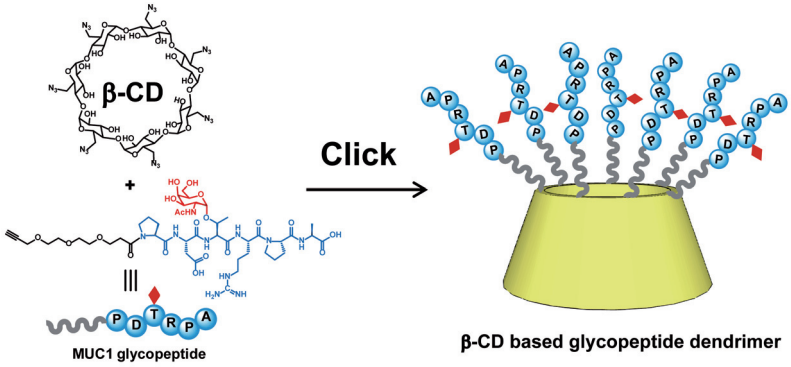
Synlett P.-G. Chen et al. Cluster

Synthesis of an MUC1 Glycopeptide Dendrimer Based on β-Cyclodextrin by Click Chemistry

Pu-Guang Chen
Zhi-Hua Huang
Zhan-Yi Sun
Qian-Qian Li
Yong-Xiang Chen
Yu-Fen Zhao
Yan-Mei Li*

Department of Chemistry, Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Tsinghua University, Beijing 100084, P. R. of China
liym@mail.tsinghua.edu.cn

Published as part of the Cluster *Recent Advances in Protein and Peptide Synthesis*



Received: 13.04.2017
Accepted after revision: 22.05.2017
Published online: 06.07.2017
DOI: 10.1055/s-0036-1590796; Art ID: st-2017-w0259-c

Abstract Glycopeptide dendrimers are attractive candidates for biomedical applications. Here, an efficient method for preparing multivalent MUC1 glycopeptide dendrimers based on β-cyclodextrin is described. By using copper(I) bromide and thioanisole as a catalyst system, precisely defined heptavalent conjugates were efficiently obtained. Using this heptavalent glycopeptide dendrimer, we observed multivalent effects in recognition and association processes in antibody and epitope interactions, which might have biomedical applications.

Key words glycopeptide dendrimers, cyclodextrin, copper catalysis, thioanisole, antibody titer, click chemistry

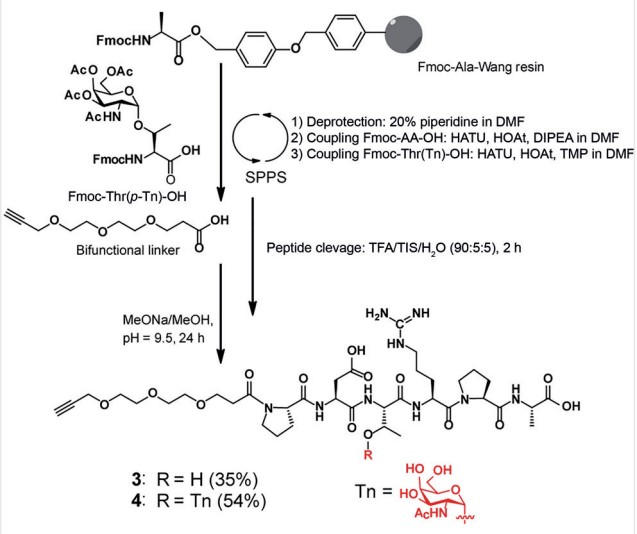
Multivalent effects occur widely in biological systems, for example in cell–cell interactions, receptor–ligand interactions, or lectin–glycan interactions.[1] Many efforts have been made to construct multivalent molecules for use as antiinfective drugs, antiinflammatory drugs, or anticancer drug carriers or for tissue-engineering applications.[2–6] Glycopeptide dendrimers, which contain both peptide and carbohydrate moieties, have shown great potential for developing inhibitors or synthetic vaccines.[7] Reymond and coworkers constructed a series of glycosylated peptide dendrimers to inhibit the biofilm formation of *Pseudomonas aeruginosa*, which might be useful as therapeutic agents for this bacterium.[8]

