

Article

## Structure-based design of potent tumor-associated antigens: modulation of peptide presentation by single atom O/S or O/Se substitutions at the glycosidic linkage

Ismael Compañón, Ana Guerreiro, Vincenzo Mangini, Jorge Castro-López, Margarita Escudero-Casao, Alberto Avenzoza, Jesús H. Busto, Sergio Castellón, Jesús Jiménez-Barbero, Juan L. Asensio, Gonzalo Jiménez-Osés, Omar Boutureira, Jesús M. Peregrina, Ramon Hurtado-Guerrero, Roberto Fiammengo, Gonçalo J.L. Bernardes, and Francisco Corzana

*J. Am. Chem. Soc.*, **Just Accepted Manuscript** • Publication Date (Web): 06 Feb 2019

Downloaded from <http://pubs.acs.org> on February 6, 2019

### Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

# Structure-based design of potent tumor-associated antigens: modulation of peptide presentation by single atom O/S or O/Se substitutions at the glycosidic linkage

Ismael Compañón,<sup>1,‡</sup> Ana Guerreiro,<sup>2,‡</sup> Vincenzo Mangini,<sup>3,‡</sup> Jorge Castro-López,<sup>4,</sup> Margarita Escudero-Casao,<sup>5</sup> Alberto Avenzoza,<sup>1</sup> Jesús H. Busto,<sup>1</sup> Sergio Castellón,<sup>5</sup> Jesús Jiménez-Barbero,<sup>6,7,8</sup> Juan L. Asensio,<sup>9</sup> Gonzalo Jiménez-Osés,<sup>1,6,7</sup> Omar Boutureira,<sup>5</sup> Jesús M. Peregrina,<sup>1</sup> Ramón Hurtado-Guerrero,<sup>4</sup> Roberto Fiammengo,<sup>3,\*</sup> Gonçalo J. L. Bernardes,<sup>2,10,\*</sup> and Francisco Corzana<sup>1,\*</sup>

<sup>1</sup> Departamento de Química, Universidad de La Rioja, Centro de Investigación en Síntesis Química, 26006 Logroño, Spain.

<sup>2</sup> Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Avenida Professor Egas Moniz, 1649-028 Lisboa, Portugal.

<sup>3</sup> Center for Biomolecular Nanotechnologies@UniLe, Istituto Italiano di Tecnologia (IIT), 73010 Arnesano, Lecce, Italy.

<sup>4</sup> Institute of Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, BIFI-IQFR (CSIC), Zaragoza, Spain; Fundación ARAID, 50018, Zaragoza, Spain.

<sup>5</sup> Departament de Química Analítica i Química Orgànica, Facultat de Química, Universitat Rovira i Virgili, 43007 Tarragona, Spain.

<sup>6</sup> CIC bioGUNE, Bizkaia Technology Park, Building 801A, 48170 Derio, Spain.

<sup>7</sup> Ikerbasque, Basque Foundation for Science, Maria Diaz de Haro 13, 48009 Bilbao, Spain.

<sup>8</sup> Department of Organic Chemistry II, Faculty of Science & Technology, University of the Basque Country, 48940 Leioa, Spain.

<sup>9</sup> Instituto de Química Orgánica General, IQOG-CSIC. 28006 Madrid.

<sup>10</sup> Department of Chemistry, University of Cambridge, Lensfield Road, CB2 1EW Cambridge, UK.

<sup>‡</sup>These authors have contributed equally.

Correspondence should be addressed to F.C., G.J.L.B. or R.F.:

[francisco.corzana@unirioja.es](mailto:francisco.corzana@unirioja.es);

[gb453@cam.ac.uk](mailto:gb453@cam.ac.uk); [gbernardes@medicina.ulisboa.pt](mailto:gbernardes@medicina.ulisboa.pt);

[roberto.fiammengo@iit.it](mailto:roberto.fiammengo@iit.it)

**Abstract**

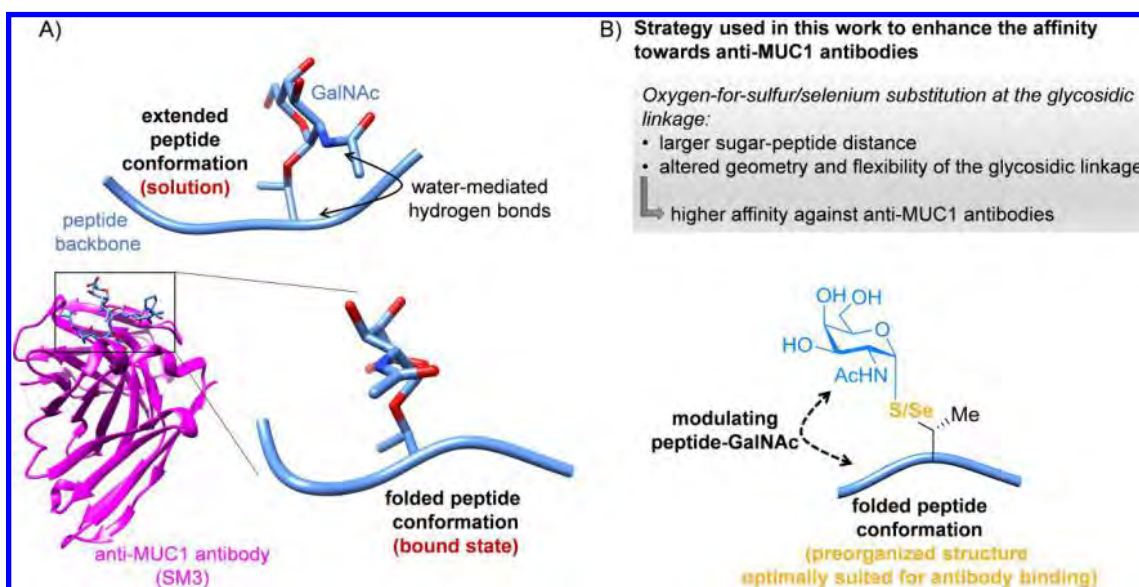
GalNAc-glycopeptides derived from mucin MUC1 are an important class of tumor-associated antigens.  $\alpha$ -O-glycosylation forces the peptide to adopt an extended conformation in solution, which is far from the structure observed in complexes with a model anti-MUC1 antibody. Herein, we propose a new strategy to design potent antigen mimics based on modulating peptide/carbohydrate interactions by means of O $\rightarrow$ S/Se replacement at the glycosidic linkage. These minimal chemical modifications bring about two key structural changes to the glycopeptide. They increase the carbohydrate-peptide distance and change the orientation and dynamics of the glycosidic linkage. As a result, the peptide acquires a preorganized and optimal structure suited for antibody binding. Accordingly, these new glycopeptides display improved binding towards a representative anti-MUC1 antibody relative to the native antigens. To prove the potential of these glycopeptides as tumor-associated MUC1 antigen mimics, the derivative bearing the S-glycosidic linkage was conjugated to gold nanoparticles and tested as an immunogenic formulation in mice without any adjuvant, that resulted in a significant humoral immune response. Importantly, the mice antisera recognize cancer cells in biopsies of breast-cancer patients with high selectivity. This finding demonstrates that the antibodies elicited against the mimetic antigen indeed recognize the naturally occurring antigen in its physiological context. Clinically, the exploitation of tumor-associated antigen mimics may contribute to the development of cancer vaccines and to the improvement of cancer diagnosis based on anti-MUC1 antibodies. The methodology presented here is of general interest for applications because it may be extended to modulate the affinity of biologically relevant glycopeptides towards their receptors.

## Introduction

MUC1 mucin is an O-glycoprotein overexpressed in many tumor tissues.<sup>1-4</sup> Although in healthy cells the backbone of this protein is decorated with complex glycans, in cancer cells this backbone carries rather simple and truncated oligosaccharides. Consequently, different tumor-associated carbohydrate antigens, such as the Tn determinant ( $\alpha$ -O-GalNAc-Ser/Thr),<sup>5</sup> are presented to the immune system and can be identified by anti-MUC1 antibodies. The peptide fragment Ala-Pro-Asp-Thr-Arg-Pro, which includes the immunodominant PDTRP region of MUC1 tandem repeats,<sup>6</sup> constitutes the minimum epitope recognized by these antibodies.<sup>7</sup> Partially glycosylated MUC1 derivatives have been used to prepare immunogenic formulations towards the development of therapeutic cancer vaccines.<sup>8-12</sup> Similarly, unnatural glycopeptides that mimic tumor-associated MUC1 can find application as biosensors for the detection of cancerous cells.<sup>13</sup> An intriguing observation about the structural characteristics of these peptides is that  $\alpha$ -O-glycosylation with GalNAc forces the underlying peptide into an extended conformation in solution as a result of stabilizing interactions, which include direct<sup>14</sup> or water-mediated hydrogen bonds, between the peptide and the carbohydrate moiety (Figure 1A).<sup>15-17</sup> In contrast, the X-ray structure of the glycopeptide epitope bound to an anti-MUC1 antibody (SM3)<sup>18</sup> revealed a folded conformation around the glycosylated Thr (Figure 1A).<sup>19</sup> In this case, the sugar shifts the structure of the peptide in solution away from that adopted upon antibody binding. This conformational entropic penalty is however compensated for by favorable enthalpic contributions (hydrogen bonds and CH/ $\pi$  stabilizing interactions)<sup>20,21</sup> between the sugar moiety and the antibody. As a result, a modest net increase in binding affinity (around 3-fold) is observed for the glycosylated versus the non-glycosylated peptide.

