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Antitumor Agents

# A Fully Synthetic Glycopeptide Antitumor Vaccine Based on Multiple Antigen Presentation on a Hyperbranched Polymer

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*Dedicated to Professor Hans-Ulrich Reißig on the occasion of his 65th birthday*

**Abstract:** For antitumor vaccines both the selected tumor-associated antigen, as well as the mode of its presentation, affect the immune response. According to the principle of multiple antigen presentation, a tumor-associated MUC1 glycopeptide combined with the immunostimulating T-cell epitope P2 from tetanus toxoid was coupled to a multi-functionalized hyperbranched polyglycerol by "click chemistry". This globular polymeric carrier has a flexible dendrimer-like structure, which allows optimal antigen presentation to the immune system. The resulting fully synthetic vaccine induced strong immune responses in mice and IgG antibodies recognizing human breast-cancer cells.

It appears particularly attractive for a tumor therapy to induce an immune response in a patient which is directed against his own tumor tissues.[1] The selectivity and efficiency of an antitumor vaccine and the quality of the induced immune response do not only depend on the selected tumor-associated antigen structure, but also on the particular presentation of the antigen to the immune system, which should be as similar to the situation on the cell surface as possible. From this point of view terminal exposition of the antigens on carriers with dendritic architecture are considered particularly promising. The tumor-associated mucin MUC1 has been identified as a promising target molecule for the development of antitumor vaccines.[2] It is expressed on most epithelial tissues and over-expressed on the corresponding tumor tissues.[3] Due to changed activities of glycosyltransferases in tumor cells, tumor-associated MUC1 shows markedly altered glycosylation profiles of prevailingly truncated glycans within the tandem repeat domains of the sequence PAHGVTSAPDTRPAPGSTAP.[1,4] As a consequence, tumor-associated MUC1 exposes peptide backbone epitopes in combination with the aberrant tumor-associated carbohydrate antigens (TACAs), which should be qualified for the development of a cancer immunotherapy.[2]

However, immunization using MUC1 sequences as sole vaccine component (B-cell epitope) failed to induce adequate immune responses because these endogenous structures are highly tolerated by the immune system. One approach to enhance the immunogenicity of these MUC1 antigens and thereby overcome the immune self-tolerance consists of covalent conjugation of synthetic MUC1 glycopeptides to immunostimulating carrier proteins, for example, tetanus toxoid (TTox).[5] This highly immunogenic protein contains numerous T-cell epitopes. In addition, it provides multivalent presentation of antigens promoting enhanced immunogenicity.[5] Such protein-based two-component vaccines can elicit strong humoral immune responses in mice affording very high titres of antibodies, which strongly bind to human cancer cells.[5] Although these protein-based vaccines are most promising candidates for antitumor vaccination, they also induce immune reactions against epitopes of the carrier protein. Unfortunately, this might suppress the desired maximum immune response towards the MUC1 glycopeptide.[6] In order to circumvent the induction of such undesired antibodies, fully synthetic two- and three-component vaccines containing a MUC1 glycopeptide as B-cell epitope and a single T-cell epitope were prepared without the use of carrier proteins.[7] Some of these fully synthetic vaccines elicited significant immune reactions in mice, in particular those containing Toll-like receptor 2 ligand structures as additional immune-stimulating components.[7b–d,f] For vaccines consisting of a combination of the MUC1 glycopeptide antigen with a single T-cell epitope peptide, the quality of the immune response distinctly depends upon the properties of the T-cell peptide. Of the T-cell epitope peptides P30 and P2, both derived from tetanus toxoid and described as universal murine and humane T-helper cell epitopes,[8] the longer P30 epitope caused a strong immune reaction,[7e] whereas the shorter amino acid sequence P2 could not entail a sufficient immune response. This may be due to the missing ability of P2 to self-assembly and, thus, to present antigens in a multivalent form;[7e] this difference sheds light on the importance of the above-mentioned presentation of the antigen.

