CSIRO PUBLISHING

Communication

www.publish.csiro.au/journals/ajc

Aust. J. Chem. **2009**, *62*, 1339–1343

Synthesis of MUC1 Peptide and Glycopeptide Dendrimers

Candy K. Y. Chun[A] *and Richard J. Payne*[A,B]

[A]School of Chemistry, The University of Sydney, NSW 2006, Australia.
[B]Corresponding author. Email: payne@chem.usyd.edu.au

Several dendrimers possessing multiple copies of peptides and glycopeptides belonging to the MUC1 eicosapeptide tandem repeat sequence have been prepared. Fmoc-strategy solid-phase peptide synthesis was used to construct the peptides and glycopeptides, which were conjugated to suitably functionalized dendrimer cores using the copper-catalyzed azide-alkyne cycloaddition reaction to produce multivalent peptide and glycopeptide dendrimers.

Manuscript received: 12 May 2009.
Manuscript accepted: 19 June 2009.

Introduction

The surfaces of human cells are decorated with glycoproteins that function as important ligands and receptors for numerous biological events, including cell adhesion, cell differentiation, immunodifferentiation, and cell growth.[1–3] Significantly, the progression of cancer is often associated with dysregulation of the enzymes responsible for protein glycosylation, thus leading to altered carbohydrate patterns at the surface of a range of tumour cell types. Specifically, in the case of several epithelial tumours (including breast, prostate, ovarian, pancreatic, and colorectal cancers), large MUC1 glycoproteins are drastically overexpressed and aberrantly glycosylated.[4] Consequently, there has been intense effort focussed on the development of synthetic and semi-synthetic cancer vaccine candidates based on MUC1 peptides and glycopeptides.[5–13]

The extracellular domain of MUC1 contains a variable number tandem repeat (VNTR) domain of 20 amino acids (GVTSAPDTRPAPGSTAPPAH), possessing five potential *O*-glycosylation sites. Incomplete glycosylation and premature sialylation of this repeat sequence leads to the presentation of several well characterized carbohydrate tumour markers on the cell surface. These include the T_N (GalNAc, **1**), T (Gal-β-1,3-GalNAc, **2**), sialyl T_N (**3**), and sialyl T (**4**) antigens (Fig. 1).[14] This notable difference in glycoprotein profile of tumour cells constitutes an important basis for therapeutic intervention, whereby a selective immunological attack of certain cancer cells can be achieved.[5,13] In the case of MUC1, the truncated glycan structures result in the exposure of additional peptide epitopes, which become accessible to the immune system.[15,16]

Synthetic peptide and glycopeptide dendrimers provide an avenue for the multivalent display of peptide and glycopeptide epitopes, a desirable feature for a strong and sustained immunological response.[16,17] Additionally, the large size of dendrimers has the potential to render them self-immunogenic, thereby precluding the need for carrier proteins traditionally employed for vaccine constructs.[18–20] Given our interest in the development of MUC1-based vaccine candidates, we set upon the synthesis of multivalent dendrimers presenting several copies of immunogenic peptides and glycopeptides of the MUC1 VNTR sequence. Our synthetic strategy was to utilize the Cu(I)-catalyzed variant