Herein, we propose a rational approach based on single-atom substitution (O $\rightarrow$ S/Se) at the glycosidic linkage to obtain potent antigens with an improved affinity towards anti-MUC1 antibodies (Figure 1B). This simple modification increases the distance between the sugar and the peptide fragment — sulfur (or selenium) is larger than oxygen — which in turn minimizes the *exo*-anomeric effect<sup>22</sup> and alters the flexibility and the most stable conformation of the glycosidic linkage towards the one optimized for the antibody. Overall, these glycopeptides adopt a distinct structure in solution, which differs markedly from their oxygenated counterparts, thus avoiding the subsequent entropic cost associated to the extended-to-folded conformational transition of the O-glycopeptide in the bound state. In this work, we describe the strong binding of these glycopeptides to a model MUC1 antibody and demonstrate the possibility of using them as tumor-associated MUC1 mimics when they are incorporated in immunogenic formulations. In

fact, the antibodies elicited in mice recognize selectively the natural occurring tumor-associated MUC1 epitopes displayed on cancer cells in biopsies of breast-cancer patients.

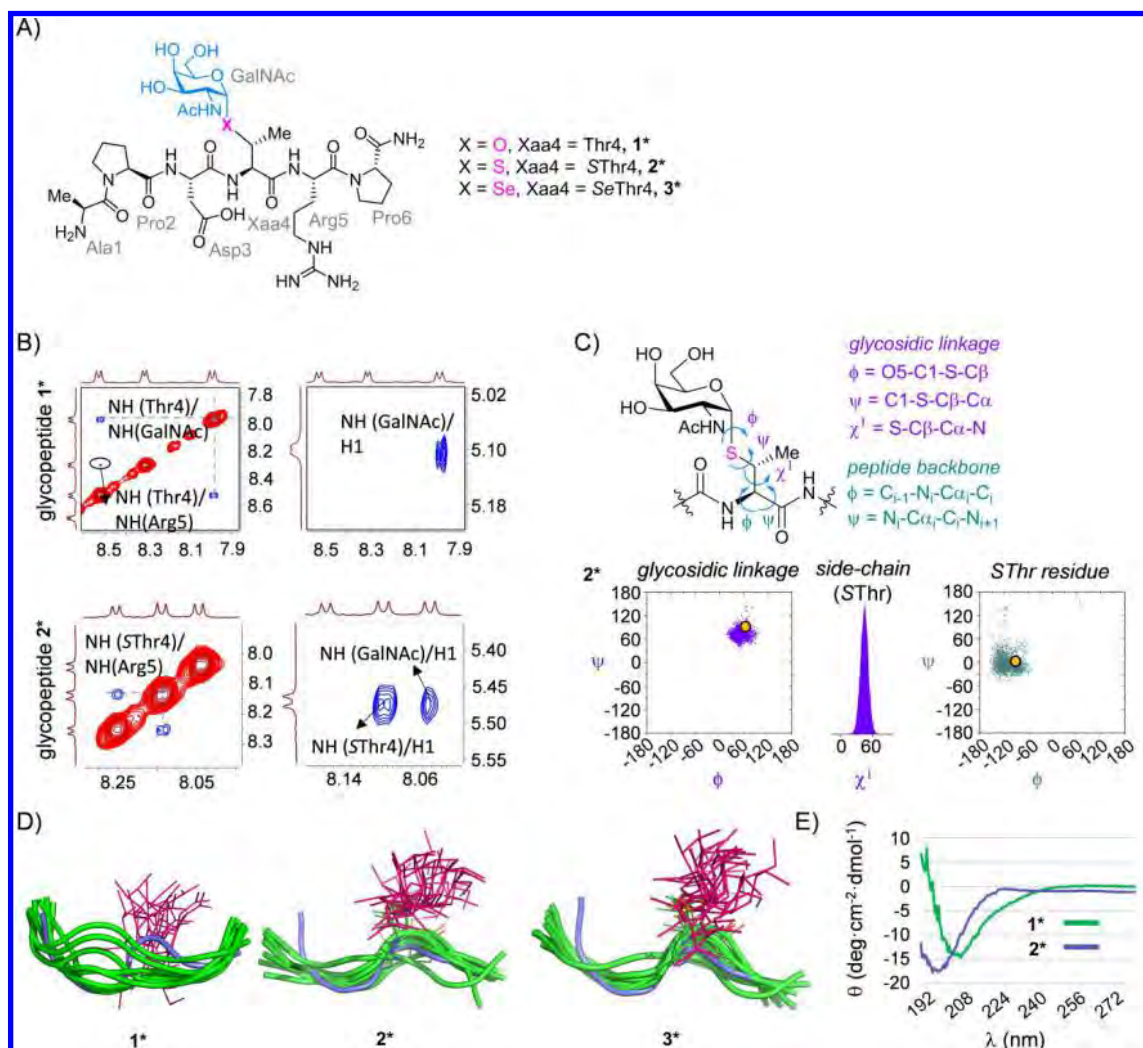


**Figure 1.** (A) Major conformation of a tumor-associated MUC1 glycopeptide in solution (top) and bound to the SM3 antibody (bottom). (B) Proposed strategy to allow the peptide backbone to adopt a preorganized structure in solution.

## Results and Discussion

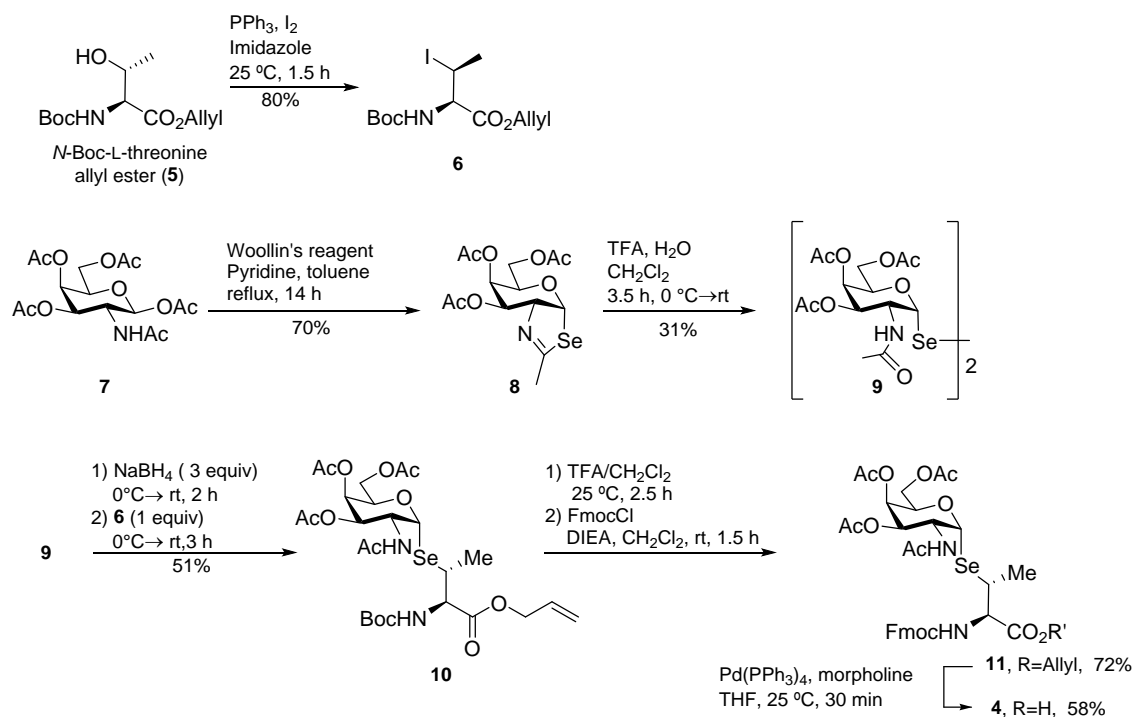
### *Synthesis and conformational analysis of the unnatural glycopeptides in solution*

Glycopeptides **2\*** and **3\*** (Figure 2A) were designed to feature S-( $\alpha$ -D-GalNAc)-thiothreonine (**SThr\***) or Se-( $\alpha$ -D-GalNAc)-selenothreonine (**SeThr\***), respectively, as the fourth residue of the Ala-Pro-Asp-Xaa-Arg-Pro epitope. Naturally occurring glycopeptide **1\***, which has a threonine residue at this position, and non-glycosylated variants **1** and **2** were prepared for comparison.