Therefore, it is considered attractive to apply the strategy introduced by J. P. Tam[9] for enhancing the immunogenicity of

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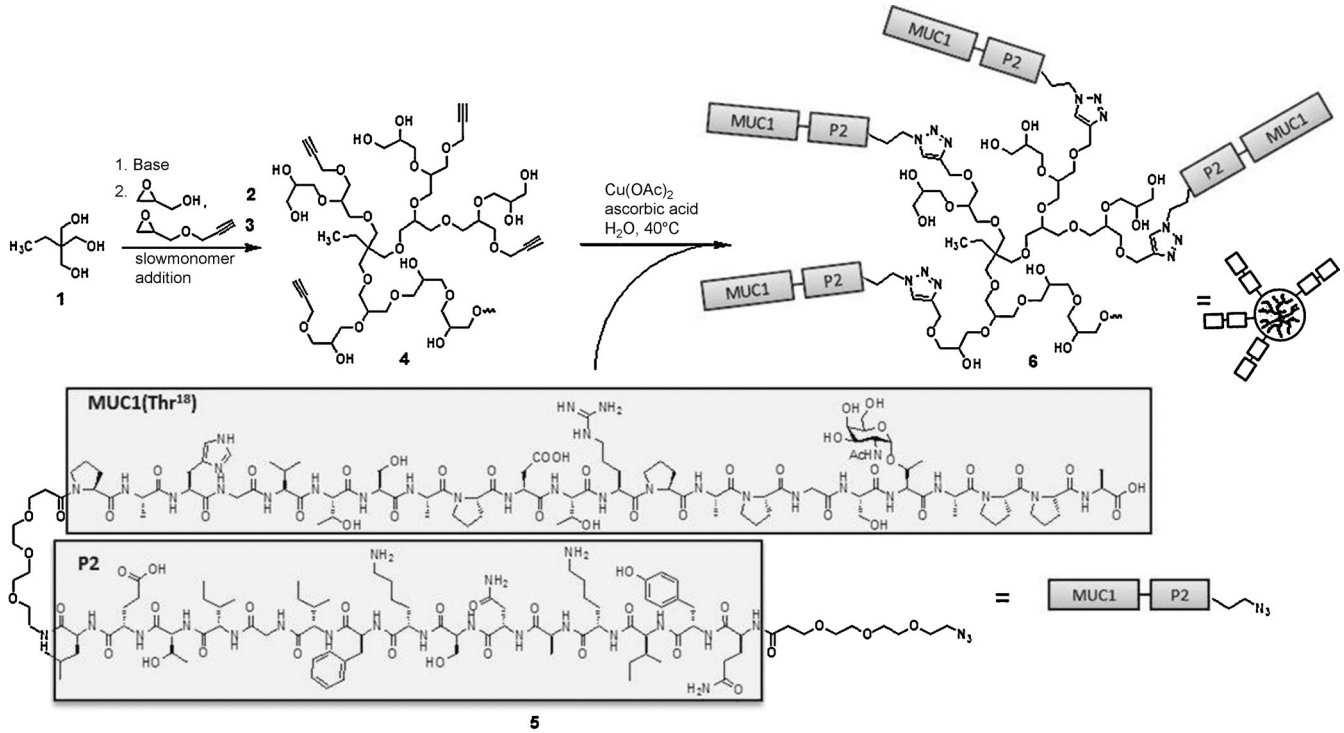
MUC1 glycopeptide antigens. In this strategy, peptide epitopes were presented in a multiple form on a single branched oligolysine core.[9] The thus achieved antigen multivalency provides an opportunity for efficient clustering of B-cell Ig receptors and stronger avidity between antigens and Ig receptors.[10] This form of antigen presentation may facilitate vaccine uptake by B-cells as antigen presenting cells (APCs) leading to enhanced immune responses.

