

# Chemoenzymatic Synthesis of Glycopeptides to Explore the Role of Mucin 1 Glycosylation in Cell Adhesion

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*In memory of Ulf Diederichsen.*

Post-translational modifications affect protein biology under physiological and pathological conditions. Efficient methods for the preparation of peptides and proteins carrying defined, homogeneous modifications are fundamental tools for investigating these functions. In the case of mucin 1 (MUC1), an altered glycosylation pattern is observed in carcinogenesis. To better understand the role of MUC1 glycosylation in the interactions and adhesion of cancer cells, we prepared a panel of homogeneously O-glycosylated MUC1 peptides by using a quantitative chemoenzymatic approach. Cell-adhesion experiments with MCF-7 cancer cells on surfaces carrying up to six

differently glycosylated MUC1 peptides demonstrated that different glycans have a significant impact on adhesion. This finding suggests a distinct role for MUC1 glycosylation patterns in cancer cell migration and/or invasion. To decipher the molecular mechanism for the observed adhesion, we investigated the conformation of the glycosylated MUC1 peptides by NMR spectroscopy. These experiments revealed only minor differences in peptide structure, therefore clearly relating the adhesion behaviour to the type and number of glycans linked to MUC1.

## Introduction

Co- and/or post-translational modifications (PTMs) diversify the proteome by modulating protein stability, structure and function. Therefore, understanding the effect of such modifications on protein function can give important insights into their biological role. Mucin 1 (MUC1) is an exemplary post-translationally modified protein. In particular, it is a membrane protein belonging to the mucin family and expressed in healthy tissues on the apical surface of epithelial cells,<sup>[1]</sup> with protection, lubrication, signalling and adhesion functions.<sup>[2]</sup> The extracellu-

lar domain of this large protein, which extends beyond the glycocalyx, contains a variable number of tandem repeats, sequences of 20 amino acids, rich in Ser, Thr, and Pro residues that repeat themselves 25–125 times. Ser and Thr residues in the tandem repeats are sites for O-glycosylation.<sup>[3]</sup> Indeed, the extracellular domain of MUC1 in healthy tissues is heavily glycosylated with complex, branched glycans that contribute to the protective role of MUC1 against infections.<sup>[4]</sup> In malignant transformations, MUC1 loses its apical polarization and is overexpressed and redistributed around the entire cell.<sup>[5,6]</sup> This phenomenon has been associated with high metastatic behav-

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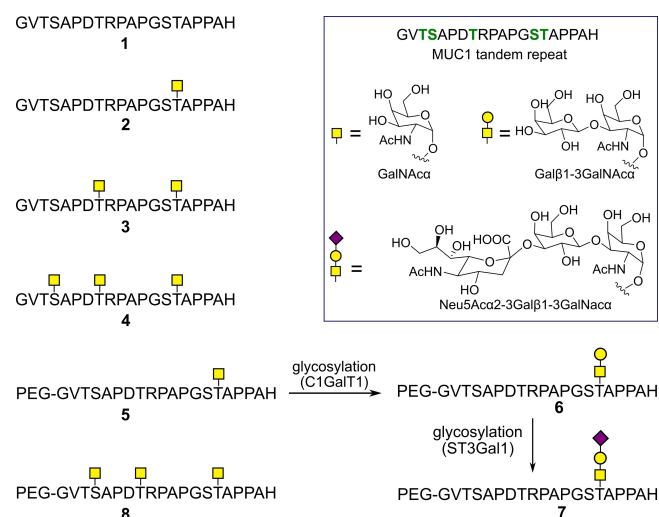
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ious and poor prognosis in cancer patients.<sup>[7]</sup> Moreover, MUC1 glycosylation on cancer cells is altered: the glycans are core 1- and not core 2-based as they are in normal cells, and mainly consist of GalNAc $\alpha$ -, Gal $\beta$ 1-3GalNAc $\alpha$ -, Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$ - (Scheme 1), and Neu5Ac $\alpha$ 2-6GalNAc $\alpha$ - on Thr or Ser residues referred as Tn, T, sialyl-T (sT) and sialyl-Tn (sTn) antigen, respectively.<sup>[8]</sup> It has been reported that MUC1 promotes cell escape and facilitates tumour dissemination by interfering with integrin-mediated cell adhesion.<sup>[2,9,10]</sup> However, the role of MUC1 and of its aberrant glycosylation in tumour progression and metastasis still remains controversial.<sup>[8,11,12]</sup>