**Figure 2.** (A) Glycopeptides synthesized and studied in this work, comprising the minimum epitope recognized by most anti-MUC1 antibodies.<sup>7</sup> (B) Sections of the 500 ms 2D-ROESY spectrum (400 MHz) in H<sub>2</sub>O/D<sub>2</sub>O (9:1) at 298 K and pH = 6.5 of glycopeptides 1\* (upper panel) and 2\* (lower panel) that show the amide region. Diagonal peaks are in red. ROE contacts are represented as blue cross-peaks. A second set of signals is observed corresponding to the *cis* configuration of the amide bond of proline residues.<sup>23</sup> (C) Geometry and flexibility at the glycosidic linkage and peptide backbone for the unnatural SThr residue of glycopeptide 2\* in solution derived from 20 ns experiment-guided MD simulations. The yellow circles correspond to the conformation found in the crystal structure of glycopeptide 1\* bound to a single-chain variable fragment of the SM3 antibody (scFv-SM3, PDB ID: 5A2K). (D) Structural ensembles derived from 20 ns experiment-guided MD simulations for compounds 1\*, 2\* and 3\* in solution, together with the conformation of the peptide backbone of 1\* (in blue) found by X-ray crystallography bound to scFv-SM3 antibody (PDB ID: 5A2K). (E) Circular dichroism (CD) spectra of compounds 1\* and 2\* (0.25 mM in sodium phosphate buffer, pH = 7.5, 20 °C).

Although the synthesis of the amino acid thiothreonine (**SThr**) has been previously described,<sup>24-26</sup> the preparation of conveniently protected thiothreonine and selenothreonine derivatives, as well as glycopeptides **2\*** and **3\***, has not been reported yet. As an example, the synthesis of building block **4**, which is ready to be used in solid phase peptide synthesis (SPPS), is shown in Scheme 1. Conveniently protected threonine **5** was reacted with triphenylphosphine and iodine with imidazole as a base to afford the iodo-derivative **6** with total inversion of configuration at the  $\beta$ -carbon.<sup>27</sup> In parallel, selenosugar **9** was prepared in two steps from peracetylated compound **7**. In the first step, **7** was treated with Woollin's reagent and pyridine to give selenazoline **8** in 70% yield. Hydrolysis of **8** with trifluoroacetic acid (TFA) in water afforded selenosugar **9** in moderate yield and as a dimer because of the formation of a diselenide bond. The key step in the synthesis of building block **4** is the nucleophilic attack of **9**, previously reduced in situ with sodium borohydride, at iodo-derivative **6**. This reaction proceeded in 51% yield and with total inversion of the configuration at the  $\beta$ -carbon of the selenothreonine surrogate and completely preserved the  $\alpha$ -configuration at the anomeric carbon as determined by <sup>1</sup>H NMR spectroscopy (see Synthesis section in Supporting Information). Subsequent deprotection steps gave the desired compound **4** in 42% overall yield from **9**. A similar strategy was used to prepare the building block of **SThr** \* (see Synthesis section in Supporting Information).



**Scheme 1.** Synthetic route followed for the preparation of building block **4**. Boc = *tert*-butyloxycarbonyl. DIEA = *N,N*-diisopropylethylamine. Fmoc = fluorenylmethoxycarbonyl.



All (glyco)peptides were synthesized with microwave-assisted SPPS (MW-SPPS) by following our reported protocol<sup>13</sup> (Supporting Information). Next, we performed a thorough conformational analysis of the unnatural glycopeptides **2\*** and **3\*** in solution, by combining NMR spectroscopic measurements with molecular dynamics (MD) simulations (Figures 2B, 2C and 2D; see also Figures S1 and S2). The lack of a ROESY cross-peak between the NH of the unnatural SThr4 residue (or SeThr4) and the NH of GalNAc (Figure 2B, left panel), characteristic of the eclipsed conformation of the glycosidic linkage in GalNAc-Thr,<sup>16,17</sup> together with the presence of a cross-peak between the NH of SThr4 (or SeThr4) and H1 of GalNAc (Figure 2B, right panel), suggest a different conformation for the S- and Se-containing glycosidic linkages in **2\*** and **3\***, respectively, with regard to GalNAc-Thr (Figure 2B). Clear structural differences between glycopeptides **1\*** and the two surrogates **2\*** and **3\*** are also observable in their peptide backbone. In particular, the sequential NH-NH ROESY cross-peak that connects residues 4 and 5 in compounds **2\*** and **3\*** hints to a folded conformation of the glycosylated SThr and SeThr residues (Figure 2B, left panel).<sup>28</sup> The relevant proton-proton distances for the conformational analysis derived from the ROESY spectrum of each compound were then used as time-averaged restraints<sup>29,30</sup> in experiment-guided MD simulations in accordance with our well-established protocol.<sup>31,32</sup> The good agreement between the experimental and theoretical derived distances validates our calculations (Table S1 and Figures S4 and S5). According to the MD simulations, the S- and Se-glycosidic linkages of **2\*** and **3\*** display a unique conformation centered at  $\phi/\psi \approx 65^\circ/70^\circ$ , which agrees with the *exo*-anomeric effect<sup>22</sup> and deviates for the more eclipsed arrangement observed for **1\*** ( $\phi/\psi \approx 65^\circ/120^\circ$ )<sup>16</sup> (Figure 2C and Figure S6). It is important to note that this conformer lies at one of the local minima calculated for methyl 4-thio- $\alpha$ -maltoside<sup>33</sup> and it is similar to that explored by an unnatural Tn antigen with a cysteine residue previously prepared in our lab.<sup>34</sup> The side chain of the unnatural residues in **2\*** and **3\*** is rather rigid in solution, with conformers characterized by  $\chi^1 = 60^\circ$ . The slightly different geometry and flexibility of S- and Se-glycosidic linkages relative to the O-glycosidic linkage, together with the larger size of the S and Se atoms, precludes an effective interaction between the peptide backbone and the carbohydrate. In fact, neither significant hydrogen bonds nor water pockets were observed between these moieties. *This finding emphasizes the synergistic roles of the methyl group of the threonine and the glycosidic oxygen atom in defining the conformational preference of the natural Tn-Thr antigen.*

Regarding the peptide backbone, compounds **2\*** and **3\*** showed conformations characterized by a folded structure around the unnatural residues SThr4 or SeThr4,



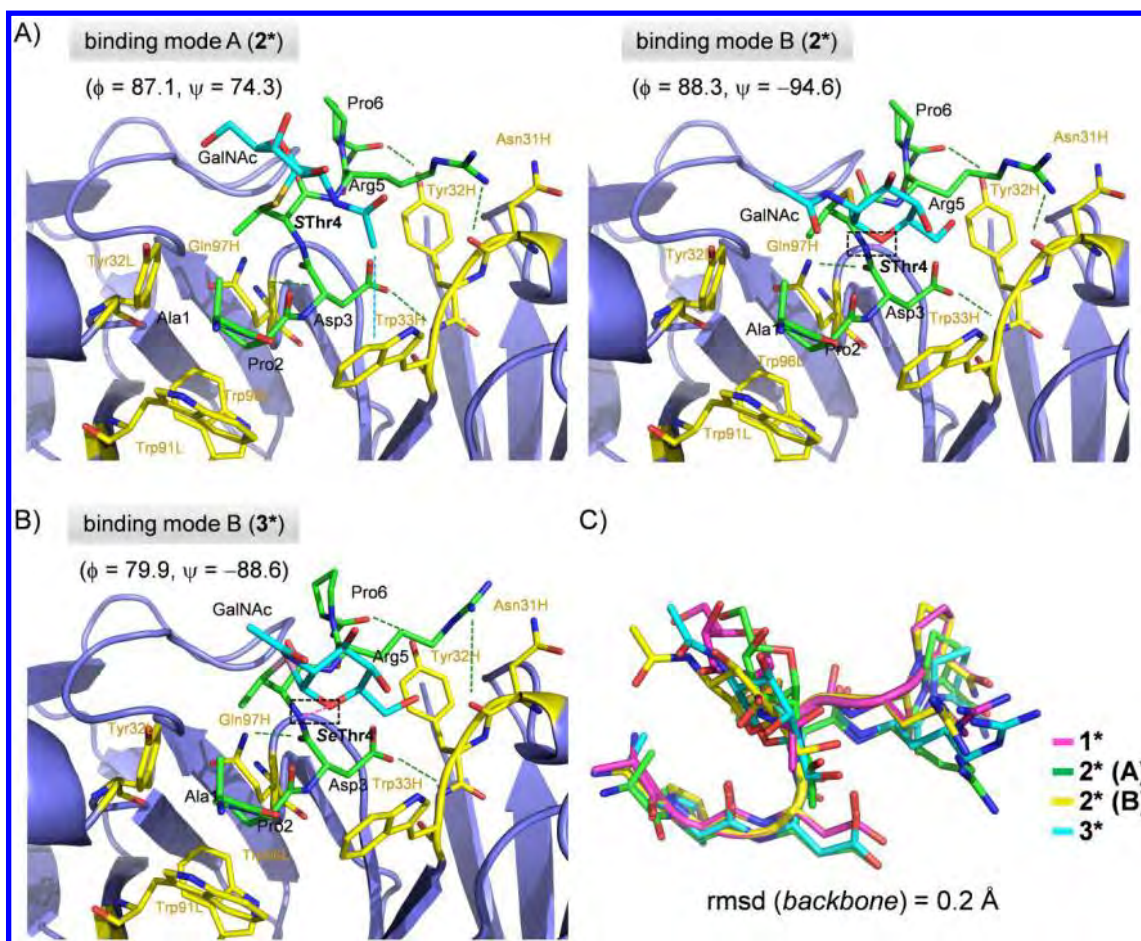
1  
2  
3 respectively (Figures 2, S4 and S5). This arrangement of the peptide differs from that  
4 previously reported for **1\*** (Figure 2D), which displays mostly a  $\beta$ -like extended  
5 conformation in solution (Figure 2D) owing to water-mediated hydrogen bonds between  
6 the peptide and GalNAc.<sup>17,32</sup> The different arrangement of the backbone was also  
7 supported by the CD spectra (Figure 2E). Furthermore, according to unrestrained 1  $\mu$ s  
8 MD simulations in explicit water, non-glycosylated peptide **2** exhibits a random coil  
9 conformation in solution (Figure S3), which is different from the structure adopted by **2\***.  
10 Thus, despite the larger distance between the carbohydrate and the peptide backbone  
11 in glycopeptides **2\*** and **3\***, our results suggest that the sugar moiety still plays a role as  
12 a structural modulator, which presumably may reduce the conformational space  
13 accessible to the peptide backbone. Overall, unnatural glycopeptides **2\*** and **3\*** display  
14 markedly different conformations in solution relative to the naturally occurring  
15 counterpart **1\*** that are induced by the replacement of a single-atom in these compounds  
16 (O $\rightarrow$ S/Se). In particular, the conformational preference both at the glycosidic linkage and  
17 the unnatural residue (SThr4 or SeThr4) are shifted towards those of **1\*** bound to an anti-  
18 MUC1 monoclonal antibody.<sup>19</sup> Thus, the energy cost associated to a conformational  
19 change of the glycopeptide from extended in solution to folded in the bound state is  
20 expected to be minimized (vide infra).  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