Taking into account both concepts (multiple antigen presentation and defined T-helper cell epitopes), we recently designed a fully synthetic polymer-based vaccine with separated MUC1 and P2 T-cell epitopes orthogonally coupled to a multistep-synthesized poly(*N*-(2-hydroxypropyl)methacrylamide) P(HPMA) as a linear non-immunogenic carrier.[11]

In the present work, a new concept is described to combine fully synthetic linear MUC1 glycopeptide–P2 T-cell epitopes in a multivalent format by coupling to a globular, water-soluble and readily available polymer. Multi-alkyne-functionalized hyperbranched polyglycerol (*hb*PG)[12] was chosen, which is accessible in a one-step copolymerization of glycidol **2** and its propargyl ether **3** (ratio 28:5), in order to achieve, for the first time, conjugation of glycopeptides to this polymer. Regarding the structural similarity to the widely used and well-studied poly(ethylene glycol), polyglycerols are considered biocompatible and non-immunogenic and, therefore, suitable as inert carriers for biomedical applications.[13] The highly branched macromolecule of globular and highly functional dendrimer-like structure provides ample space on its "surface" for multivalent presentation of relevant compounds, as for example, antigens. In contrast to epitope coupling to the linear P(HPMA),[11] the coupling to hyperbranched polyglycerol as the carrier prevents an entanglement of the vaccine. The nano-sized dendrimeric particles provide a presentation of the glycopeptide on the surface and, thus, ensure a better accessibility of the bound antigens to the immune system. Furthermore, the polydiversity of the dendritic macromolecules reflects the multiple arrangements on a cell surface. Consequently, these dendrimer-type polymer vaccines appear more cell surface-like than all vaccines based on linear polymers. In addition, any mutually hindering interactions of covalently bound antigens are reduced by the flexibility of the hydrophilic polyether arms allowing for an optimal exposure of each antigen to the immune system. Due to the hydrophilic properties of *hb*PG, this core also enhances the solubility of synthetic vaccines in aqueous environment. The attachment of the antigens was conveniently realized by $Cu^{I}$-catalyzed Huisgen cycloaddition ("click chemistry") between the MUC1–P2 conjugate carrying an azido-terminated triethyleneglycol acyl spacer[14] and the alkyne-functionalized *hb*PG-polymers. The numbers of linked antigens can be adjusted in this one-step reaction (Scheme 1).[12]

A section of the tandem repeat domain from MUC1 including the immunodominant motifs PDTRP and GSTA was chosen as the B-cell epitope, as recent studies have revealed that anti-MUC1 antibodies bind to these motifs preferentially.[15,16] Since glycosylation influences the conformation of the MUC1 sequence and is important for the tumor selectivity of the induced antibodies,[16,17] a Tn antigen was linked to threonine-18 within the GSTA region. There are three proline residues adjacent to this region of MUC1, which have significant conformational influence[17b,18] and consequently, may affect the binding




**Scheme 1.** One-step synthesis of poly(glycerol-*co*-propargyl glycidyl ether) (*hb*P(G-GPE)) **4** and subsequent coupling of MUC1–P2 glycopeptide **5** by copper-catalyzed azide–alkyne cycloaddition to the hyperbranched polyglycerol-based antitumor vaccine **6**.

affinity of the antibodies to the GSTA region. Hence, the 20 amino acid MUC1 tandem repeat sequence was extended by two further amino acids (proline and alanine) resulting in the 22-mer peptide B-cell epitope PAHGVTSAPDTRPAPGSTAPPA.[19] As second component for the vaccine design, the T-cell epitope P2 (QYIKANSKFIGITEL)[8] of tetanus toxoid was coupled to the MUC1 B-cell epitope. In order to minimize mutual conformational distortion, an immunogenically silent oligoethyleneglycol-spacer was inserted between the components.[14]