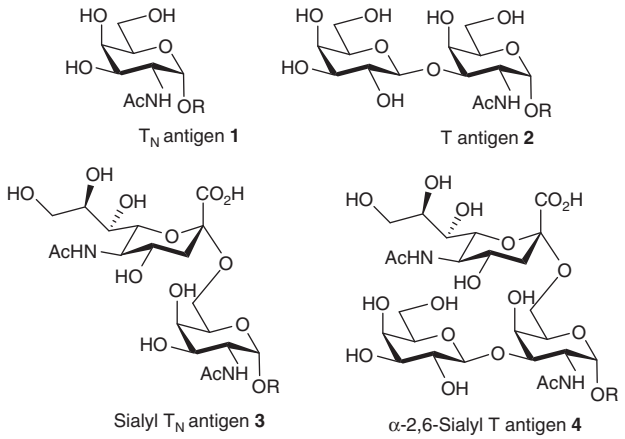
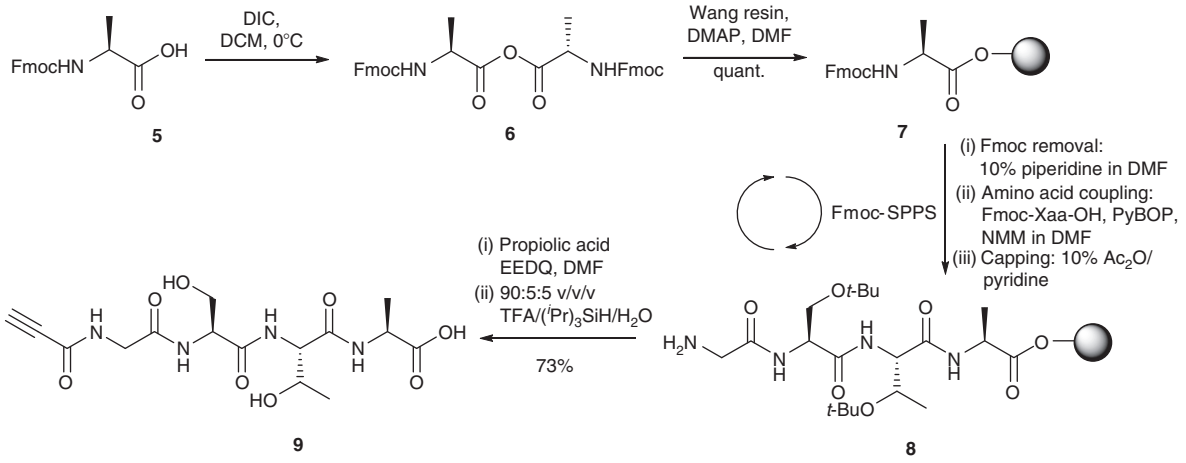


Fig. 1. Tumour-associated glycans.

