Synthesis of MUC1-derived glycopeptide as potential antitumor vaccine

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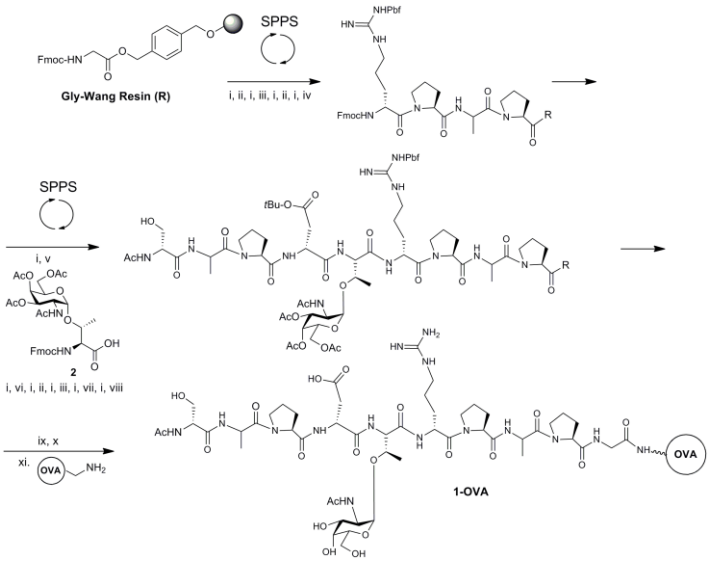
Introduction

Mucins are highly glycosylated *O*-glycoproteins, containing typical tandem repeat motifs rich in Ser and Thr, which represent potential sites for *O*-glycosylation. In tumor-related mucins the glycosylation process is deregulated, resulting in abnormal glycans, where the overexpression of αGalNAc-Ser/Thr (Tn antigen) is a common feature.[1] Among different classes of tumor mucins, MUC1 is one of the most extensively studied, being constituted by a variable number (25–125) of tandem repeat sequences (HGVTSAPDTRPAPGSTAPPA) with five potential O-glycosylation (*O*-αGalNAc) sites.[2] Due to its functional properties of adhesion, invasion and metastasis, besides association to several types of cancer, such as breast, prostate and lung, it can be considered a valuable target for immunotherapy against cancer. Therefore, this work presents the synthesis of the MUC1-derived glycopeptide NHAc-Ser-Ala-Pro-Asp-[αGalNAc]-Thr-Arg-Pro-Ala-Pro-Gly-OVA **1** and its biological evaluation as an antitumor vaccine.

Results and Discussion

The synthesis of glycopeptide **1** in solid phase (SPPS-Gly-Wang resin) was performed by sequential coupling reactions of the amino acids FmocProOH, FmocAlaOH, FmocArg(Pbf)OH, FmocThrOH, FmocAsp(tBu)OH and FmocSerOH, and the glycosyl-amino acid αGalNAc-ThrOH (Tn) **2**, in the presence of the coupling reactants benzotriazol-1-yloxytris-pyrrolidino phosphonium hexafluorophosphate (PyBOP) and 1-hydroxybenzotriazole (HOBt), and the base diisopropylethylamine (DIPEA) in DMF,[2] as outlined in Scheme 1. After cleavage from the resin with aqueous TFA, followed by *N*-acetylation (Py and Ac_2O) and *O*-deacetylation (NaOMe) reactions, glycopeptide **1** was purified by gel filtration chromatography, being obtained in the overall yield of 30%. Subsequently, glycopeptide **1** was conjugated to the carrier protein ovalbumin (OVA) and then submitted to immunization assays in murine models. For the detection of glycopeptide **1**-OVA specific antibodies, ELISA assays were performed utilizing increasingly diluted sera resultant from 4 immunizations, being verified the satisfactory titer of 16000.



Scheme 1. Solid-phase synthesis of glycopeptide **1**. i. 20% piperidine-DMF; ii. FmocProOH, PyBOP, HOBt, DIPEA, 3h; iii. FmocAlaOH; iv. FmocArg(Pbf)OH, v. αGalNAc-ThrOH **2**, 48h, vi. FmocAsp(tBu)OH, vii. FmocSerOH, viii. Py, Ac_2O, ix. TFA 80%, x. NaOMe, MeOH, xi. EDCI, NHS, OVA.

Conclusions

Glycopeptide **1** was obtained in reasonable yield by combination of *in solution* and solid phase synthesis, and according to the results obtained by ELISA, show potential for development of an antitumor vaccine

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[1] Buscaglia, C. A.; Campo, V. A.; Frasch, A. C. C.; DiNoia, J. M. *Nat. Rev. Microbiol.* **2006**, *4*, 229.
[2] Campo, V. L; Riul, T. B.; Carvalho, I.; Baruffi, M-D. *ChemBioChem* **2014**, *15*, 1495.