The conjugation of the MUC1 glycopeptide with the defined T-cell epitope can directly be integrated into antigen assembly by solid-phase (glyco)peptide synthesis (SPPS). To this end, the MUC1 glycopeptide, the T-cell epitope P2 and the two spacers were prepared in a linear solid-phase synthesis according to the established 9-fluorenylmethoxycarbonyl (Fmoc) protocol[5] starting from a TentaGel resin preloaded with a trityl-anchored[20] Fmoc-protected alanine. Couplings of the spacers as well as that of the Tn antigen threonine were carried out under modified conditions (see the Supporting Information). The completed MUC1–P2 peptide was detached from resin with concomitant removal of all amino acid protecting groups using trifluoroacetic acid (TFA), triisopropylsilane (TIS) and water to afford the glycopeptide–peptide conjugate after purification by semipreparative HPLC. Subsequently, the *O*-acetyl groups of the carbohydrate were removed using sodium methoxide/methanol at pH $<10$ to avoid β-elimination. The completely deprotected glycopeptide **5** was isolated after additional purification by HPLC in an overall yield of 11%.

To obtain the vaccine, conjugation of MUC1–P2 **5** with the polymeric carrier *hb*PG **4** was performed by copper-catalyzed azide–alkyne click-type cycloaddition (Scheme 1).[21] To this end, an multi-alkyne functionalized *hb*PG with an average molecular weight of $M_n = 2770\ g\,mol^{-1}$ was synthesized. It carried approximately five alkyne groups per polymer as calculated by $^1H$ NMR spectroscopy (according to published procedure).[12] The alkyne moieties at the polymer were treated with the terminal azido-functionalized spacer of the MUC1–P2 antigen **5** in degassed water using copper(II) acetate and ascorbic acid at 40 °C for three days.

The coupling yielded the fully synthetic glycopeptide-hyperbranched polyglycerol vaccine **6**, which was purified by semipreparative size exclusion chromatography (SEC). The copper-free fraction collected at 2.8–3.9 min (see the Supporting Information) was purified by dialysis. The structure of **6** was confirmed by matrix-assisted laser desorption and ionization mass spectrometry (MALDI-TOF) and by analytical SEC with hexafluoro-2-propanol (HFIP) as the eluent. Compared to the SEC volumes of the starting compounds (MUC1–P2 peptide **5** and alkyne-functional *hb*PG **4**), the SEC elugram clearly showed a shift to shorter elution times indicating covalent attachment of the glycopeptides to the carrier (Figure 1a). MALDI-TOF mass spectrometry (intensity is no measure of amounts of the compounds) detected molecular masses of 16000–20000 $g\,mol^{-1}$, which correspond to three and four bound MUC1–P2 antigens ($M_n = 4395.26\ g\,mol^{-1}$) per polymer. In addition, masses of approximately 11000 $g\,mol^{-1}$ were recorded, which correspond to two (glyco)peptide antigens per polymer

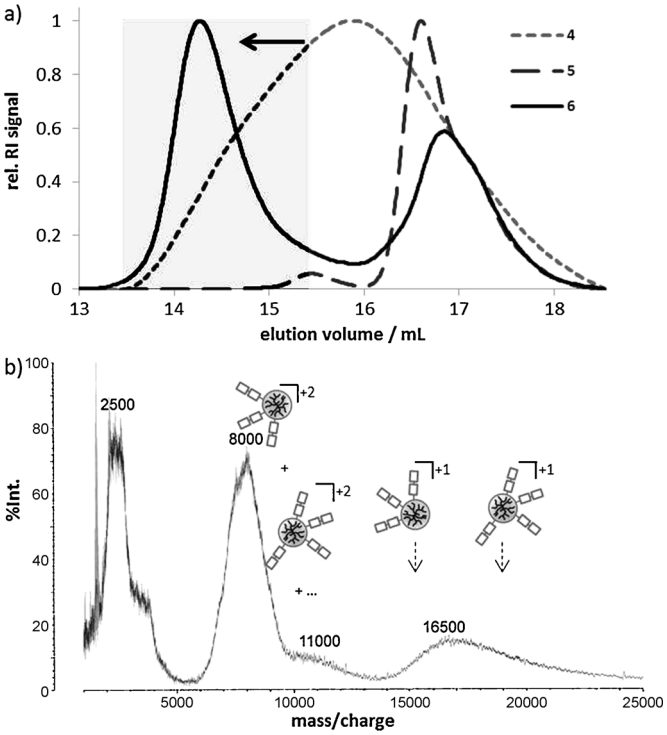



**Figure 1.** a) SEC elugrams of *hb*P(G-GPE) **4**, MUC1–P2 antigen **5** and vaccine **6** (eluent: HFIP). b) MALDI-TOF analysis of **6** (giving no indication of amounts).