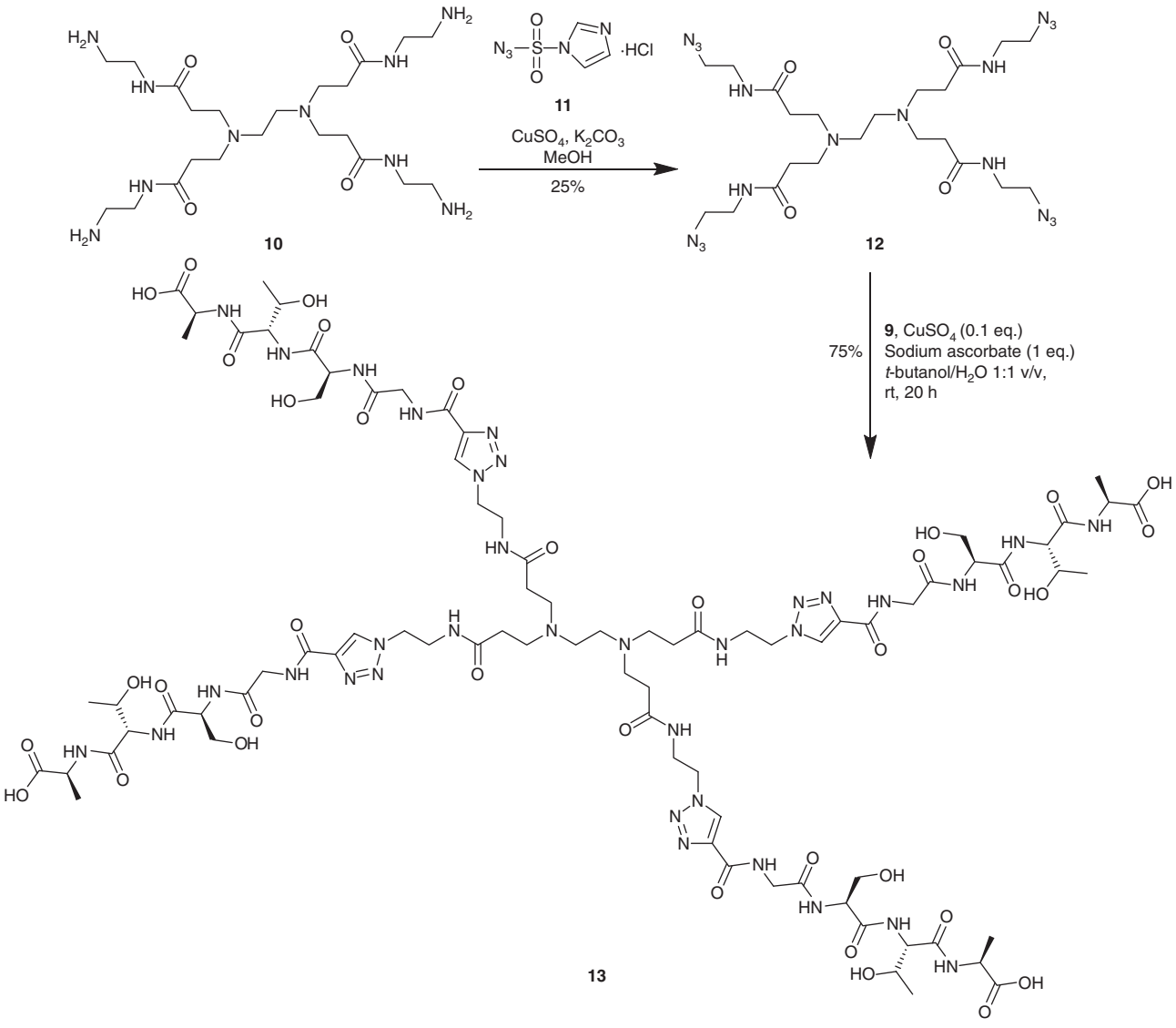
of the Huisgen 1,3-dipolar cycloaddition reaction,[21] namely the Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC),[22,23] to generate these constructs in an efficient and concise manner.

In order to synthesize the desired MUC1-based peptide and glycopeptide dendrimers, it was initially necessary to prepare a suitable peptide substrate which could be coupled to a dendrimer scaffold. We first chose to prepare the immunodominant glycinylserinylthreoninylalanine (GSTA) tetrapeptide epitope of MUC1, possessing an alkyne functionalized *N*-terminus. Treatment of Fmoc-Ala-OH (**5**) with *N*,*N*′-diisopropylcarbodiimide produced symmetrical anhydride **6**, which was next coupled to Wang resin to generate the functionalized solid support **7** in quantitative yield (as determined by measuring the piperidine-fulvene adduct at $\lambda = 301$ nm generated upon treatment of **7** with 10% piperidine/DMF). Peptide assembly was then achieved by Fmoc-strategy solid-phase peptide synthesis (SPPS) to give resin bound tetrapeptide **8** which, following Fmoc deprotection, was initially treated with propiolic acid and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) followed by acidolytic deprotection and cleavage from the resin to afford the desired alkynyl peptide **9** in 73% yield after purification by preparative HPLC (Scheme 1).