### Conformational analysis of the unnatural glycopeptides **2\*** and **3\*** bound to scFv-SM3

33 Crystals suitable for X-ray diffraction analysis of a recombinantly expressed single-chain  
34 variable fragment of the SM3 antibody (scFv-SM3) complexed with **2\*** and **3\*** were  
35 obtained. The X-ray structure of these complexes, solved at high resolution (< 2.0 Å,  
36 Table S2 and Figure 3, and Figure S7, PDB IDs: 5N7B and 6FRJ) revealed that the  
37 conformation of the bound peptide was nearly identical to that adopted by **1\*** when bound  
38 to scFv-SM3 (Figure 3C). This result demonstrates that the antibody recognizes a well-  
39 defined epitope conformation, regardless of the nature of the glycosylated amino acid,  
40 characterized by torsion angles at the glycosylated residue typical of folded structures ( $\phi$   
41 and  $\psi$  close to  $-88^\circ$  and  $10^\circ$ , respectively). As detailed above, this conformation is also  
42 adopted in solution by the peptide backbone of glycopeptides **2\*** and **3\*** (Figure 2D and  
43 Figures S4 and S5). As for glycopeptide **1\***, the stabilizing contacts in complexes  
44 **2\*/scFv-SM3** and **3\*/scFv-SM3** involve several hydrogen bonds, some of which are  
45 mediated by water molecules, as well as several stacking interactions (Figures 3A and  
46 3B).  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56

57 Of note, two distinct binding modes are observed for glycopeptide **2\*** in complex with  
58 scFv-SM3 that differ solely in the geometry of the glycosidic linkage. Binding mode A is  
59  
60

1  
2  
3 characterized by a glycosidic linkage with  $\phi/\psi = 87^\circ/74^\circ$ . This conformer corresponds to  
4 the structure adopted by **2\*** in solution (Figures 2C and 3A). Alternatively, in binding  
5 mode B, with glycosidic linkage angles  $\phi/\psi \approx 90^\circ/-90^\circ$ , the glycopeptide structure is  
6 stabilized by an intramolecular hydrogen bond between the NH of SThr4 and the  
7 endocyclic oxygen (O5) of GalNAc (Figure 3A). This binding mode was also found for  
8 the serine and a cysteine variants of the immunodominant PDTRP region of MUC1.<sup>19</sup>  
9 Although binding mode A allows the *N*-acetyl group of GalNAc to stack with the aromatic  
10 ring of a tryptophan residue (Trp33H) of scFv-SM3, mode B impedes any direct contact  
11 between the sugar and the antibody. The electron density observed for the GalNAc  
12 moiety of glycopeptide **3\*** is rather weak, which may suggest the simultaneous presence  
13 of both binding modes observed for derivative **2\*** (Figure 3B and Figure S7). Extensive  
14 MD simulations performed on the **2\***/scFv-SM3 complex supported that both binding  
15 modes (A and B) are stable in solution (Figures S8 and S9).  
16 Interestingly, quantum mechanical (QM) calculations performed on abbreviated models  
17 of glycopeptides **1\*** and **2\*** (compounds **1'** and **2'**, respectively; see Tables S3 and S4  
18 and Figure S10) indicate that the larger repulsion between the  $\beta$ -methyl group of Thr and  
19 H1 of GalNAc in glycopeptide **1\*** –as a result of the smaller size of the oxygen atom–  
20 together with the more distorted geometry of the intramolecular hydrogen bond O5  
21 (GalNAc) and NH (Thr), leads to the lack of binding mode B in the natural occurring  
22 glycopeptide.  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

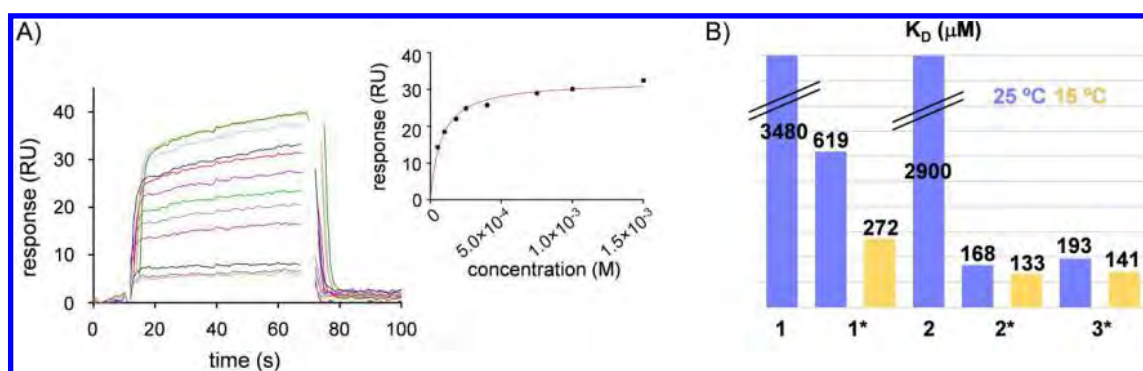


**Figure 3.** X-ray structures of glycopeptides **2\*** (A) and **3\*** (B) bound to the scFv-SM3 antibody (PDB IDs: 5N7B and 6FRJ). Glycopeptides carbon atoms are shown in green. Carbon atoms of key residues of scFv-SM3 are colored yellow. Green dashed lines indicate hydrogen bonds between peptide backbones and scFv-SM3 antibody. Pink dashed lines indicate the hydrogen bond between the NH of SThr (or SeThr) and O5 (see dashed boxes). Blue dashed line indicates a CH/ $\pi$  interaction between the *N*-acetyl group of GalNAc and Trp33H in binding mode A. Note that the density corresponding to the GalNAc moiety in glycopeptide **3\*** is only partial (Figure S7), strongly suggesting the existence of local flexibility. (C) Superposition of glycopeptides **1\***, **2\*** and **3\*** in complex to the scFv-SM3 antibody, which shows that the antibody recognizes the same conformation for the peptide backbone, regardless of the nature of the glycosylated residue.

#### Affinity of the unnatural glycopeptides **2\*** and **3\*** towards scFv-SM3

Detailed conformational analysis of glycopeptides **2\*** and **3\*** both in solution and bound to scFv-SM3 in comparison to that assumed in solution indicates that the structure of these peptides is preorganized for binding, which this is not the case for **1\***. Accordingly,

a tighter binding would be expected for the unnatural derivatives (vide supra). To confirm this hypothesis, their binding affinities ( $K_D$ ) to scFv-SM3 were measured by using surface plasmon resonance (SPR) assays (Figure 4 and Figures S11-S15). The highest affinities were observed for the unnatural glycopeptides **2\*** and **3\*** (with  $K_D = 168$  and  $193 \mu\text{M}$  at  $25 \text{ }^\circ\text{C}$ , respectively). Notably, an improved affinity ( $\sim 20$ -fold) was obtained relative to unglycosylated epitope **1**. The variation in the affinity of natural glycopeptide **1\*** with temperature is higher than for the unnatural counterparts. This result may indicate the existence of an extra entropic penalty associated with the binding of **1\*** (Figure 4B) and highlights in this respect the inherently different conformational behavior of unnatural glycopeptides **2\*** and **3\***, as already concluded through NMR experiments and MD simulations.



**Figure 4.** (A) SPR curves and response-concentration fit obtained for the binding of **2\*** towards scFv-SM3. (B)  $K_D$  constants derived from SPR experiments for the studied (glyco)peptides.