MUC1 glycopeptide, which is the variable number tandem repeats (VNTR) of MUC1 glycoprotein in the extracellular domain, has been identified as a potent tumor-associated antigen for cancer immunotherapy.[9] Many groups have tried to construct multivalent MUC1 glycopeptide architectures with unique geometric characteristics. Several multivalent MUC1 glycopeptide dendrimers based on a dendritic lysine core have been synthesized by means of the Fmoc solid-phase peptide-synthesis strategy,[10] the *N*-alkylcysteine-assisted sequential-segment-coupling strategy,[11] and the copper-catalyzed azide–alkyne cycloaddition reaction (CuAAC) strategy.[12] A series of multivalent MUC1 glycopeptides have constructed on various polymer scaffolds, such as poly[*N*-(2-hydroxypropyl)methacrylamide],[13] polyamidoamine,[14] poly(*N*-isopropylacrylamide),[15] or poly(*tert*-butyl acrylate).[16] Other multivalent neoglyconjugates have been constructed by using GlcNAc-centered glycoclusters, glycocyclopeptides, or tribranched glycopeptide mimics, among others.[17] Moreover, Spadaro and colleagues conjugated multiple units of the MUC1 core sequence with a calix[4,8]arene platform modified with Toll-like receptor 2 ligands, to form self-adjuvant vaccine candidates.[18]

β-Cyclodextrin (β-CD) is a naturally occurring cyclic oligosaccharide that has proved to be useful as a well-defined multivalent molecular scaffold.[6] Many functional molecules, such as amines, sugars, amino acids, polymers, or drugs, have been conjugated with β-CD for use in pharmaceuticals, supramolecular chemistry, or material science.[6,19] However, due to the complexity of peptides and β-CD, only few multivalent peptide dendrimers based on β-CD have been developed.[20] To the best of our knowledge, no glycopeptide dendrimers based on β-CD have been developed. We successfully synthesized a multivalent MUC1 glycopeptide dendrimer based on β-CD by means of click chemistry. By using CuBr/thioanisole as a catalyst system, precisely defined heptavalent conjugates were effectively obtained through azide–alkyne cycloaddition reaction. Moreover, by using this heptavalent glycopeptide dendrimer, we observed multivalent effects in the recognition and interaction process of antibody with its epitopes. We demonstrated that antibody avidity to a multivalent epitope was al-

most five times higher than that to the corresponding monovalent epitope, a result that might have biomedical applications.

MUC1 glycopeptide consists of 20 amino acid residues associated with various tumor-associated carbohydrate antigens such as Tn, T, STn, or ST at Ser/Thr residues.[21] It had been demonstrated that the peptide sequence PDTRP derived from the MUC1 glycopeptide is highly immunogenic.[9b,21] Glycosylation on the Thr residue of the PDTRP domain can stabilize the β-turn conformation and improve the immunogenicity of the MUC1 glycopeptide.[22] We used the glycosylated PDTRP domain as a model glycopeptide to construct the glycopeptide dendrimer. To suppress the formation of diketopiperazine during the solid-phase peptide synthesis (SPPS), we extended the PDTRP sequence with alanine at the C-terminus. First, we synthesized native and Tn-antigen-glycosylated PDTRPA peptides by Fmoc SPPS. A bifunctional poly(ethylene glycol)-based linker, which contained a carboxy group and an alkynyl group, was attached to the N-terminus of each of the two peptides. After cleavage from the resins, the crude peptides were purified by reverse-phase HPLC (RP-HPLC). The GalNAc protective groups were subsequently removed by treatment with 1% NaOMe in MeOH to give compounds **3** and **4** (Scheme 1).[23]



Scheme 1 Synthesis of alkyne-spacer-modified peptide **3** and glycopeptide **4**. Detailed conditions and RP-HPLC and ESI-MS analyses are given in the Supporting Information.