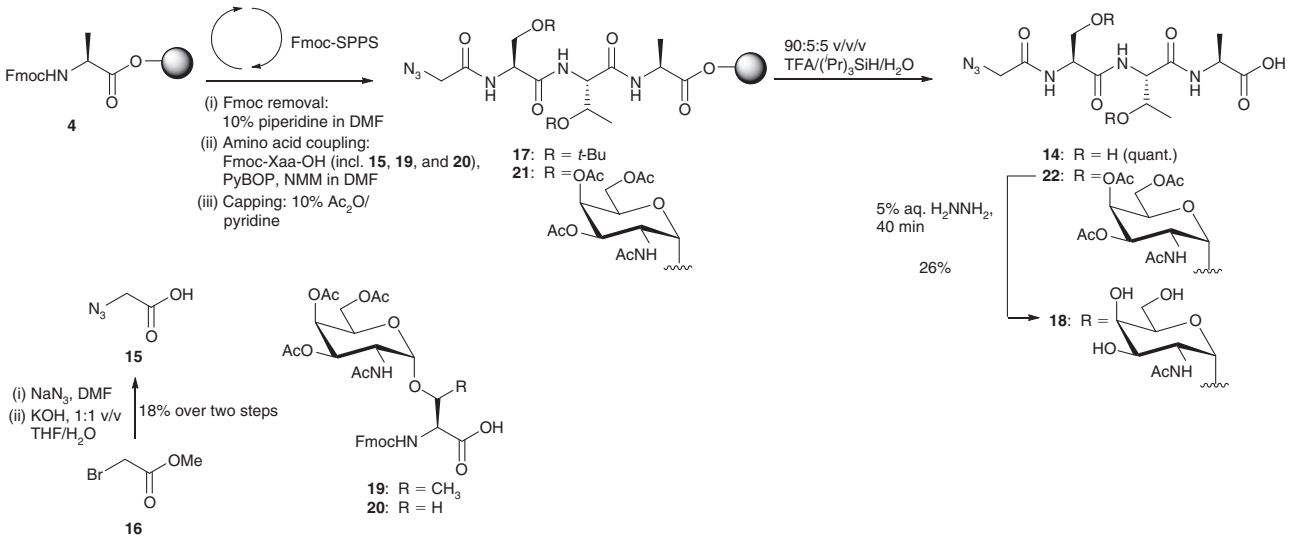
 10.1071/CH09282 0004-9425/09/101339



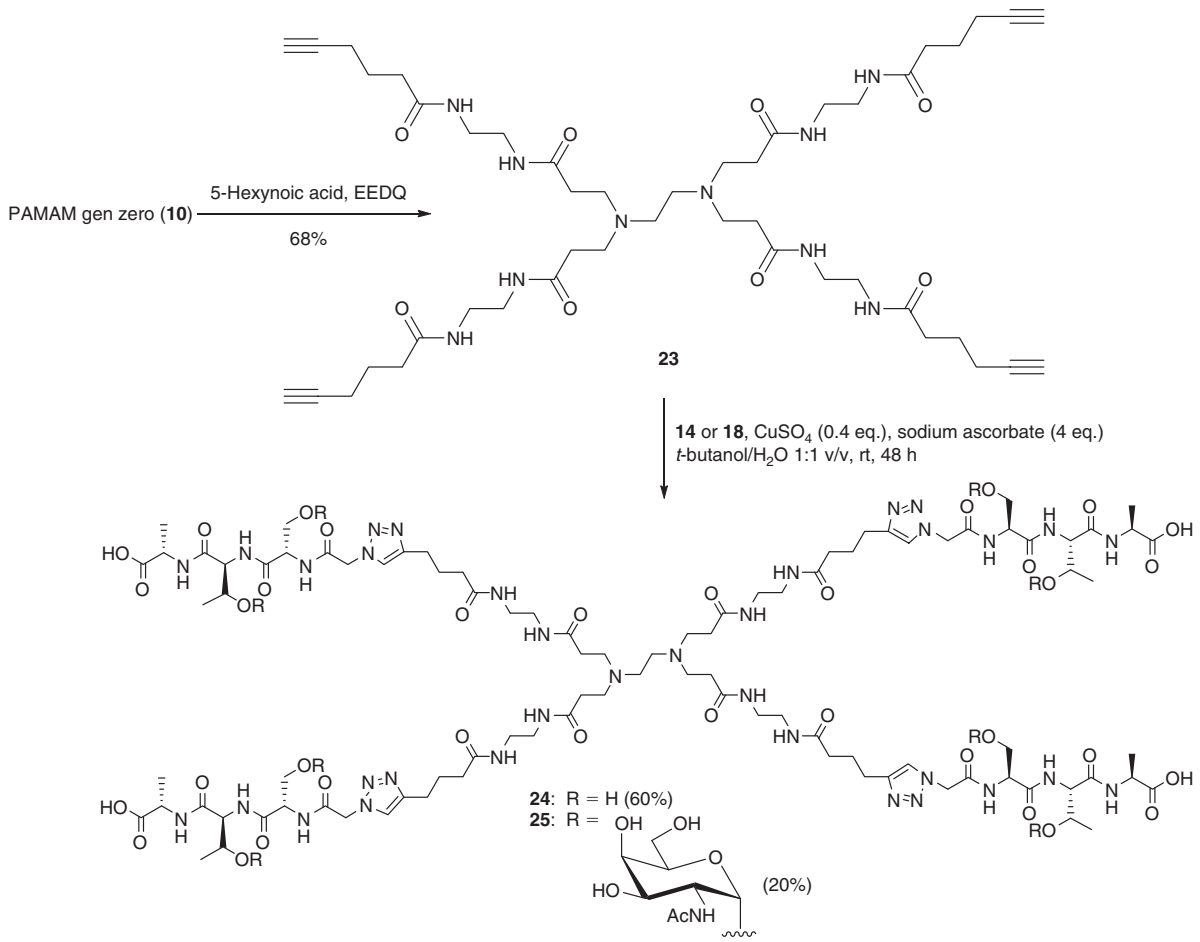
Scheme 1.