Investigating the precise role of MUC1 aberrant glycosylation in cancer cell adhesion is complicated by the heterogeneous nature of this type of PTM, and by the difficulties in investigating carbohydrate interactions.<sup>[13,14]</sup> Indeed, the non-templated nature of glycan biosynthesis and the high variability of glycosylation,<sup>[15]</sup> make them practically impossible to predict based on genetic information and difficult to analyse by classical methods. Moreover, carbohydrate-mediated interactions generally display low affinity<sup>[16,17]</sup> and can be specific for certain organelles or cell compartments,<sup>[16]</sup> contributing to the complexity of the system.

Synthetic glycopeptides have been already successfully used to demonstrate the glycosylation function in different applications.<sup>[18–23]</sup> Therefore, mucin peptides carrying homogeneous glycosylation can be effective tools to investigate the role of MUC1 glycosylation patterns in cell-cell adhesion. Using a chemoenzymatic method that we previously developed for the synthesis of homogeneous, site-selectively glycosylated peptides,<sup>[24]</sup> we prepared a set of synthetic MUC1 glycopeptides carrying well defined glycans at specific positions to explore the impact of the type and the number of tumour-associated O-linked glycans on peptide conformation and cell adhesion.



**Scheme 1.** Schematic representation of the MUC1 tandem repeat peptides with GalNAc monosaccharides at selected positions. PEGylated MUC1 peptides carrying a disaccharide (6) or a trisaccharide (7) were synthesized by enzymatic elongation of GalNAc on the PEGylated glycopeptide 5. Amino acid residues highlighted in green in the sequence of the tandem repeat (see box) are the potential glycosylation sites.

## Results and Discussion

### Chemoenzymatic glycosylation enables the efficient synthesis of a panel of homogeneously glycosylated MUC1 peptides

The tandem repeat sequence of MUC1 consists of 20 amino acids and contains five potential O-glycosylation sites (Scheme 1). In this proof of concept study, we selected three sites, thus limiting the number of variants to a small set of homogeneous MUC1 (glyco)peptides. Among the three sites Thr15 is one of the preferential glycosylation sites of the most ubiquitous transferases (GalNAcT1 and GalNAcT2), thus glycosylation at this site has a very high probability of natural occurrence,<sup>[25,26]</sup> and Thr8 is part of the so-called “protective epitope” of MUC1.<sup>[27,28]</sup> The third site, that is, Ser4, was chosen as part of the other antigenic region of the MUC1 tandem repeat. We applied Fmoc/tBu/OAc solid-phase peptide synthesis (SPPS) for the efficient preparation of the MUC1 tandem repeat aglycone 1 and of the three differently glycosylated variants 2, 3, and 4, carrying one, two, and three GalNAc monosaccharides, respectively, at specific Thr and/or Ser residues in the sequence (Scheme 1 and Figures S1–S4 in the Supporting Information). Fmoc–Thr[GalNAc(OAc)<sub>3</sub>]–OH and Fmoc–Ser[GalNAc(OAc)<sub>3</sub>]–OH were used as building blocks in SPPS to introduce O-glycosylation directly with complete control over position.<sup>[29]</sup>

Further enzymatic glycosylation on Thr(GalNAc) was possible thanks to the removable monodispersed polyethylene glycol linker (PEG<sub>27</sub>) at the N-terminus, which we successfully demonstrated to facilitate further glycosylation and recovery of the desired glycosylated peptides.<sup>[24,30,31]</sup> In fact, the presence of PEG<sub>27</sub> conveys full solubility to the peptides in the experimental conditions required for quantitative enzymatic glycosylation with the specific glycosyltransferases. In a subsequent step, the PEG linker enables selective precipitation of the glycopeptide conjugate by simple addition of appropriate organic solvents and recovery by centrifugation. In previous studies PEG<sub>27</sub> was successfully removed via enzymatic release using tobacco etch virus (TEV) protease to allow traceless modifications.<sup>[24]</sup>