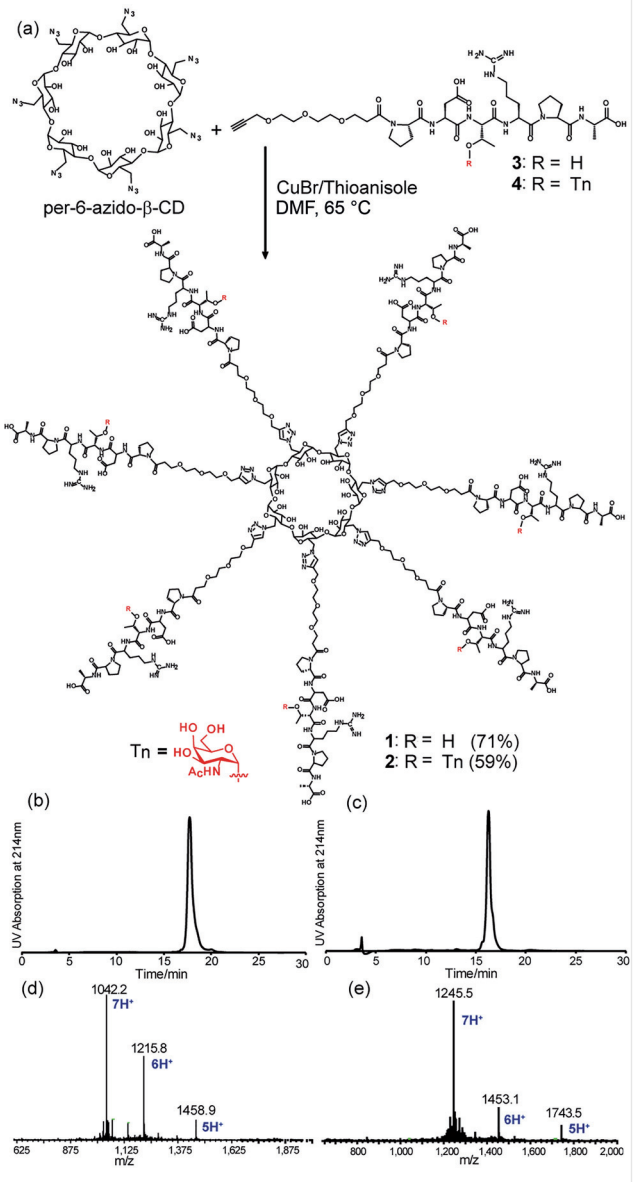
β-CD is a unique multivalent molecular scaffold with well-defined geometrical characteristics, so that many β-CD-based conjugates have been developed for various applications through selective functionalizations of β-CD. Most functionalizations of β-CDs were based on the hydroxy groups on the upper rim (the 6-OHs), due to the different chemical reactivities of the 2-OH, 3-OH, and 6-OH groups.

Azidation of β-CD on its upper rim was achieved by the previously reported method.[24] The β-CD was first substituted with iodine to afford **per-6-iodo-β-CD** and then directly substituted with NaN_3 to give **per-6-azido-β-CD** (see Supporting Information; Scheme S1). Because of the poor solubility of azido-modified β-CD, $CuSO_4$ and sodium ascorbate, the standard catalyst system for CuAAC, is unsuitable for this conjugation. CuI/triethylamine was also found to be an inefficient catalyst system for peptide conjugation with β-CD, as the heptavalent β-CD-peptide conjugates could only be obtained with 80% purity.[20c] Previously, CuBr/thioanisole has been identified as a rapid and highly efficient catalyst system for click chemistry of water-insoluble substrates.[25] We therefore attempted to perform the click reaction of the nonglycosylated peptide **3** with **per-6-azido-β-CD** by using CuBr/thioanisole as a catalyst system in DMF at 65 °C. RP-HPLC with ESI-MS detection showed that the conjugation reaction was nearly complete within two hours, and incompletely converted compounds such as hexavalent, pentavalent, or tetravalent glycopeptide dendrimers were not obtained (Schemes 2a, 2b, and 2d). The target molecule **1** was obtained by RP-HPLC in high purity and further lyophilized.[26] For glycopeptide **4**, the same catalyst system was used and the target β-CD-based glycopeptide dendrimer **2** was obtained with high purity by RP-HPLC after ten hours of reaction (Schemes 2a, 2c, and 2e).[27]