Scheme 2.



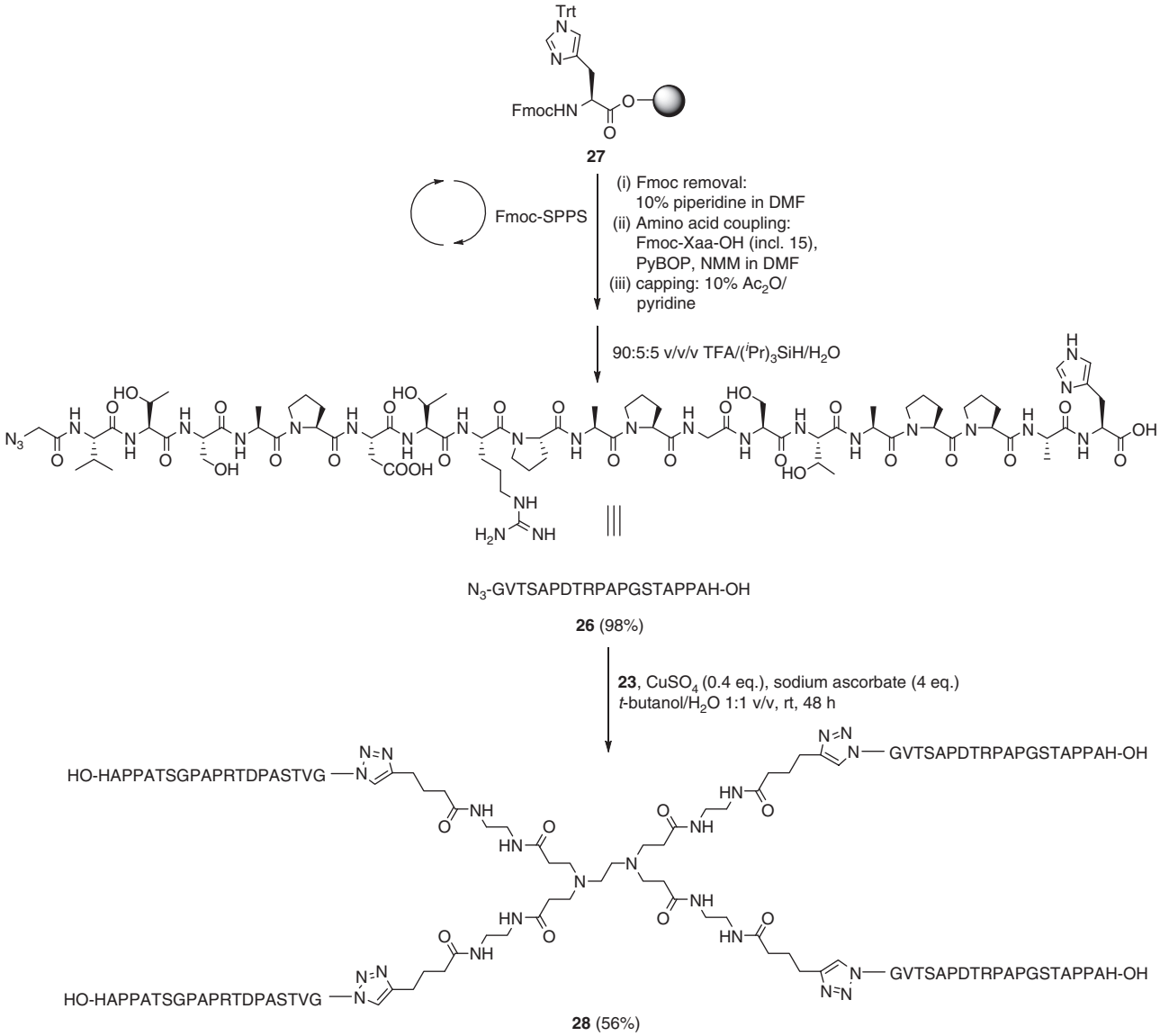
Scheme 3.