(Figure 1b). Signals with lower *m/z* ratio are probably caused by multiply charged conjugates (e.g., the peaks at about 8000 $g\,mol^{-1}$ relate to doubly charged three and four bound antigens per polymer). The analytical SEC elugram also showed remaining glycopeptide–peptide conjugate **5**, which does not affect the immunological effects.[7e]

In order to evaluate the immunological properties of the fully synthetic dendritic polymer vaccine, three wild-type BALB/c mice were immunized subcutaneously together with complete Freund's adjuvant. Two further immunizations with incomplete Freund's adjuvant at intervals of 21 days were performed by intraperitoneal applications. Five days after the second boost, blood was drawn from tail veins of the mice, and the three obtained sera were analyzed by enzyme-linked immunosorbent assay (ELISA) in order to identify vaccine-induced antibodies. The microtitre plates were coated with the corresponding MUC1 glycopeptide **5** conjugate to bovine serum albumin (BSA; see the Supporting Information). The ELISA results of all three mice confirmed that the fully synthetic multivalent vaccine induced a significant specific immune response against the MUC1 B-cell epitope (Figure 2). Antibody subtype analysis after the second immunization revealed prevailing IgG1 isotype antibodies indicating MHC II-mediated immune responses (Figure 2b). Interestingly, mouse 2 (showing the highest titre) and mouse 3 additionally produced IgG2 antibodies. The binding of the induced antibodies to human breast- tumor cells (MCF-7)[22] was determined by flow cytometry (FACS). All sera exhibited significant binding to MCF-7 cells up to a recognition of 85% of the tumor cells in case of mouse 3 (Figure 2c).