To further simplify the previously described strategy, we investigated the possibility to directly use the PEGylated glycopeptides in cell-adhesion experiments, one of the aims of the present study. To test this hypothesis, we prepared conjugates 5 and 8, corresponding to the PEGylated versions of glycopeptides 2 and 4, respectively, by linking PEG<sub>27</sub> directly to the N-terminus of the corresponding peptidyl resins via HATU-mediated coupling, before cleavage from the resin. Then, after deprotection of the amino acid side chains and of the hydroxyl functions of the sugar moieties and cleavage from the resin, final conjugates were purified by RP-HPLC. PEGylated glycopeptide 5 (Scheme 1 and Figure S5) was used as substrate in enzymatic glycosylation reactions to elongate the Tn antigen to the T and subsequently to the sT antigen. These stepwise reactions were used to prepare two different variants of the MUC1 peptide to investigate the effects of the sequential elongation of the GalNAc on its properties. These reactions were performed following the same procedure previously applied.<sup>[30]</sup> Galactosylation of 5 with UDP–Gal as glycan donor

and C1GalT1 transferase as catalyst, gave conjugate **6** bearing a Gal $\beta$ 1-3GalNAc $\alpha$ - on Thr15 (T antigen, Scheme 1 and Figure S7), and sialylation of **6** with CMP-Neu5Ac in the presence of ST3Gal1 transferase lead to conjugate **7** carrying a Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$  trisaccharide on Thr15 (sT antigen, Scheme 1 and Figure S8).

Unfortunately, preliminary cell-adhesion experiments conducted with PEGylated glycopeptides **5** and **8** showed that PEG<sub>27</sub> strongly interferes, decreasing cell adhesion in a non-reproducible manner, thus making impossible to evaluate the effect of the glycosylation density on adhesion (data not shown). Thus, we investigated our original methodology based on enzymatically removable PEG<sub>27</sub>-TEV to prepare two new glycopeptides carrying a T and an sT antigen, respectively, focusing on the MUC1 analogue carrying the glycan on Thr15 (Scheme 2). To this end, PEG conjugate glycopeptide **9** (Scheme 2 and Figure S9) was prepared via on-resin elongation of peptide **2** with the recognition sequence of TEV protease<sup>[32]</sup> and PEGylation at the N-terminus. Product **9** was used in an enzymatic glycosylation reaction using UDP-Gal as saccharide donor and C1GalT1 glycosyltransferase as catalyst to elongate the GalNAc on Thr15 with a galactose moiety (Figure S10). After completion of the reaction, the mixture containing the PEGylated peptide **10** was split in two portions. One-pot enzymatic removal of PEG<sub>27</sub> on one portion, followed by RP-HPLC purification, lead to pure glycopeptide **11** in 64% yield over the two steps (Scheme 2).

PEGylated glycopeptide **10** was recovered from the second portion of the reaction mixture after precipitation and centrifugation, and directly used in the next glycosylation step: reaction with CMP-Neu5Ac as glycosyl donor and ST3Gal1 sialyltransfer-

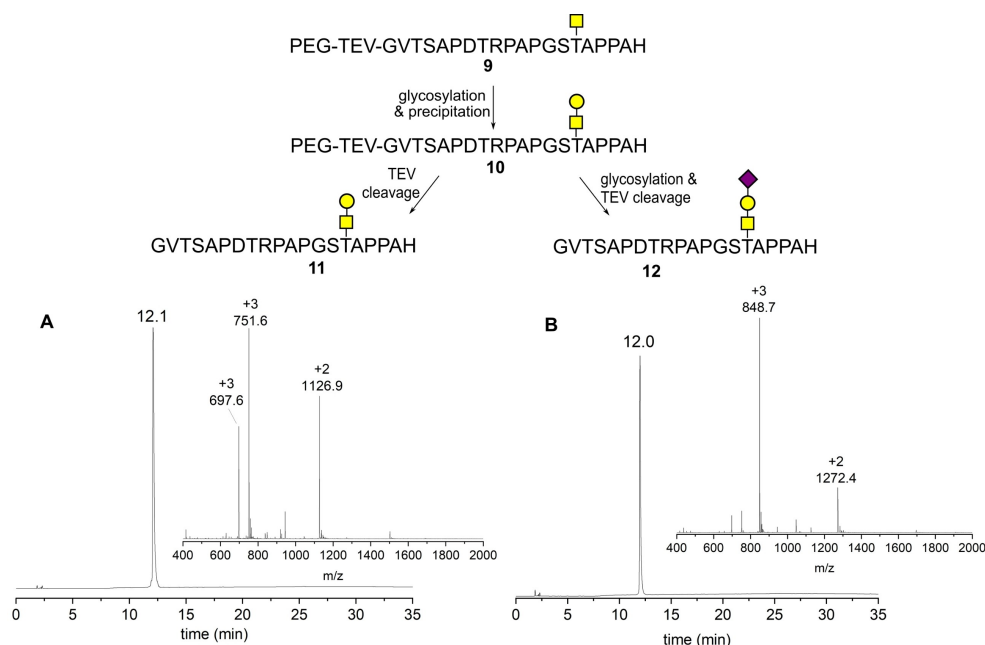
ase gave **10'** carrying the sT antigen (Figure S10). Removal of the PEG polymer via proteolytic digestion was carried out in one-pot after sialylation. Final RP-HPLC purification gave **12** in 77% yield over the two steps (Scheme 2).