Scheme 4.

Polyamidoamine (PAMAM) generation zero (**10**), possessing an ethylenediamine core and four amino groups on the periphery, was chosen as a suitable scaffold from which to construct our multivalent MUC1-based dendrimers. To facilitate functionalization of the chosen dendrimer core, it was initially necessary to modify the surface amino groups by conversion to the corresponding azides, thereby enabling subsequent conjugation to alkynyl peptide **9** using CuAAC chemistry. In the event, conversion to the corresponding azide moiety was readily achieved by treating **10** with the imidazole-based Goddard–Borger–Stick diazotransfer reagent **11** (Scheme 2).[24]

While it was possible to successfully prepare the desired azido-PAMAM **12**, we were disappointed to find that the isolated yield (after preparative reverse-phase HPLC) was only



Scheme 5.

25%. This was attributed to the instability and consequent degradation of the tetra-azide moiety during purification. Indeed, **12** also proved unstable to storage at low temperatures (−20°C) for extended periods. Despite these difficulties, **12** was successfully implemented as a scaffold in the preparation of peptide dendrimer **13**, bearing four copies of the immunogenic GSTA segment of the MUC1 repeat. Treatment of PAMAM tetra-azide **12** with 6 molar equivalents of alkynylpeptide **9** (1.5 equivalents per azide), 10 mol-% of $CuSO_4$ and 1 molar equivalent of sodium ascorbate in water and *t*-butanol[23] gave the desired peptide dendrimer product **13** in 75% yield after HPLC purification (Scheme 2).

While gratified to have successfully completed preparation of the first member of our planned PAMAM dendrimer library, the inherent instability of tetra-azide **12** prompted a revision of our synthetic strategy. Thus, it was decided to alter the functionality on the PAMAM scaffold to incorporate terminal alkynes in place of the azide moieties. It was anticipated that this revised PAMAM core could then be reacted with MUC1-based peptides and glycopeptides possessing *N*-terminal azide moieties under analogous CuAAC conditions to generate multivalent dendrimers.