#### Preparation and in vivo studies of an anticancer vaccine based on an engineered glycopeptide.

As discussed above, partially glycosylated peptides with sequences derived from MUC1 are an exciting niche of research for the development of therapeutic anticancer vaccines. As yet, none of them have so far succeeded in clinical trials, underlining the difficulty of inducing effective and durable immunological responses to a self-antigen such as tumor-associated-MUC1.<sup>12</sup> Additional research is needed to understand how to break tolerance to self-antigens, which includes knowledge on how vaccine formulation are processed by the immune system and (glyco)peptide antigens are presented by the major histocompatibility complex (MHC) I and II.<sup>35-38</sup>

The results presented in this work prove that a single atom substitution at the glycosidic linkage has a remarkable impact on the structure of the glycopeptide in solution, especially at the glycosylated residue, which in turn may significantly affect peptide presentation and overall vaccine efficacy. Additionally, natural glycopeptides may suffer

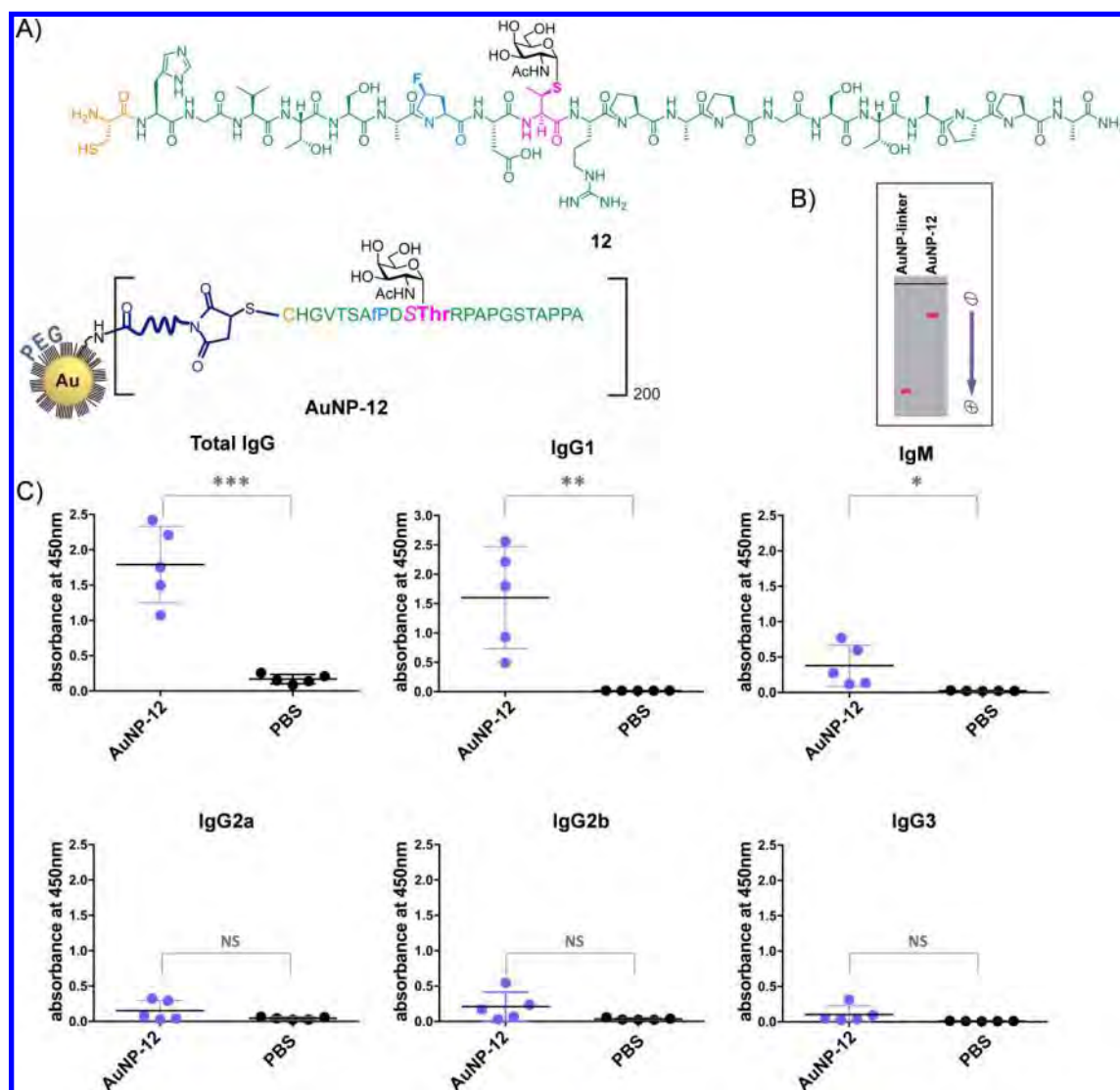
1  
2  
3 degradation from endogenous glycosidases,<sup>39,40</sup> which in turn alters their effectiveness  
4 as immunizing antigens,<sup>41</sup> while S-glycoside analogs have improved stability.<sup>42,43</sup> These  
5 two considerations prompted us to test whether structurally engineered glycopeptide **12**  
6 could be used as tumor-associated antigen mimic through a nanoparticle-based  
7 immunogenic formulation (Figure 5A). Glycopeptide **12** comprises the complete tandem  
8 repeat sequence of MUC1 and features the **SThr\*** residue described above. Additionally,  
9 this glycopeptide displays a (4S)-4-fluoro-L-proline (fPro) residue that replaces the Pro  
10 moiety at the beginning of the PDTRP epitope sequence. The motivation to select this  
11 doubly engineered glycopeptide was to combine the entropic benefit induced by **SThr\***  
12 by preorganizing the epitope structure for optimal binding, and the beneficial enthalpic  
13 effect produced by the fPro by enhancing antigen-antibody interactions.<sup>13</sup> Moreover, one  
14 of us has been previously shown that PEGylated AuNPs could be used as efficient  
15 antigen carriers to establish humoral immunity against the tumor-associated form of  
16 MUC1 in mice, and the elicited antibodies did recognize the natural antigen on human  
17 breast cancer cells.<sup>44</sup> These promising results led us to conjugate the MUC1 antigen  
18 mimic glycopeptide **12** with AuNPs in accordance with the strategy previously described  
19 (**AuNP-12**, Figure 5A, Figure S16 and Table S5).<sup>44</sup> In this occasion, the synthetic effort  
20 was greatly reduced by omitting the extension of the glycopeptide with a CD4 T-cell  
21 peptide epitope and the immunogenic formulation was administered to the mice without  
22 any additional adjuvant.

23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35 The success of the conjugation reactions was easily confirmed through gel  
36 electrophoresis analysis, in which the conjugated **AuNP-12** is characterized by a  
37 reduced electrophoretic mobility relative to the precursor, linker-functionalized AuNPs  
38 (Figure 5B). Additionally, a significant increase in hydrodynamic diameter was observed  
39 with dynamic light scattering (DLS, Table S5) upon conjugation. Peptide loading was  
40 determined by amino acid analysis to be ~200 glycopeptides/AuNP.

41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
Next, a standard immunization strategy was followed to test *in vivo* the immunogenic  
potential of **AuNP-12**. Thus, a group of five balb/c mice were immunized with a prime  
dose followed by three equal booster doses of **AuNP-12** (each dose corresponds to 2  
 $\mu\text{g}$  of the glycopeptide) at 21-days intervals whereas a control group was treated with  
phosphate buffered saline (PBS) as shown in Figure 5B. A week after the last booster  
dose, the mice were sacrificed, and the serum harvested. Analysis of the antisera  
showed that **AuNP-12** can elicit a significant anti-MUC1 IgG antibody response. The  
total antibody endpoint titers (Figure S18) were better than those observed for the  
previously reported AuNP-based vaccine candidate in the presence of complete  
Freund's adjuvant.<sup>44</sup> This result demonstrates that this adjuvant is fully dispensable for  
administration of our AuNP-based vaccine candidate, which are therefore self-adjuvating



in their own right. Non-negligible IgM titers were also observed (although these were significantly lower than IgG titers), which suggests that glycopeptide **12** on AuNPs can induce class-switch recombination even without the use of a “universal” CD4 T-cell peptide. Next, the antibody isotypes in the antisera were evaluated. IgG1 was the predominant antibody in all antisera (Figure 5C), which suggests that Th2 type immune responses were predominantly induced by **AuNP-12** in these mice. Finally, IgG2a, IgG2b and carbohydrate-related IgG3 antibodies<sup>45</sup> were detected in all animals albeit weakly.