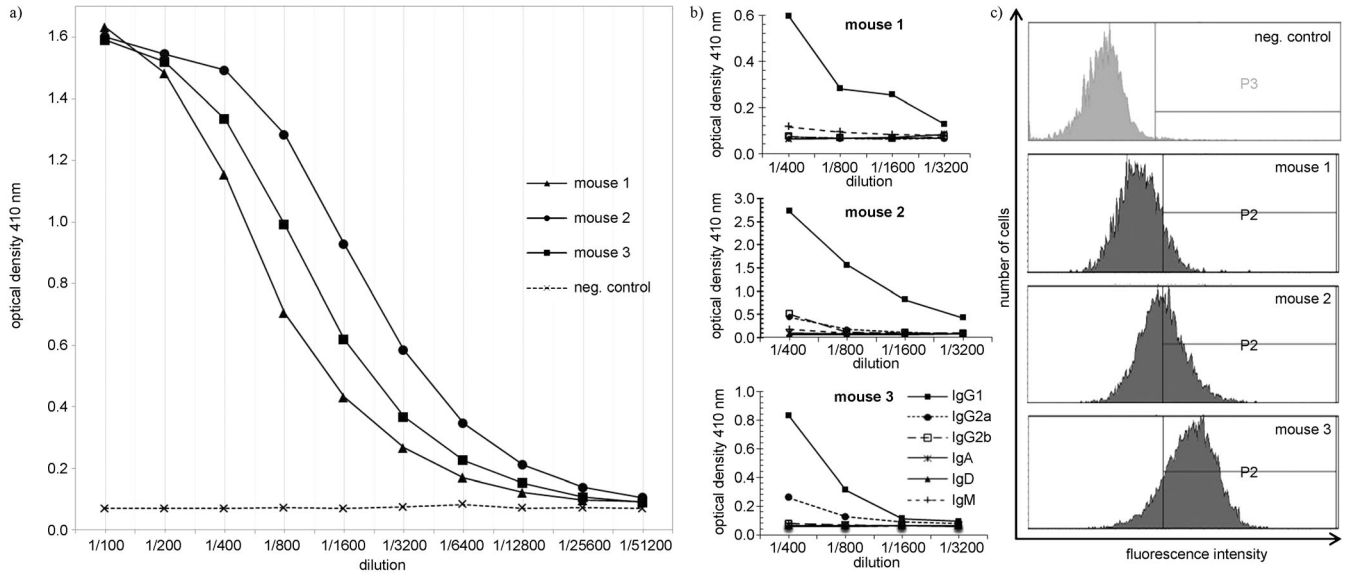



**Figure 2.** a) ELISA analysis of the antisera induced by vaccine **6**; microtitre plates were coated with the corresponding MUC glycopeptide–BSA conjugate. b) Antibody subtype analysis five days after the second immunization. c) FACS analysis of the binding of antisera to MCF-7 breast- tumor cells; negative control was obtained from mice treated with PBS buffer.

In conclusion, a novel efficient fully synthetic vaccine has been prepared, which is based on biocompatible hyperbranched polyglycerol as polymeric carrier. The globular structure of the macromolecular carrier ensures the presentation of both the MUC1 glycopeptide B-cell epitope and the tetanus toxoid T-cell epitope peptide on the surface of these nanosized compounds. The vaccine containing a tumor-associated MUC1 glycopeptide antigen and the P2 T-cell helper epitope induced significant immune responses in mice and antibodies of the IgG isotype, which recognize human tumor cells. The multivalent and branched architecture of dendritic polymer vaccines provides numerous opportunities for further variation and optimization and ensures sufficient water solubility for immunological applications. Furthermore, in contrast to the use of tetanus toxoid as the carrier the immune stimulation through the P2 peptides prevents the formation of undesired antibodies. Therefore, this concept of adjustable multivalent antigen presentation on hyperbranched polyglycerols as carrier simplifies current approaches to obtain fully synthetic antitumor vaccines and appears promising for further developments in synthetic vaccine technology.

Mice were used at 6–10 weeks of age. All mice used for this study were bred and housed in a specific pathogen-free colony at the animal facility of Johannes Gutenberg University by using institutionally approved protocols (permission was obtained from the Landesuntersuchungsamt Koblenz, reference number: 23 177-07/G 08-1-019).

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**Keywords:** antitumor agents · glycopeptides · hyperbranched polymers · polyglycerol · tumor-associated MUC1

[1] a) I. Brockhausen, J. M. Yang, J. Burchell, C. Whitehouse, J. Taylor-Papadimitriou, *Eur. J. Biochem.* **1995**, *233*, 607–617; b) T. F. Orntoft, N. Harving, N. C. Langkilde, *Int. J. Cancer* **1990**, *45*, 666–672; c) G. F. Springer, *Science* **1984**, *224*, 1198–1206.

[2] M. A. Cheever, J. P. Allison, A. S. Ferris, O. J. Finn, B. M. Hastings, T. T. Hecht, I. Mellman, S. A. Prindiville, J. L. Viner, L. M. Weiner, L. M. Matrisian, *Cli. Cancer Res.* **2009**, *15*, 5323.

[3] a) R. Singh, D. Bandyopadhyay, *Cancer Biol. Ther.* **2007**, *6*, 481–486; b) J. Taylor-Papadimitriou, J. Burchell, D. W. Miles, M. Dalziel, *Biochim. Biophys. Acta* **1999**, *1455*, 301; c) A. Girling, J. Bartkova, J. Burchell, S. Gendler, C. Gillett, J. Taylor-Papadimitriou, *Int. J. Cancer* **1989**, *43*, 1072–1076.

[4] a) S. Gendler, J. Taylor-Papadimitriou, T. Duhig, J. Rothbard, J. Burchell, *J. Biol. Chem.* **1988**, *263*, 12820; b) K. O. Lloyd, J. Burchell, V. Kudryashov, B. W. T. Yin, J. Taylor-Papadimitriou, *J. Biol. Chem.* **1996**, *271*, 33317.