To this end, azidopeptide **14** was synthesized by Fmoc-strategy SPPS starting from preloaded Wang resin **7** (Scheme 3). Azidoglycine **15** (synthesized in two steps from methylbromoacetate **16**) was coupled to generate resin-bound tetrapeptidyl azide **17**. Side chain deprotection and cleavage from the resin then furnished **14** in quantitative yield (as determined by the Fmoc loading of the penultimate serine residue). A similar sequence of reactions was followed for the preparation of glycopeptide **18**, with glycosylthreonine **19** and glycosylserine **20** coupled to the growing chain in place of the standard side chain protected Fmoc-amino acids. Incorporation of azidoglycine as the final amino acid generated the resin bound glycopeptide **21** which, after acidic deprotection and cleavage from the resin, gave **22**, bearing two acetylated copies of the T_N antigen. Treatment of **22** with an aqueous hydrazine solution to remove the acetate groups, followed by HPLC purification, then gave the desired glycopeptide **18** in 26% yield based on the Fmoc loading of the glycosylserine residue.

The next stage in the synthesis involved the preparation of a suitably functionalized revised dendrimer core, which could subsequently be conjugated to both **14** and **18**. PAMAM core **23**, bearing four terminal alkyne moieties, was generated in 68% yield by treatment of **10** with 5-hexynoic acid and EEDQ (Scheme 4). Azidopeptide **14** was now reacted with **23** under the CuAAC conditions described previously. In this instance, however, these conditions returned only starting materials. Postulating that this may be a consequence of non-productive copper chelates being formed with our new substrates, the reaction was repeated with additional equivalents of both $CuSO_4$ (40 mol-%) and sodium ascorbate (4 eq.). Under these revised conditions, we were delighted to find that dendrimer **24**, bearing four copies of the immunogenic GSTA peptide, was formed in 48 h at ambient temperature and was isolated in 60% yield. In a similar manner, glycopeptide dendrimer **25**, possessing four copies of the fully glycosylated GSTA fragment, was synthesized by reaction between **23** and azidoglycopeptide **18**. In this case, the desired product **25** was isolated in a reduced yield of 20% yield, due in major part to difficulties in separating the product from **18** by reverse-phase HPLC.

Having established that PAMAM peptide and glycopeptide dendrimers could be synthesized bearing four copies of unglycosylated and glycosylated GSTA fragments, we next turned our attention to the construction of multivalent dendrimers possessing four copies of the full eicosapeptide MUC1 tandem repeat sequence. To this end, we embarked on the synthesis of peptide **26** bearing an *N*-terminal azidoglycine residue. Starting from Wang resin preloaded with Fmoc-His(Trt)-OH (**27**), the desired peptide was assembled by SPPS according to the Fmoc-strategy. Azidoglycine (**15**) was coupled as the final amino acid residue, to afford azidopeptide **26** in 98% yield after side chain deprotection and cleavage from the resin followed by HPLC purification (Scheme 5). Synthesis of dendrimer **28** bearing four copies of the MUC1 eicosapeptide repeat was readily achieved under the same conditions described for the shorter peptide analogues **24** and **25**. We were delighted to find that the reaction proceeded to completion after 48 h at ambient temperature and purification by reverse HPLC gave the 8.5 kDa peptide dendrimer in 56% yield.

In summary, we have utilized solid- and solution-phase chemistry in combination with the CuAAC reaction to construct a small library of peptide and glycopeptide dendrimers in a fast and efficient manner. We found that the optimal strategy involved coupling peptides and glycopeptides bearing an *N*-terminal azide with a dendrimer scaffold presenting multiple alkynes. The constructs synthesized in this study are currently undergoing immunological evaluation for their potential to generate antibodies specific for peptide and glycopeptide fragments of the MUC1 tandem repeat. Future directions within this laboratory will also aim to utilize the synthetic strategy outlined here for the synthesis of more complex MUC1-based dendrimers possessing other cancer-associated glycans.

Accessory Publication

For synthesis details please access the Accessory Publication available on the Journal's website.

Acknowledgements

We would like to thank the Australian Research Council for funding and Yves Hsieh for assistance with MALDI-TOF mass spectrometry.