### Conformation and cell-adhesion studies to correlate adhesion behaviour to the type and number of glycans

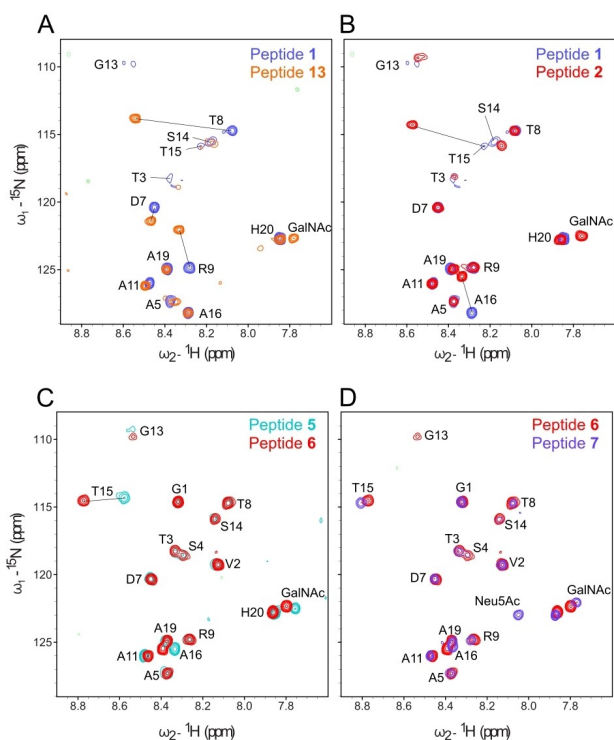
The synthetic glycopeptides were analysed by NMR to investigate the impact of different glycans in different positions on the conformations of the tandem repeat segment (Figures 1 and S11). The conformational preference of MUC1-derived glycopeptides has been previously investigated, particularly with the aim of identifying a potential preferred epitope conformation to develop MUC1-based anticancer vaccines.<sup>[33]</sup> The main conclusions were that the investigated glycosylations did not lead to a well-defined MUC1 backbone conformation, but they affected the conformational equilibrium of the backbone, favouring extended conformations around the Thr residue. Short-range NOE correlations between GalNAc and the peptide indicate an orientation of the GalNAc-ring perpendicular to the extended peptide chain.

To evaluate the effect of glycosylation on the backbone conformations, we first compared glycopeptide **2**, bearing one Tn antigen at position 15, with the aglycone **1** and with the previously described MUC1 glycopeptide **13** bearing the Tn antigen at position 8 (Figures 1A,B and S11A–C).

The combined <sup>1</sup>H and <sup>15</sup>N chemical shift deviations (Figure S11A,B), a very sensitive measure for alterations in conformations and in their populations, and the <sup>13</sup>C chemical shift



**Scheme 2.** Synthesis of glycopeptides **11** and **12** carrying a Gal $\beta$ 1-3GalNAc $\alpha$  disaccharide and a Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$  trisaccharide on Thr15, respectively. A) RP-HPLC and MS analysis of glycopeptide **11**; calcd. mass: 2252. The MS spectrum shows sugar fragmentation during analysis (–Gal):  $[M + 3H]^{3+}$  697.6 (found),  $[M + 3H]^{3+}$  697.7 (calcd). B) RP-HPLC and MS analysis (direct infusion) of peptide **12**; calcd. mass: 2542. TEV=TEV protease recognition sequence = GDENLYFQ.



