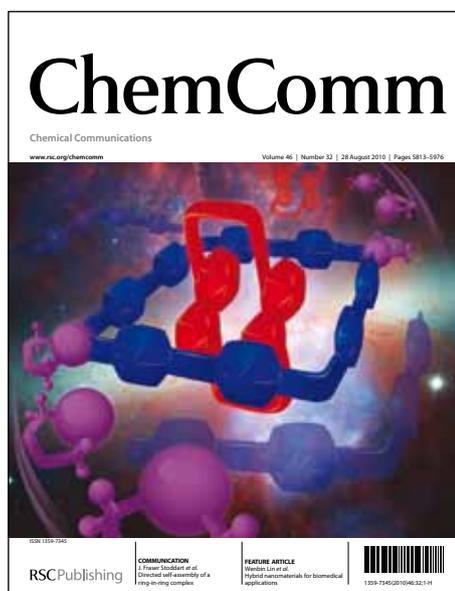


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COMMUNICATION

Automated Glycopeptide Assembly by Combined Solid-Phase Peptide and Oligosaccharide Synthesis

Cite this: DOI: 10.1039/x0xx00000x

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Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Current strategies for the synthesis of glycopeptides require multiple manual synthetic steps. Here, we describe a synthesis concept that merges solid phase peptide and oligosaccharide syntheses and can be executed automatically using a single instrument.

Most human proteins are glycosylated. Glycan structure as well as the exact location and linkage to the protein influence glycoprotein function. Establishing structure-activity relationships of glycoproteins is difficult since these molecules that are typically obtained as mixtures of glycoforms. Synthesis produces homogenous glycopeptides that serve to elucidate the biological significance of specific glycoprotein fragments.¹ In addition, synthetic glycopeptides have been explored as diagnostic tools and vaccine candidates.^{1d, 2}

Glycopeptide synthesis has been extensively developed in recent years.³ *O*-glycopeptides are usually synthesized via two main routes based on early work by Kaifu and Osawa.⁴ One strategy incorporates glycosylated amino acid building blocks during solid phase peptide synthesis (SPPS) (Figure 1 A).^{1b, c, 5} Alternatively, a shortened glycan is grafted onto the peptide backbone and after completion of peptide synthesis, the glycan is further elongated by chemical or chemoenzymatic means (Figure 1 B).⁶ In both strategies, glycosylated amino acids are required for glycopeptide assembly by SPPS. The synthesis of glycosylated amino acids is time consuming, as it typically requires multistep solution phase procedures. Most building blocks are not commercially available or extremely expensive to purchase in large quantities. Lack of accessibility limits the use of these building blocks in solid phase peptide synthesis where large access of building block is used for each coupling step. Moreover, glycosylated amino acids may epimerize during SPPS and the purity of the synthesized glycopeptide needs to be ascertained.⁷ Consequently, a method that minimizes synthetic effort involved in glycopeptides synthesis is sought. An alternative synthetic route based on oligosaccharide assembly on a

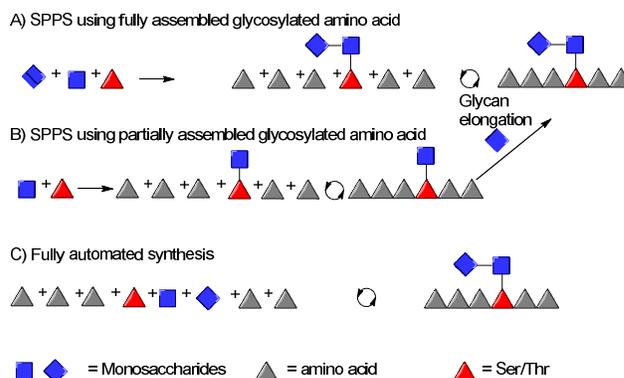


Figure 1. Strategies for glycopeptide synthesis. Routes A and B utilizes glycosylated amino acid as building block for the assembly of glycopeptide by SPPS. Route C requires only monomeric building blocks for automated assembly of glycopeptides by SPPS and SPOS.

solid supported peptide platform was outlined but did not get reduced to automated practice.⁸ This route is attractive as it may circumvent the use of glycosylated amino acids.