[5] a) A. Kaiser, N. Gaidzik, U. Westerlind, D. Kowalczyk, A. Hobel, E. Schmitt, H. Kunz, *Angew. Chem.* **2009**, *121*, 7688; *Angew. Chem. Int. Ed.* **2009**, *48*, 7551; b) A. Hoffmann-Röder, A. Kaiser, S. Wagner, N. Gaidzik, D. Kowalczyk, U. Westerlind, B. Gerlitzki, E. Schmitt, H. Kunz, *Angew. Chem.* **2010**, *122*, 8676; *Angew. Chem. Int. Ed.* **2010**, *49*, 8498.

[6] a) T. Buskas, Y. Li, G.-J. Boons, *Chem. Eur. J.* **2004**, *10*, 3517; b) J. Ni, H. Song, Y. Wang, N. M. Stamatos, L. X. Wang, *Bioconjugate Chem.* **2006**, *17*, 493.

[7] a) S. Dziadek, A. Hobel, E. Schmitt, H. Kunz, *Angew. Chem.* **2005**, *117*, 7803–7808; *Angew. Chem. Int. Ed.* **2005**, *44*, 7630–7635; b) S. Ingale, M. A. Wolfert, J. Gaekwad, T. Buskas, G.-J. Boons, *Nat. Chem. Biol.* **2007**, *3*, 663–667; c) B. L. Wilkinson, S. Day, L. R. Malins, V. Apostolopoulos, R. J. Payne, *Angew. Chem.* **2011**, *123*, 1673–1677; *Angew. Chem. Int. Ed.* **2011**, *50*, 1635–1639; d) V. Lakshminarayanan, P. Thompson, M. A. Wolfert, T. Buskas, J. M. Bradley, L. B. Pathangey, C. S. Madsen, P. A. Cohen, S. J. Gendler, G.-J. Boons, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 261–266; e) H. Cai, M.-S. Chen, Z.-Y. Sun, Y.-F. Zhao, H. Kunz, Y.-M. Li, *Angew. Chem.* **2013**, *125*, 6222–6226; *Angew. Chem. Int. Ed.* **2013**, *52*, 6106–6110; f) H. Cai, Z.-Y. Sun, Z.-H. Huang, L. Shi, Y.-F. Zhao, H. Kunz, Y.-M. Li, *Chem. Eur. J.* **2013**, *19*, 1962–1970.

[8] a) D. Valmori, A. Pessi, E. Bianchi, G. Corradin, *J. Immunol.* **1992**, *149*, 717; b) P. Panina-Bordignon, A. Tan, A. Termijtelen, S. Demotz, G. Corradin, A. Lanzavecchia, *Eur. J. Immunol.* **1989**, *19*, 2237.

