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Chemical Synthesis of Human Syndecan-3 Glycopeptides Bearing Two Heparan Sulfate Glycan Chains

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Abstract

Despite the ubiquitous presence of proteoglycans in mammalian systems, methodologies to synthesize this class of glycopeptides with homogeneous glycans are not well developed. Herein, we report the first synthesis of glycosaminoglycan family glycopeptides containing two different heparan sulfate chains from human syndecan-3. With the large sizes and tremendous structural complexities, multiple unexpected obstacles were encountered in synthesis, which include the high sensitivity to base treatment and the instability of glycopeptides with two glycan chains towards catalytic hydrogenation conditions. To overcome these challenges, after many trials, a successful strategy was established by constructing the partially deprotected single glycan chain containing glycopeptides first followed by union of the glycan bearing fragments and cleavage of ester type protecting groups. This work has laid the foundation to prepare other members of this important class of molecules.

Keywords

glycopeptides; glycosylation; synthetic design; sulfation; total synthesis

Glycopeptides and glycoproteins play important roles in many biological events such as cellular proliferation, neuron development and inflammation.^[1] There are two major classes of glycopeptides/glycoproteins, i.e., *N*-linked glycans and *O*-linked glycans. *O*-glycans can be further divided into two main classes, i.e., the mucin type and the glycosaminoglycan family proteoglycans. Many innovative strategies have been designed to synthesize glycopeptides bearing *N*-glycans and mucin type *O*-glycans^[2–3] with successful preparation of molecules approaching the complexities of native glycoproteins.^[4–5]

In sharp contrast to peptides bearing *N*-glycans and mucin type *O*-glycans, glycosaminoglycan glycopeptide syntheses are much less developed, despite their ubiquitous presence and many important biological functions.^[6] Synthesis in this area has

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mainly focused on the glycosaminoglycan oligosaccharides^[7–23] or the tetrasaccharide linker.^[24–27] Recently, we reported the first synthesis of a proteoglycan family glycopeptide, i.e., a syndecan-1 glycopeptide bearing one heparan sulfate chain.^[28] An additional level of complexity of proteoglycans is that many carry more than one glycosaminoglycan chains.^[6] To address this, we have begun to establish a viable route towards homogeneous glycopeptides bearing multiple heparan sulfates with human syndecan-3 as the target. Serious difficulties were encountered as the presence of multiple glycan chains significantly increased the structural complexity and instability of the targeted molecules. Herein, we report the lessons we have learned and the eventual establishment of a successful approach to access these highly complex molecules.

Our synthetic target is human syndecan-3 extracellular domain glycopeptide **1**, which contains the typical structural features of heparan sulfate proteoglycans including peptide backbone, different heparan sulfate chains, the full tetrasaccharide linkers, 2-*O* sulfation, 6-*O* sulfation, glucosamine α linked to both glucuronic acid and iduronic acid, and *N*-acetylation. In order to prepare this molecule, we adapted a cassette approach^[29] where glucuronic acid containing octasaccharide serine cassette **2** and iduronic acid cassette **3**^[28] were produced first and then incorporated into the glycopeptide.

There are multiple possible reaction sequences to connect the glycosyl units in the octasaccharide modules. After much exploration, we established a 3+2+3 strategy using building blocks consisted of ABC trisaccharide, DE disaccharide and FGH trisaccharide to access the octasaccharide modules **2** and **3**. To prepare the ABC trisaccharide, the glucoside donor **4** was pre-activated by *p*-TolSCI/AgOTf,^[30] which subsequently glycosylated disaccharide **5**^[28] generating ABC trisaccharide **6** in 85% yield (Scheme 1). The 3+2 glycosylation between trisaccharide **6** and DE disaccharide **5** went smoothly producing pentasaccharide **7**. **7** reacted with the trisaccharide serine unit **8**^[28] generating the

octasaccharide cassette **9** in an excellent 87% yield. The TBDPS silyl ether groups in **9** were removed by HF/pyridine to expose the three primary hydroxyls, which were oxidized to carboxylic acids^[31] and subsequently converted to methyl esters (compound **11**). The two azide groups in **11** were transformed to *N*-acetyl moieties through a one pot reduction/acetylation procedure with zinc, copper sulfate and acetic anhydride to afford octasaccharide **2**.

A serious challenge in heparan sulfate glycopeptide assembly is the compatibility of the protective group removal conditions with the sulfated glycopeptide. Due to the high sensitivity of sulfates to acid, commonly used acid cleavable amino acid side chain protective groups such as Boc and trityl are to be avoided. Furthermore, cautions need to be taken as the glycoside-serine linkage is prone to base promoted β -elimination.^[28,32] Thus, the sequence of deprotection and reagents applied need to be carefully designed and established.

Previously, we showed that the ester protective groups (Ac, Bz) on glycopeptide **13** could be successfully removed under transesterification condition using NaOMe.^[28] The free C-terminal of the glycopeptide **13** was crucial to prevent base promoted β -elimination of the glycan chain. This route was applied to the glucoside containing octasaccharide cassette **15**, which was produced from octasaccharide module **2** (Scheme 2). However, NaOMe treatment of **15** at room temperature led to multiple products due to backbone cleavage at the glucuronic acid sites with only trace amount of the desired product obtained. Lowering the pH or reaction temperature led to incomplete removal of the Bz groups. The high lability of glycopeptide **15** to base treatment compared to glycopeptide **13** was possibly due to neighboring group assisted glycan cleavages^[11,33] (Scheme S1).