**Figure 1.** Effect of the position and length of *O*-glycans on the peptide backbone sensed by NMR spectroscopy. A) Superposition of fingerprint  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of the Thr8-glycosylated peptide 13 and the aglycone peptide 1. B) Comparison of  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of the Thr15-glycosylated peptide 2 and the aglycone peptide 1. C) Comparison of the  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of the peptides glycosylated on Thr15, that is, peptide 5 with a single GalNAc monosaccharide and peptide 6 with a Gal–GalNAc disaccharide. D) Effect of sialylation by comparing  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of peptides 6 and 7.

deviations from random coil values (Figure S11C), a measure for secondary structure and torsion angles, revealed only small changes as a consequence of glycosylation for the amino acid residues at the +1 and –1 positions of the modified Thr residue, in addition to the large expected influence on the Thr residue itself. That reflects the restriction of the backbone conformational space to a more extended conformation compared to the aglycone.<sup>[33]</sup> This result is in agreement with previous observations reported by Conibear et al.<sup>[22]</sup> Our data clearly indicate that there is no stabilization of a specific secondary structure. In our measurements we do not observe any other NOEs than sequential NOE correlations and local contacts between GalNAc and the peptide. As these NOE patterns resemble previously observed ones,<sup>[25,34–37]</sup> we did not perform 3D structure calculations. In support to our findings, a detailed investigation by Corzana et al.<sup>[35]</sup> on short model peptides carrying GalNAc on Thr demonstrated that extended structures dominate, but  $\alpha$ -helical and other structures are also populated. All these structures interchange, with no single structure induced by the glycosylation on the peptide backbone.

Comparison of the NMR data from glycopeptides 2 and 5 demonstrated that the presence of the PEG polymer does not

affect peptide conformation, especially around Thr residues (Figure S12). Therefore, the PEGylated peptides 6 and 7, carrying a di- and a trisaccharide on Thr15, respectively, were compared with the corresponding non-PEGylated variants. Thus, peptides bearing different glycan structures on Thr15 (corresponding to Tn, T, and sT antigen, respectively) all exhibited extended peptide conformations, in agreement with data from the literature (Figures 1C,D and S11D,E).<sup>[25,38]</sup>

Previous reports indicate that linear extension of the glycan has no influence on the peptide backbone conformation,<sup>[37,39]</sup> although the conformational equilibrium of glycopeptides with different structure and multiple glycosylation is complex, as demonstrated for MUC1-derived glycopeptides containing several bulky branched *O*-glycans.<sup>[40]</sup>

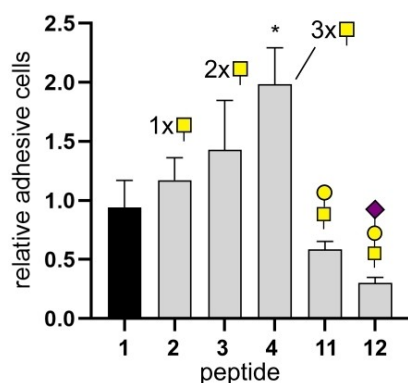
In our case, we observe small and local  $^1\text{H}/^{15}\text{N}$  chemical shift deviations when the monosaccharide in the glycopeptide is elongated to disaccharide and subsequently to trisaccharide by sialylation, affecting the threonine signal and, less strongly, the signal of next neighbouring alanine residue (Figure 1C). The N-acetyl group NH signal in GalNAc is also affected. Considering that no other changes were observed (Figure S11D and E), we conclude that glycan extension has no effect on the overall peptide backbone conformation.

After excluding a significant effect of glycans in inducing a well-defined peptide structure, we pursued experiments on breast cancer cell line MCF-7 to evaluate cell adhesion. A comparative study of adhesion properties of the non-glycosylated MUC1 with the different glycosylated variants was performed starting from the observation that MUC1 is aberrantly expressed in human breast cancer.<sup>[41,42]</sup>

We first performed a cell viability assay in the absence and presence of the glycopeptides to evaluate their effect on MCF-7 cell survival. None of the MUC1 (glyco)peptides, independently of their glycosylation pattern, showed toxicity towards MCF-7 breast cancer cells (Figure S13).