Recent advances in automated solid phase oligosaccharides synthesis (SPOS)⁹ set the stage to combining both solid phase peptide and oligosaccharide assembly platforms to prepare glycopeptides. Here, we describe an automated, stepwise solid-phase approach to glycopeptide synthesis that relies exclusively on monosaccharide and amino acid building blocks (Figure 1 C).

The choice of linker to tether the growing glycopeptide to the solid support is the crucial strategic decision for syntheses that require a multitude of protective groups. Photo-cleavable linkers are compatible with a variety of functional groups and were used for previous automated solid phase glycopeptide syntheses.¹⁰ By

the presence of TMSOTf. Cleavage from the resin and HPLC analysis revealed that the glycosylation reaction had proceeded with good selectivity (4:1, major α). The α -anomeric configuration of **5** was confirmed by comparison of the NMR spectra of **5** with Fmoc-Thr(Ac₃GalN₃)-Ot-Bu.^{12b}

After establishing that automated peptide glycosylation is possible, glycopeptide **6** bearing a (Gal β 1-3GalNAc β -O) disaccharide was prepared. Commercially available amino acids were used along with glycosylating agents **2** (prepared in three steps from a known intermediate)¹³ and **3**.¹⁴ The tetrapeptide backbone of **6** was assembled using SPPS. Selective removal of the *t*-Bu group was followed by glycosylation with glycosyl imidate **2** and TMSOTf as activator at -10 °C. The glycosylation reaction proceeded with high conversion and yielded one major product. Removal of the levulinoyl protecting group was followed by formation of a second glycosidic linkage using galactosyl thioglycoside **3** activated by N-iodosuccinimide (NIS) and triflic acid (TfOH). Progress of the automated synthesis of **6** was monitored by cleaving small amounts of material from the solid support at different stages of the synthesis followed by HPLC-MS.

Deprotection of the galactosamine by reduction of the azide using AcSH,¹⁵ and benzylidene removal using TFA were carried out manually after completion of the automated synthesis. Finally, glycopeptide **6** was cleaved from the resin under UV irradiation. Desired product **6** was obtained in 5.7% isolated yield after HPLC purification. ¹H-NMR coupling constants and coupled CH-HSQC analysis clearly indicates that two β -linkages were formed.

Since many glycopeptides harbor more than one glycosylation site, bis-glycosylated peptide **7** was targeted to challenge the method. Fmoc-Thr(Trt)-OH and mannose thioglycoside **4**¹⁶ were employed twice. Placement of threonine on the solid support was followed by Trt removal. Thioglycoside **4** was attached using the NIS/TfOH activator mixture. Fmoc removal and repetition of the assembly cycle gave glycopeptide **7** after light-induced cleavage from the solid support in 14% yield after HPLC purification. NMR analysis proved that both threonines were glycosylated α -selectively.

An automated procedure for the synthesis of *O*-glycopeptides based on the combination of solid phase peptide and oligosaccharide syntheses protocols can be executed on a single instrument. Employing Merrifield polystyrene resin with a photo-cleavable linker allowed for the use of different leavings groups and activation systems. The examples presented in this study demonstrate the potential of monomer based strategy for synthesizing glycopeptides. This conceptual advance, combined with improved synthetic procedures, is expected to provide more expedient access to homogeneous glycopeptides in the future.

Notes and references

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† Electronic Supplementary Information (ESI) available: Detailed description of building block synthesis and characterization, automated synthesis protocols and modules, intermediates HPLC characterization and final compound NMR, HPLC and MS analysis. See DOI: 10.1039/c000000x/

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