[9] J. P. Tam, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 5409.
[10] M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908 – 2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754 – 2794.
[11] L. Nuhn, S. Hartmann, B. Palitzsch, B. Gerlitzki, E. Schmitt, R. Zentel, H. Kunz, *Angew. Chem.* **2013**, *125*, 10846; *Angew. Chem. Int. Ed.* **2013**, *52*, 10652.
[12] C. Schüll, T. Gieshoff, H. Frey, *Polym. Chem.* **2013**, *4*, 4730.
[13] a) D. Wilms, S.-E. Stiriba, H. Frey, *Acc. Chem. Res.* **2010**, *43*, 129 – 141; b) R. K. Kainthan, J. Janzen, E. Levin, D. V. Devine, D. E. Brooks, *Biomacromolecules* **2006**, *7*, 703; c) R. K. Kainthan, E. B. Muliawan, S. G. Hatzikiriakos, D. E. Brooks, *Macromolecules* **2006**, *39*, 7708 – 7717; d) M. Calderón, M. A. Quadir, S. K. Sharma, R. Haag, *Adv. Mater.* **2010**, *22*, 190 – 218; e) R. K. Kainthan, D. E. Brooks, *Bioconjugate Chem.* **2008**, *19*, 2231 – 2238; f) R. K. Kainthan, D. E. Brooks, *Biomaterials* **2007**, *28*, 4779 – 4787.
[14] S. Keil, C. Claus, W. Dippold, H. Kunz, *Angew. Chem.* **2001**, *113*, 379; *Angew. Chem. Int. Ed.* **2001**, *40*, 366.
[15] a) O. Blixt, E. Cló, A. S. Nudelman, K. K. Sørensen, T. Clausen, H. H. Wandall, P. O. Livingston, H. Clausen, K. J. Jensen, *J. Proteome Res.* **2010**, *9*, 5250; b) J. Burchell, J. Taylor-Papadimitriou, M. Boshell, S. Gendler, T. Duhig, *Int. J. Cancer* **1989**, *44*, 691; c) M. A. Tarp, A. L. Sørensen, U. Mandel, H. Paulsen, J. Burchell, J. Taylor-Papadimitriou, H. Clausen, *Glycobiology* **2007**, *17*, 197 – 209.
[16] U. Westerlind, H. Schröder, A. Hobel, N. Gaidzik, A. Kaiser, C. M. Niemeyer, E. Schmitt, H. Waldmann, H. Kunz, *Angew. Chem.* **2009**, *121*, 8413; *Angew. Chem. Int. Ed.* **2009**, *48*, 8263.
[17] a) M. J. Scanlon, S. D. Morley, D. E. Jackson, M. R. Price, S. J. B. Tendler, *Biochem. J.* **1992**, *284*, 137; b) P. Braun, G. Davies, M. R. Price, P. M. Williams, S. J. Tendler, H. Kunz, *Bioorg. Med. Chem.* **1998**, *6*, 1531 – 1545; c) J. A. Mollick, F. S. Hodi, R. J. Soiffer, L. M. Nadler, G. Dranoff, *Cancer Immunity* **2003**, *3*, 3.
[18] J. D. Fontenot, N. Tjandra, B. Dawen, H. Chien, R. C. Montelaro, O. J. Finn, *Cancer Res.* **1993**, *53*, 5386.
[19] N. Gaidzik, A. Kaiser, D. Kowalczyk, U. Westerlind, B. Gerlitzki, H. P. Sinn, E. Schmitt, H. Kunz, *Angew. Chem.* **2011**, *123*, 10153; *Angew. Chem. Int. Ed.* **2011**, *50*, 9977.
[20] J. M. J. Fréchet, K. E. Haque, *Tetrahedron Lett.* **1975**, *16*, 3055.
[21] a) V. V. Rostovtsev, L.-G. Green, V. V. Fokin, K. G. Sharpless, *Angew. Chem.* **2002**, *114*, 2708; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596; b) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057.
[22] H. D. Soule, J. Vazquez, J. Long, S. Albert, M. Brennan, *J. Natl. Cancer Inst. (1940 – 1978)* **1973**, *51*, 1409.

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**Antitumor Agents**

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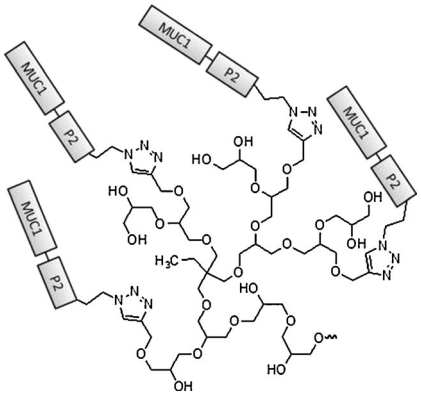
## A Fully Synthetic Glycopeptide Antitumor Vaccine Based on Multiple Antigen Presentation on a Hyperbranched Polymer




**Branching out**: A tumor-associated MUC1 glycopeptide as B-cell epitope combined with a tetanus toxoid T-cell epitope was coupled to hyperbranched polyglycerol (see figure). Due to the dendritic carrier structure, the antigen is presented in an optimal multiple display to the immune system. The fully synthetic and water-soluble vaccine induced antibodies that recognize human breast-tumor cells.