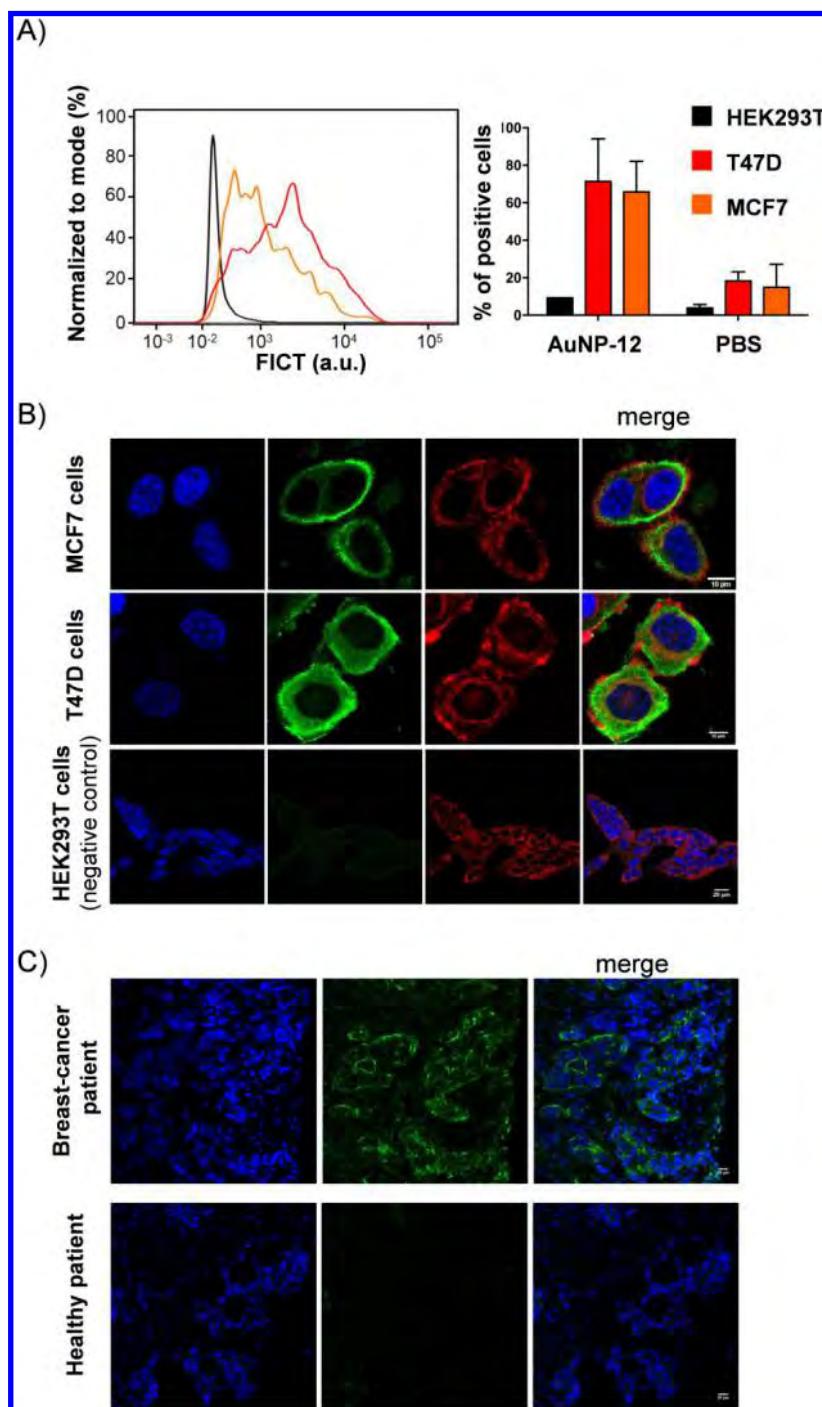


**Figure 5.** (A) Schematic representation of the vaccine candidate containing the engineered MUC1-like glycopeptide **12** attached to the surface of gold nanoparticles (**AuNP-12**). (B) Agarose gel electrophoresis of **AuNP-linker** (loaded with the SM(PEG)<sub>2</sub>-linker. See Supporting Information) and **AuNP-12**. (C) Total and sub-typing (IgG1, IgG2a/b, IgG3 and IgM) anti-MUC1 antibodies after immunizing mice (n=5) with **AuNP-12**. The ELISA plates were coated with glycopeptide **12** conjugated to bovine serum

1  
2  
3 albumin. Horizontal lines indicate the mean for the group of 5 mice. Asterisks indicate  
4 statistically significant difference ( $*** P < 0.005$ ,  $** P < 0.01$ ,  $* P < 0.05$ ) and NS indicates  
5 no significant difference.  
6  
7

8  
9 To confirm that the elicited antibodies were able to recognize selectively the native  
10 tumor-associated MUC1 antigen on human cancer cells, two human cancer cell lines  
11 (MCF-7, T47D) and the human embryonic kidney cell line (HEK293T) were stained with  
12 the mice antisera and analyzed by flow cytometry (Figure 6A). Indeed, the antisera  
13 reacted strongly with MCF-7 and T47D cells, which express tumor-associated MUC1 on  
14 their surface. Conversely, negligible low binding was observed for HEK293T cells, which  
15 is consistent with the lack of MUC1 on their surface. These results are in good agreement  
16 with those obtained from confocal microscopy (Figure 6B) that show the presence of the  
17 MUC1 antigen on the surface of MCF-7 and T47D cells (green color), but not on  
18 HEK293T cells. Notably, the antisera also positively stained cancer cells from biopsies  
19 of breast-cancer patients (right panel in Figure 6C and Figure S19), but no staining is  
20 observed in case of cells from healthy patients. Thus, these results demonstrate the  
21 antigen mimic potential of unnatural glycopeptide **12**.  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60





**Figure 6.** (A) Staining of living cells with the antisera of mice immunized with **AuNP-12** analyzed by flow cytometry: HEK293T (black line), MCF7 (orange line) and T47D (red line). Staining with 1:100 dilution of sera and visualization with a mouse secondary  $\alpha$ -IgG-488 antibody. (B) Confocal microscopy images show that mice antisera after vaccination with **AuNP-12** do not stain HEK293T cells as expected because these cells do not express tumor-associated MUC1 on their surface. On the contrary, breast cancer cells MCF7 and T47D expressing tumor-associated MUC1 are positively stained by mice antisera. Blue = Hoechst (nuclei); Green = secondary anti-mouse IgG Alexa 488 (tumor-associated MUC1); Red = CellMask Deep Red (membrane dye). (C) The antisera of

1  
2  
3 mice vaccinated with **AuNP-12**, positively stain tissue biopsies from breast-cancer  
4 patients. Blue = Hoechst (nuclei); Green = secondary anti-mouse IgG Alexa 488 (tumor-  
5 associated MUC1).  
6  
7

## 8 9 **Conclusions**

10  
11 Our experimental evidence strongly suggests that it is possible to fine-tune the  
12 conformational preferences of GalNAc-containing glycopeptides in solution by  
13 employing a simple oxygen-for-sulfur or oxygen-for-selenium substitution at the  
14 glycosidic linkage. These simple chemical modifications have a significant structural  
15 impact allowing the peptide backbone to adopt a preorganized structure that is optimally  
16 suited for antibody binding, as confirmed by the improved binding affinity to a model anti-  
17 MUC1 antibody. Additionally, the potential of a dually modified glycopeptide (fPro and  
18 SThr) as tumor-associated MUC1 antigen mimic has been demonstrated *in vivo*.  
19 Significantly, the antisera of mice vaccinated with **AuNP-12** recognize cancer cells with  
20 high selectivity in biopsies of breast-cancer patients. This result confirms that the  
21 antibodies generated against the engineered antigen are able to recognize the naturally  
22 occurring antigen in its physiological context. Finally, we envision the strategy presented  
23 here to be of general interest because it may be applied to modulate the affinity of  
24 biologically relevant glycopeptides towards their receptors.  
25  
26  
27  
28  
29  
30  
31  
32  
33

## 34 **Acknowledgements**

35  
36 We thank the *Ministerio de Economía y Competitividad* (projects CTQ2015-67727-R,  
37 CTQ2013-44367-C2-2-P, CTQ2015-64597-C2-1P, CTQ2017-89750-R, CTQ2017-  
38 90088-R, CTQ2015-70524-R, and BFU2016-75633-P) and the Italian Ministry of  
39 Education, University and Research (MIUR) (PRIN 2015 contract nr. 2015RNWJAM).  
40 I.C. thanks Universidad de La Rioja for a FPI grant. A.G. and G.J.L.B. thank FCT  
41 Portugal (PhD studentship, SFRH/BD/115932/2016, and FCT Investigator  
42 IF/00624/2015, respectively). G.J.L.B. holds a Royal Society URF (UF110046 and  
43 URF\R\180019) and an ERC StG (TagIt, GA No. 676832). F.C. and G. J. L. B thank the  
44 EU (Marie-Sklodowska Curie ITN, Protein Conjugates, GA No. 675007). O.B. and G.J.-  
45 O. are Ramón y Cajal Fellows (RYC-2015-17705 and RYC-2013-14706, respectively).  
46 R.H-G. thanks *Agencia Aragonesa para la Investigación y Desarrollo* (ARAID) and the  
47 *Diputación General de Aragón* (DGA, E34\_R17) for financial support. The research that  
48 led to these results also attracted funding from the FP7 (2007-2013) under BioStruct-X  
49 (grant agreement N°283570 and BIOSTRUCTX\_5186). We also thank the ALBA  
50 synchrotron radiation source; in particular the beamline XALOC facility. We thank  
51 Emanuele Papini and Regina Tavano (University of Padua, Italy) for insightful  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 discussions about the immunogenicity of glycopeptide-functionalized AuNPs, and Vito  
4 Maggi and Valentina Rosato (IIT-Lecce) for expert technical assistance. Computational  
5 resources of CESGA (Santiago de Compostela) and BERONIA (Universidad de La  
6 Rioja) were used in this work. The authors thank Vikki Cantrill for her help with the  
7 preparation and editing of this manuscript.  
8  
9  
10