References

[1] A. Varki, *Glycobiology* **1993**, *3*, 97. doi:10.1093/GLYCOB/3.2.97
[2] R. G. Spiro, *Glycobiology* **2002**, *12*, 43R. doi:10.1093/GLYCOB/12.4.43R
[3] R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683. doi:10.1021/CR940283B
[4] F.-G. Hanisch, S. Muller, *Glycobiology* **2000**, *10*, 439. doi:10.1093/GLYCOB/10.5.439
[5] H. Kunz, *J. Pept. Sci.* **2003**, *9*, 563. doi:10.1002/PSC.477
[6] H. Herzner, T. Reipen, M. Schultz, H. Kunz, *Chem. Rev.* **2000**, *100*, 4495. doi:10.1021/CR990308C
[7] T. Buskas, S. Ingale, G. J. Boons, *Glycobiology* **2006**, *16*, 113R. doi:10.1093/GLYCOB/CWJ125
[8] S. J. Danishefsky, J. R. Allen, *Angew. Chem. Int. Ed.* **2000**, *39*, 836. doi:10.1002/(SICI)1521-3773(20000303)39:5<836::AID-ANIE836>3.0.CO;2-I
[9] S. Dziadek, H. Kunz, *Chem. Rec.* **2004**, *3*, 308. doi:10.1002/TCR.10074
[10] O. Ouerfelli, J. D. Warren, R. M. Wilson, S. J. Danishefsky, *Expert Rev. Vaccines* **2005**, *4*, 677. doi:10.1586/14760584.4.5.677
[11] C. Brocke, H. Kunz, *Bioorg. Med. Chem.* **2002**, *10*, 3085. doi:10.1016/S0968-0896(02)00135-9
[12] M. Meldal, P. M. St Hilaire, *Curr. Opin. Chem. Biol.* **1997**, *1*, 552. doi:10.1016/S1367-5931(97)80052-X
[13] S. Dziadek, C. G. Espinola, H. Kunz, *Aust. J. Chem.* **2003**, *56*, 519. doi:10.1071/CH02241
[14] G. F. Springer, *Science* **1984**, *224*, 1198. doi:10.1126/SCIENCE.6729450
[15] S. Dziadek, C. Griesinger, H. Kunz, U. M. Reinscheid, *Chem. Eur. J.* **2006**, *12*, 4981. doi:10.1002/CHEM.200600144
[16] A. L. Sorensen, C. A. Reis, M. A. Tarp, U. Mandel, K. Ramachandran, V. Sankaranarayanan, T. Schwientek, R. Graham, J. Taylor-Papadimitriou, M. A. Hollingsworth, J. Burchell, H. Clausen, *Glycobiology* **2006**, *16*, 96. doi:10.1093/GLYCOB/CWJ044
[17] A. Liakatos, H. Kunz, *Curr. Opin. Mol. Ther.* **2007**, *9*, 35.
[18] P. Niederhafner, M. Reinis, J. Sebestik, J. Jezek, *J. Pept. Sci.* **2008**, *14*, 556. doi:10.1002/PSC.1011
[19] C. Ozawa, H. Katayama, H. Hojo, Y. Nakahara, *Org. Lett.* **2008**, *10*, 3531. doi:10.1021/OL801340M
[20] C. Ozawa, H. Hojo, Y. Nakahara, H. Katayama, K. Nabeshima, T. Akahane, *Tetrahedron* **2007**, *63*, 9685. doi:10.1016/J.TET.2007.07.023
[21] R. Huisgen, G. Szeimies, L. Mobius, *Chem. Ber.* **1967**, *100*, 2494. doi:10.1002/CBER.19671000806
[22] C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057. doi:10.1021/JO011148J
[23] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596. doi:10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4
[24] E. D. Goddard-Borger, R. V. Stick, *Org. Lett.* **2007**, *9*, 3797. doi:10.1021/OL701581G