The failure of the previously established acyl removal strategy prompted us to examine alternatives. We envision a less basic yet strong nucleophile such as hydrazine^[34] could potentially remove the Ac and Bz groups without damaging the glycopeptide linkage. To incorporate hydrazinolysis, the full length glycopeptide **17** is designed, which would be assembled from glycopeptides **18** and **19**. The uronic acids in the glycan chains of **18** and **19** are protected as methyl esters, which can be converted to free carboxylic acids by mild base treatment laying the stage for hydrazinolysis to cleave all the acyl protective groups.

Synthesis of glycopeptide **18** started from acetylation of **3**. Subsequent conversion of azides to acetamides, Lev group removal by hydrazine acetate and sulfation afforded octasaccharide **20** (Scheme 3). The Fmoc group in **20** was removed and the resulting free amine was coupled to dipeptide **21** to produce glycopeptide **22** in 56% yield over two steps. Selective removal of the benzyl ester in glycopeptide **22** under hydrogenation in the presence of NH_4OAc generated glycopeptide **18** with a free carboxylic acid terminal (Scheme 3).

Synthesis of glycopeptide **19** began with hydrogenation of octasaccharide **2** in the presence of NH_4OAc affording glycopeptide **23** containing the free C-terminal carboxylic acid (Scheme 4). Coupling of **23** with tripeptide **24** gave glycopeptide **25**, which was treated with piperidine and then joined with tripeptide **26**. The resulting product **27** was deprotected by piperidine leading to the free amine bearing glycopeptide **19**.

With glycopeptides **18** and **19** in hand, they were united as promoted with *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) to produce glycopeptide **28** bearing two different glycans. To remove the benzyl ethers, *p*-methoxybenzyl (PMB) and benzylidene groups, glycopeptide **28** was subjected to global hydrogenation using Pearlman's reagent or Pd/C under atmospheric pressure of hydrogen in mixed solvents of CH_2Cl_2 and methanol. However, this reaction failed to yield any desired product **17** (Scheme 5). As it is possible that the partially deprotected glycopeptides formed during hydrogenation of **28** could undergo aggregation preventing access to the palladium catalyst, colloidal Pd nanoparticles were tested for the hydrogenation of **28** as these nanoparticles could remove benzyl ethers from solid phase bound glycans.^[35] Various solvents, pH values, reaction temperature and elevated hydrogen pressure as well as low acidity triflic acid condition for benzyl removal^[36] were also examined. However, none of these endeavors yielded the desired compounds with decomposition products observed in mass spectrum analysis.

Due to the unexpected difficulty in the hydrogenation of **28**, an alternative route was envisioned where hydrogenation was to be performed on glycopeptides **22** and **27** bearing a single heparan sulfate chain. The iduronic acid containing non-sulfated glycopeptide **29** was

tested first, which was prepared analogously to **22**. Catalytic hydrogenation of compound **29** under slightly acidic condition (pH ~ 5.5) using Pearlman's catalyst went smoothly and gave the desired product **30** in quantitative yield (Scheme 6a). In a similar manner, sulfated glycopeptide **22** and glycopeptide **27** were successfully hydrogenated yielding **31** and **32** quantitatively (Scheme 6b). The reason for the increase in fragility of glycopeptides bearing two heparan sulfate chains towards hydrogenation is not clear.

The two partially deprotected glycopeptides **30** and **32** were united through HATU mediated coupling affording glycopeptide **33** (Scheme 7). To complete the deprotection, the methyl esters in **33** were cleaved by LiOH (pH 9.5), which was followed by hydrazine treatment. The hydrazinolysis procedure successfully removed all acyl protective groups affording the fully deprotected glycopeptide **1a**.

With the success in preparing the non-sulfated glycopeptide **1a**, we moved on to the synthesis of the sulfated glycopeptide **1**. Coupling of glycopeptides **31** and **32** afforded compound **34** (Scheme 8). The sulfated glycan chain turned out to be very sensitive to base as pH 9.5 LiOH led to partial chain cleavage. Instead, the methyl ester removal was performed at pH 9.0 with frequent monitoring by mass spectroscopy, which was followed by hydrazinolysis to afford glycopeptide **1** in 61% yield.

In conclusion, a successful strategy was developed for the assembly of syndecan-3 glycopeptides bearing two heparan sulfate chains. Many obstacles were encountered during the syntheses and the previous synthetic route established for syndecans-1 with one glycan chain could not be directly applied to glycopeptide **1**. This is because as the size of the molecule grows larger, unique reactivity and stability problems emerged. To overcome the challenges, the hydrogenation reaction was performed on the glycopeptide bearing single glycan chain followed by union of the partially deprotected fragments. The final deprotections required the cleavage of all the ester protective groups, which was accomplished by mild base treatment followed by hydrazinolysis. The hydrazinolysis procedure was critical to ensure complete removal of the benzoyl moieties without undesired β -elimination or cleavage of the highly sensitive glycan chain. The knowledge gained in the current study will be valuable to the synthesis of other glycosaminoglycan family glycopeptides bearing multiple heparan sulfate chains.

Supplementary Material

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Acknowledgments

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Scheme 1.

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