### 11 12 **Competing financial interests**

13  
14 The authors declare no competing financial interests.  
15  
16

### 17 **Supporting Information**

18  
19 Synthesis and characterization of the glycopeptides **2\*** and **3\*** and the AuNP-based  
20 vaccine, conformational analysis in solution of glycopeptides **2\*** and **3\***, details of the X-  
21 ray structure of **2\*** and **3\*** bound to scFv-SM3, SPR curves and response-concentration  
22 fit obtained for the binding of the (glyco)peptides, cartesian coordinates, electronic  
23 energies, Gibbs free energies and lowest frequencies of the DFT calculated structures,  
24 additional molecular dynamics simulations figures, immunization protocol, antibodies  
25 titers and antibody isotypes, antibodies reactivity towards human cancer cell lines  
26 determined by flow cytometry analysis and analyzed by confocal microscopy, and  
27 studies on cancer cell from breast-cancer patients. This material is available free of  
28 charge via the Internet at <http://pubs.acs.org>  
29  
30  
31  
32  
33  
34  
35

### 36 **References**

- 37  
38 (1) Hollingsworth, M. A.; Swanson, B. J. Mucins in Cancer: Protection and Control  
39 of the Cell Surface. *Nat. Rev. Cancer* **2004**, *4*, 45–60.  
40  
41 (2) Kufe, D. W. Mucins in Cancer: Function, Prognosis and Therapy. *Nat. Rev.*  
42 *Cancer* **2009**, *9*, 874–885.  
43  
44 (3) Senapati, S.; Das, S.; Batra, S. K. Mucin-Interacting Proteins: From Function to  
45 Therapeutics. *Trends Biochem. Sci.* **2010**, *35*, 236–245.  
46  
47 (4) Taylor-Papadimitriou, J.; Burchell, J. M. *Mucins and Cancer*, Taylor-  
48 Papadimitriou, J., Burchell, J. M., Eds.; Future Medicine: London, 2013.  
49  
50 (5) Ju, T.; Otto, V. I.; Cummings, R. D. The Tn Antigen-Structural Simplicity and  
51 Biological Complexity. *Angew. Chem. Int. Ed.* **2011**, *50*, 1770–1791.  
52  
53 (6) Schuman, J.; Campbell, A. P.; Koganty, R. R.; Longenecker, B. M. Probing the  
54 Conformational and Dynamical Effects of O-Glycosylation Within the  
55 Immunodominant Region of a MUC1 Peptide Tumor Antigen. *J. Pept. Res.*  
56 **2003**, *61*, 91–108.  
57  
58  
59  
60