Adhesion experiments were then performed by plating MCF-7 cells on cell culture dishes pre-coated with the differently glycosylated MUC1 peptides and assessing the number of attached cells after 30 min of incubation. Adsorption of glycopeptides on the plastic surface, mediated mainly by hydrophobic interactions, was verified by HPLC analysis (see Adhesion Assay in the Supporting Information)

Adhesion of MCF-7 cells was clearly promoted by the presence of an increasing number of Tn antigens on the peptide backbone (Figure 2), probably through MUC1 self-interaction,<sup>[43]</sup> thus suggesting a role for GalNAc glycosylation in tumour migration/invasion. Comparing the effect of MUC1 aglycone 1 with glycopeptide 2 carrying only one GalNAc monosaccharide, we observed a positive effect on cell adhesion for this type of glycosylation, despite not very pronounced probably because of the low abundance of the sugar moieties in this peptide. In stark contrast, elongation of the glycan as in variant 11 carrying the disaccharide Gal $\beta$ 1-3GalNAc $\alpha$ - on Thr15 (T antigen), severely impaired cell adhesion. This behaviour was clearly enforced by addition of sialic acid as third sugar moiety to obtain the sT antigen (glycopeptide 12). Our results are in agreement with the fundamental role of sialic acid in inhibiting



**Figure 2.** Adhesion experiments. MCF-7 cells were seeded for 30 min on cell culture dishes pre-coated with MUC1 glycopeptides with different glycosylation patterns (the number corresponding to each peptide is reported on the x-axis). The nature and abundance of glycosylation have a clear impact on cell adhesion. Results are reported relative to the number of cells adhering to uncoated plates. Data are represented as mean  $\pm$  SEM of three replicates. \* $p < 0.05$  for 4 vs 1;  $p < 0.5$  for 12 vs 1 (One-way Anova with Dunnett's post hoc test). ■ = GalNAc $\alpha$ ; -■-● = Gal $\beta$ 1-3GalNAc $\alpha$ ; -■-●-◆ = Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$ .

cell-cell self-interaction, as demonstrated in the case of sialic acid on Tn (sTn).<sup>[44]</sup>

## Conclusion

Deciphering the role of protein glycosylation remains an intriguing challenge due to the complexity and intrinsic heterogeneity of this relevant post-translational modification. Our chemoenzymatic approach enabled us to efficiently synthesize a set of homogeneous MUC1 glycopeptides as specific tools to explore the relationship between glycosylation type and cell adhesion. The glycopeptides were characterized by NMR spectroscopy to exclude any indirect larger conformation effects on the backbone influencing cell adhesion. All glycopeptides showed an extended conformation that was only locally influenced by the position and length of the glycan, particularly at the glycosylation site. We then used these glycopeptides to probe the effect of specific glycosylation on cell adhesion. Our results clearly show that GalNAc glycosylation both on serine and threonine residues promotes adhesion of MCF-7 breast cancer cells, while elongation of the glycan, in particular by the addition of sialic acid (sT antigen), demonstrated the opposite effect. These findings support previously reported data that suggest a contribution of GalNAc glycosylation to tumour migration/invasion by promoting MUC1 self-interaction and an inhibitory effect induced by sialylation. In fact, it is known that glycosylation has an influence on various recognition phenomena, such as receptor-ligand interactions, protein-protein interactions, cell recognition and cell adhesion.<sup>[45–48]</sup> Sugar-mediated interactions are influenced by the nature of the glycan, which does not affect the conformational propensity of the peptide backbone, and by avidity effects, as found for carbohydrate-lectin interactions.<sup>[49]</sup> Thus,

the number and frequency of glycosylation sites often results in different biological activities.<sup>[50]</sup> Given its size and dense glycosylation, MUC1 strongly influences cell adhesion and recognition events involving epithelial cells, and has been pursued as a valuable biomarker and therapeutic target for several types of cancer.<sup>[5]</sup> Based on these observations, strategies involving glycopeptides bearing more complex carbohydrates might be an alternative to currently pursued efforts to use glycans for specific targeting or as biomarkers.<sup>[51]</sup> The usefulness and efficiency of the chemoenzymatic approach presented herein could be further exploited for the preparation of tailored glycopeptides to tackle biological questions.

## Experimental Section

Material and methods used in this work, together with experimental details and compound analyses can be found in the Supporting Information. General procedures are reported below.