- 1  
2  
3 (7) Karsten, U.; Serttas, N.; Paulsen, H.; Danielczyk, A.; Goletz, S. Binding Patterns  
4 of DTR-Specific Antibodies Reveal a Glycosylation-Conditioned Tumor-Specific  
5 Epitope of the Epithelial Mucin (MUC1). *Glycobiology* **2004**, *14*, 681–692.  
6  
7 (8) Wilson, R. M.; Danishefsky, S. J. A Vision for Vaccines Built from Fully Synthetic  
8 Tumor-Associated Antigens: From the Laboratory to the Clinic. *J. Am. Chem.*  
9 *Soc.* **2013**, *135*, 14462–14472.  
10  
11 (9) Buskas, T.; Thompson, P.; Boons, G.-J. Immunotherapy for Cancer: Synthetic  
12 Carbohydrate-Based Vaccines. *Chem. Commun.* **2009**, *105*, 5335–5349.  
13  
14 (10) Martínez-Sáez, N.; Peregrina, J. M.; Corzana, F. Principles of Mucin Structure:  
15 Implications for the Rational Design of Cancer Vaccines Derived from MUC1-  
16 Glycopeptides. *Chem. Soc. Rev.* **2017**, *46*, 7154–7175.  
17  
18 (11) Gaidzik, N.; Westerlind, U.; Kunz, H. The Development of Synthetic Antitumour  
19 Vaccines from Mucin Glycopeptide Antigens. *Chem. Soc. Rev.* **2013**, *42*, 4421–  
20 4442.  
21  
22 (12) Hossain, M. K.; Wall, K. A. Immunological Evaluation of Recent MUC1  
23 Glycopeptide Cancer Vaccines. *Vaccines* **2016**, *4*, 25–13.  
24  
25 (13) Somovilla, V. J.; Bermejo, I. A.; Albuquerque, I. S.; Martínez-Sáez, N.; Castro-  
26 López, J.; Garcia-Martin, F.; Compañón, I.; Hinou, H.; Nishimura, S.-I.; Jiménez-  
27 Barbero, J.; Asensio, J. L.; Avenoza, A.; Busto, J. H.; Hurtado-Guerrero, R.;  
28 Peregrina, J. M.; Bernardes, G. J. L.; Corzana, F. The Use of Fluoroproline in  
29 MUC1 Antigen Enables Efficient Detection of Antibodies in Patients with  
30 Prostate Cancer. *J. Am. Chem. Soc.* **2017**, *139*, 18255–18261.  
31  
32 (14) Coltart, D. M.; Royyuru, A. K.; Williams, L. J.; Glunz, P. W.; Sames, D.; Kuduk,  
33 S. D.; Schwarz, J. B.; Chen, X.-T.; Danishefsky, S. J.; Live, D. H. Principles of  
34 Mucin Architecture: Structural Studies on Synthetic Glycopeptides Bearing  
35 Clustered Mono-, Di-, Tri-, and Hexasaccharide Glycodomains. *J. Am. Chem.*  
36 *Soc.* **2002**, *124*, 9833–9844.  
37  
38 (15) Corzana, F.; Busto, J. H.; Jiménez-Oses, G.; Asensio, J. L.; Jiménez-Barbero,  
39 J.; Peregrina, J. M.; Avenoza, A. New Insights into  $\alpha$ -GalNAc-Ser  
40 Motif: Influence of Hydrogen Bonding *versus* Solvent Interactions on the  
41 Preferred Conformation. *J. Am. Chem. Soc.* **2006**, *128*, 14640–14648.  
42  
43 (16) Corzana, F.; Busto, J. H.; Jiménez-Oses, G.; García de Luis, M.; Asensio, J. L.;  
44 Jiménez-Barbero, J.; Peregrina, J. M.; Avenoza, A. Serine Versus Threonine  
45 Glycosylation: The Methyl Group Causes a Drastic Alteration on the  
46 Carbohydrate Orientation and on the Surrounding Water Shell. *J. Am. Chem.*  
47 *Soc.* **2007**, *129*, 9458–9467.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 (17) Bermejo, I. A.; Usabiaga, I.; Compañón, I.; Castro-López, J.; Insausti, A.;  
4 Fernández, J. A.; Avenoz, A.; Busto, J. H.; Jiménez-Barbero, J.; Asensio, J. L.;  
5 Peregrina, J. M.; Jiménez-Oses, G.; Hurtado-Guerrero, R.; Cocinero, E. J.;  
6 Corzana, F. Water Sculpts the Distinctive Shapes and Dynamics of the Tumor-  
7 Associated Carbohydrate Tn Antigens: Implications for Their Molecular  
8 Recognition. *J. Am. Chem. Soc.* **2018**, *140*, 9952–9960.  
9  
10  
11  
12 (18) Burchell, J.; Gendler, S.; Taylor-Papadimitriou, J.; Girling, A.; Lewis, A.; Millis,  
13 R.; Lampion, D. Development and Characterization of Breast Cancer Reactive  
14 Monoclonal Antibodies Directed to the Core Protein of the Human Milk Mucin.  
15 *Cancer Res.* **1987**, *47*, 5476–5482.  
16  
17  
18 (19) Martínez-Sáez, N.; Castro-López, J.; Valero-González, J.; Madariaga, D.;  
19 Compañón, I.; Somovilla, V. J.; Salvadó, M.; Asensio, J. L.; Jiménez-Barbero,  
20 J.; Avenoz, A.; Busto, J. H.; Bernardes, G. J. L.; Peregrina, J. M.; Hurtado-  
21 Guerrero, R.; Corzana, F. Deciphering the Non-Equivalence of Serine and  
22 Threonine O-Glycosylation Points: Implications for Molecular Recognition of the  
23 Tn Antigen by an anti-MUC1 Antibody. *Angew. Chem. Int. Ed.* **2015**, *54*, 9830–  
24 9834.  
25  
26  
27 (20) Asensio, J. L.; Ardá, A.; Cañada, F. J.; Jiménez-Barbero, J. Carbohydrate-  
28 Aromatic Interactions. *Acc. Chem. Res.* **2013**, *46*, 946–954.  
29  
30  
31 (21) Jiménez-Moreno, E.; Gómez, A. M.; Bastida, A.; Corzana, F.; Jiménez-Oses,  
32 G.; Jiménez-Barbero, J.; Asensio, J. L. Modulating Weak Interactions for  
33 Molecular Recognition: A Dynamic Combinatorial Analysis for Assessing the  
34 Contribution of Electrostatics to the Stability of CH/π Bonds in Water. *Angew.*  
35 *Chem. Int. Ed.* **2015**, *54*, 4344–4348.  
36  
37  
38 (22) García-Herrero, A.; Montero, E.; Muñoz, J. L.; Espinosa, J. F.; Vián, A.; García,  
39 J. L.; Asensio, J. L.; Cañada, F. J.; Jiménez-Barbero, J. Conformational  
40 Selection of Glycomimetics at Enzyme Catalytic Sites: Experimental  
41 Demonstration of the Binding of Distinct High-Energy Distorted Conformations  
42 of C-, S-, and O-Glycosides by E. Coli β-Galactosidases. *J. Am. Chem. Soc.*  
43 **2002**, *124*, 4804–4810.  
44  
45  
46 (23) Dziadek, S.; Griesinger, C.; Kunz, H.; Reinscheid, U. M. Synthesis and  
47 Structural Model of an α(2,6)-Sialyl-T Glycosylated MUC1 Eicosapeptide under  
48 Physiological Conditions. *Chem. Eur. J.* **2006**, *12*, 4981–4993.  
49  
50  
51 (24) Gracia-Vitoria, J.; Osante, I.; Cativiela, C. Stereoselective Synthesis of Modified  
52 Cysteines. *Tetrahedron: Asymmetry* **2017**, *28*, 215–245.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 (25) Chen, S.; Fahmi, N. E.; Nangreave, R. C.; Mehellou, Y.; Hecht, S. M. Synthesis  
4 of pdCpAs and Transfer RNAs Activated with Thioreonine and Derivatives.  
5 *Bioorg. Med. Chem.* **2012**, *20*, 2679–2689.  
6  
7 (26) Narayan, R. S.; VanNieuwenhze, M. S. Versatile and Stereoselective Syntheses  
8 of Orthogonally Protected  $\beta$ -Methylcysteine and  $\beta$ -Methylanthionine. *Org. Lett.*  
9 **2005**, *7*, 2655–2658.  
10  
11 (27) Jobron, L.; Hummel, G. Solid-Phase Synthesis of New S-Glycoamino Acid  
12 Building Blocks. *Org. Lett.* **2000**, *2*, 2265–2267.  
13  
14 (28) Dyson, H. J.; Wright, P. E. Defining Solution Conformations of Small Linear  
15 Peptides. *Annu. Rev. Biophys. Chem.* **1991**, *20*, 519–538.  
16  
17 (29) Pearlman, D. A.; Kollman, P. A. Are Time-Averaged Restraints Necessary for  
18 Nuclear Magnetic Resonance Refinement? *J. Mol. Biol.* **1991**, *220*, 457–479.  
19  
20 (30) Pearlman, D. How Is an NMR Structure Best Defined? An Analysis of Molecular  
21 Dynamics Distance-Based Approaches. *J. Biomol. NMR* **1994**, *4*, 1–16.  
22  
23 (31) Fernández-Tejada, A.; Corzana, F.; Busto, J. H.; Jiménez-Osés, G.; Jiménez-  
24 Barbero, J.; Avenoza, A.; Peregrina, J. M. Insights into the Geometrical Features  
25 Underlying  $\beta$ -O-GlcNAc Glycosylation: Water Pockets Drastically Modulate the  
26 Interactions Between the Carbohydrate and the Peptide Backbone. *Chem. Eur.*  
27 *J.* **2009**, *15*, 7297–7301.  
28  
29 (32) Madariaga, D.; Martínez-Sáez, N.; Somovilla, V. J.; Coelho, H.; Valero-  
30 González, J.; Castro-López, J.; Asensio, J. L.; Jiménez-Barbero, J.; Busto, J. H.;  
31 Avenoza, A.; Marcelo, F.; Hurtado-Guerrero, R.; Corzana, F.; Peregrina, J. M.  
32 Detection of Tumor-Associated Glycopeptides by Lectins: the Peptide Context  
33 Modulates Carbohydrate Recognition. *ACS Chem. Biol.* **2015**, *10*, 747–756.  
34  
35 (33) Mazeau, K.; Tvaroska, I. PCILO Quantum-Mechanical Relaxed Conformational  
36 Energy Map of Methyl 4-Thio- $\alpha$ -Maltoside in Solution. *Carbohydr. Res.* **1992**,  
37 *225*, 27–41.  
38  
39 (34) Aydillo, C.; Compañón, I.; Avenoza, A.; Busto, J. H.; Corzana, F.; Peregrina, J.  
40 M.; Zurbano, M. M. S-Michael Additions to Chiral Dehydroalanines as an Entry  
41 to Glycosylated Cysteines and a Sulfa-Tn Antigen Mimic. *J. Am. Chem. Soc.*  
42 **2014**, *136*, 789–800.  
43  
44 (35) Lakshminarayanan, V.; Thompson, P.; Wolfert, M. A.; Buskas, T.; Bradley, J. M.;  
45 Pathangey, L. B.; Madsen, C. S.; Cohen, P. A.; Gendler, S. J.; Boons, G.-J.  
46 Immune Recognition of Tumor-Associated Mucin MUC1 Is Achieved by a Fully  
47 Synthetic Aberrantly Glycosylated MUC1 Tripartite Vaccine. *Proc. Natl. Acad.*  
48 *Sci. U.S.A.* **2012**, *109*, 261–266.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 (36) Lakshminarayanan, V.; Supekar, N. T.; Wei, J.; McCurry, D. B.; Dueck, A. C.;  
4 Kosiorek, H. E.; Trivedi, P. P.; Bradley, J. M.; Madsen, C. S.; Pathangey, L. B.;  
5 Hoelzinger, D. B.; Wolfert, M. A.; Boons, G.-J.; Cohen, P. A.; Gendler, S. J.  
6 MUC1 Vaccines, Comprised of Glycosylated or Non-Glycosylated Peptides or  
7 Tumor-Derived MUC1, Can Circumvent Immunoediting to Control Tumor  
8 Growth in MUC1 Transgenic Mice. *PLoS ONE* **2016**, *11*, e0145920.  
9  
10  
11  
12 (37) Wu, X.; Yin, Z.; McKay, C.; Pett, C.; Yu, J.; Schorlemer, M.; Gohl, T.;  
13 Sungsuwan, S.; Ramadan, S.; Baniel, C.; Allmon, A.; Das, R.; Westerlind, U.;  
14 Finn, M. G.; Huang, X. Protective Epitope Discovery and Design of MUC1-Based  
15 Vaccine for Effective Tumor Protections in Immunotolerant Mice. *J. Am. Chem.*  
16 *Soc.* **2018**, *140*, 16596–16609.  
17  
18  
19  
20 (38) Wolfert, M. A.; Boons, G.-J. Adaptive Immune Activation: Glycosylation Does  
21 Matter. *Nat. Chem. Biol.* **2013**, *9*, 776–784.  
22  
23  
24 (39) Richichi, B.; Thomas, B.; Fiore, M.; Bosco, R.; Qureshi, H.; Nativi, C.; Renaudet,  
25 O.; BenMohamed, L. A Cancer Therapeutic Vaccine Based on Clustered Tn-  
26 Antigen Mimetics Induces Strong Antibody-Mediated Protective Immunity.  
27 *Angew. Chem. Int. Ed.* **2014**, *53*, 11917–11920.  
28  
29  
30 (40) Nativi, C.; Renaudet, O. Recent Progress in Antitumoral Synthetic Vaccines.  
31 *ACS Med. Chem. Lett.* **2014**, *5*, 1176–1178.  
32  
33 (41) Rich, J. R.; Bundle, D. R. S-Linked Ganglioside Analogues for Use in Conjugate  
34 Vaccines. *Org. Lett.* **2004**, *6*, 897–900.  
35  
36 (42) Huo, C.-X.; Zheng, X.-J.; Xiao, A.; Liu, C.-C.; Sun, S.; Lv, Z.; Ye, X.-S. Synthetic  
37 and Immunological Studies of N-Acyl Modified S-Linked STn Derivatives as  
38 Anticancer Vaccine Candidates. *Org. Biomol. Chem.* **2015**, *13* (12), 3677–3690.  
39  
40 (43) Bousquet, E.; Spadaro, A.; Pappalardo, M. S.; Bernardini, R.; Romeo, R.;  
41 Panza, L.; Ronsisvalle, G. Synthesis and Immunostimulating Activity of a  
42 Thioglycolipopeptide Glycomimetic as a Potential Anticancer Vaccine Derived  
43 From Tn Antigen. *J. Carbohydr. Chem.* **2000**, *19*, 527–541.  
44  
45 (44) Cai, H.; Degliangeli, F.; Palitzsch, B.; Gerlitzki, B.; Kunz, H.; Schmitt, E.;  
46 Fiammengo, R.; Westerlind, U. Glycopeptide-Functionalized Gold Nanoparticles  
47 for Antibody Induction Against the Tumor Associated Mucin-1 Glycoprotein.  
48 *Bioorg. Med. Chem.* **2016**, *24*, 1132–1135.  
49  
50 (45) Haji-Ghassemi, O.; Blackler, R. J.; Martin Young, N.; Evans, S. V. Antibody  
51 Recognition of Carbohydrate Epitopes. *Glycobiology* **2015**, *25*, 920–952.  
52  
53  
54  
55  
56  
57  
58  
59  
60



## TOC