**Solid-phase peptide synthesis: general procedure:** Peptides were synthesized manually in solid-phase employing Fmoc/tBu orthogonal protection strategy. The syntheses were performed on Fmoc-His(Trt) TentaGel® R PHB resin (0.19 mmol/g). After swelling of the resin in DMF for 30 min, the peptide was elongated via repeating cycles of deprotection and coupling with the required amino acids orthogonally protected as follows: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH, Fmoc-Thr(tBu)-OH, Fmoc-Val-OH. Briefly: 1) deprotection of the N-terminal Fmoc protecting group by treating the peptidyl resin twice with a solution of piperidine in DMF (20% v/v, 3 + 7 min); 2) flow wash for 1 min; 3) coupling of the amino acid by addition of a solution of the protected amino acid, HBTU and DIEA in DMF (3 equiv., 2.75 equiv. and 6 equiv., respectively); 4) shaking of the suspension for 30 min at room temperature; 5) solvent removal and washing of the peptidyl resin with DMF. Double couplings were performed for proline residues and the amino acids after proline residues. Couplings were monitored via Kaiser test. Fmoc-Thr[GalNAc(OAc)<sub>3</sub>]-OH and Fmoc-Ser[GalNAc(OAc)<sub>3</sub>]-OH were introduced using HATU-mediated coupling (1.5 equiv. amino acid, 1.35 equiv. HATU (0.3 M solution in DMF), 2.5 equiv. DIEA, 40 min; double coupling). Peptidyl resins bearing fully protected glycopeptides 2, 3, and 4 were prepared starting from the same batch of resin (0.3 mmol) and split in correspondence of the addition of the modified amino acid. For detailed procedures, see the Supporting Information. For the synthesis of peptide 13, further elongation with the TEV protease recognition sequence was performed using orthogonally protected amino acids, that is, Fmoc-Asp(OtBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Tyr(tBu)-OH, and HBTU coupling reagent as described above. Final Fmoc removal with 20% v/v piperidine in DMF was performed for all peptidyl resins.

**Peptide conjugation with monodispersed PEG: general procedure:** PEGylation of the peptides was performed treating the peptidyl resin with a 0.3 M solution of Fmoc-NH-(PEG)<sub>27</sub>-OH (2 equiv.), 0.3 M HATU (1.75 equiv.) and 0.7 M DIEA (5 equiv.) in DMF/ACN 3:2 overnight at room temperature. After filtering the solvent, the peptidyl resin was washed extensively with DMF. Removal of the Fmoc protecting group on PEG was performed by treatment with a 20% v/v solution of piperidine in DMF, as described in the general procedure for solid-phase peptide synthesis.

**Deprotection, cleavage and purification of peptides: General procedure:** Peptide cleavage from the resin and simultaneous side-chain deprotection was carried out by shaking the resin for 3 h with a solution of TFA/triisopropylsilane/water (92.5:5:2.5 v/v/v; 1 mL/100 mg peptidyl resin). The resin was then filtered and washed with TFA. The crude peptide was precipitated by addition of cold diethyl ether and recovered after centrifugation. In the case of the glycopeptides, deprotection of the acetyl groups on GalNAc was carried out by dissolving the lyophilized glycopeptides in a 5% v/v solution of hydrazine monohydrate in methanol. The solution was shaken for 1.5 h, then the reaction was quenched with acetic acid and the solvent was evaporated. The crude was dissolved in water and freeze-dried. Purification was carried out by semipreparative RP-HPLC or by Flash chromatography using a reversed-phase C<sub>18</sub> SNAP Cartridge. Details about purification conditions are reported in the Supporting Information.

**Adhesion assay:** Plates were coated by adding 100 μL of a solution containing 10 μg/mL MUC1 peptides in PBS and let to adhere for 16 h at RT. 30.000 MCF-7 cells were seeded onto 24-multiwell coated-plates for 30 min. Adherent cells were stained with crystal violet solution (0.5% in 20% methanol). After 5 min of staining, photos of the wells were taken. Cells were counted in randomly chosen fields using the ImageJ software (NIH, USA). Data are represented as mean ± SEM of three biological replicates. One-way ANOVA with Dunnett's post hoc test, \**p* < 0.05 for 8 vs 5; *p* < 0.5 for 17 vs 5.

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## Conflict of Interests

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:** cell adhesion · glycopeptides · glycosylation · mucin 1 · NMR spectroscopy

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