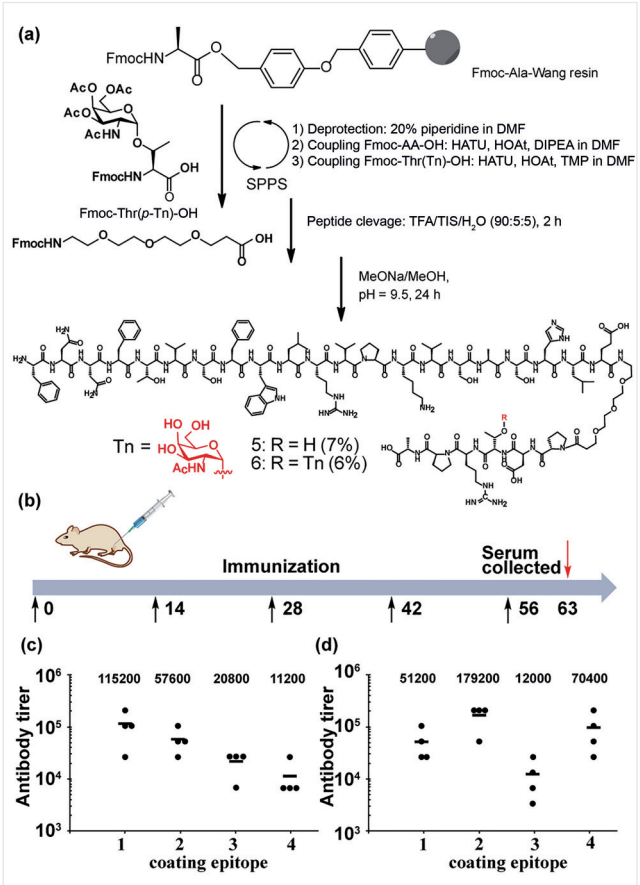
To investigate differences in the recognition and binding by antibodies of multivalent antigens and monovalent antigens, we synthesized a pair of two-component vaccines to provide MUC1-glycopeptide-specific antibodies (Scheme 3). A two-component vaccine consisting of a B-cell epitope and a T-helper-cell epitope effectively activates the immune system and elicits a high titer of antigen-specific antibodies.[28] Tn-antigen-glycosylated and nonglycosylated PDTRPA peptides were extended with a T-helper cell epitope, consisting of 21 amino-acid residues derived from tetanus toxin, which has been shown to be a potent T-helper cell epitope for synthetic chemical vaccines,[28b,29] to form a pair of two-component vaccines (compounds **5** and **6**; Scheme 3a) Female Balb/c mice were immunized with the two-component vaccines without an adjuvant through intraperitoneal injection at days 0, 14, 28, 42, and 56. At day 63, the mice were sacrificed, and the serum was collected (Scheme 3b).

Through enzyme-linked immunosorbent assay (ELISA), the antibody titers of the collected serum were measured for the peptide dendrimer **1**, glycopeptide dendrimer **2**, native PDTRPA peptide **3**, and glycosylated PDTRPA peptide **4** as coating epitopes, respectively. For nonglycosylated-peptide-specific antibody, the mean antibody titers of native PDTRPA peptide **3** and glycosylated PDTRPA peptide **4** were 20800 and 11200, respectively (Scheme 3c), which indicated that the two-component vaccines containing nonglycosylated MUC1 peptide induced a particular antibody for specific binding with native PDTRPA peptide **3**. Moreover, the mean antibody titers for peptide dendrimer **1** and na-

tive PDTRPA peptide **3** were 57600 and 11200, respectively (Scheme 3c). This indicates that the antibody titer for the peptide dendrimer was five time higher than that for the monovalent peptide, implying that the multivalent effect has an important roles in antibody–epitope recognition and interaction. For the glycosylated peptide specific antibody, the mean antibody titers for compounds **1**–**4** were 51200, 179200, 12000, and 70400, respectively (Scheme 3d). The results proved that two-component vaccines consisting of glycosylated mucin peptide specifically induced an antibody that bound preferentially with the glycosylated PDTRPA peptide **4**. What is more, the mean antibody titer of glycopeptide dendrimer **2** was about 2.5 times higher than that is of the glycosylated PDTRPA peptide **4**, which is consistent with data for a nonglycosylated-peptide-specific antibody, and confirmed the existence of a multivalent effect in the antibody–epitope recognition and association process (Scheme 3c). The multivalent effect in antibody recognition and binding with epitopes might have biomedical applications, for example in antibody detection, serum analysis, or disease diagnosis.



Scheme 2 (a) Syntheses of peptide dendrimer **1** and glycopeptide dendrimer **2**. The compound **3** was conjugated with **per-6-azido-β-CD** by using CuBr/thioanisole as a catalyst in DMF at 65 °C for 2 h, whereas compound **4** was conjugated with **per-6-azido-β-CD** by using CuBr/thioanisole in DMF at 65 °C for 10 h. More details are provided in the Supporting Information. RP-HPLC (b) and ESI-MS analyses (d) of peptide dendrimer **1**. RP-HPLC (c) and ESI-MS analyses (e) of glycopeptide dendrimer **2**.



Scheme 3 (a) The synthesis of the two-component vaccines **5** and **6**. Detail information is given in Supporting Information. (b) Immunization schedule for the synthetic vaccines. Compounds **5** and **6** were injected intraperitoneally into Balb/c mice at days 0, 14, 28, 42, and 56. The serum was collected at day 63. The antibody titers of compounds **5** (c) and **6** (d) were measured by ELISA. The coating epitope were peptide dendrimer **1**, glycopeptide dendrimer **2**, nonderivatized PDTRPA peptide **3**, and glycosylated PDTRPA peptide **4**. The antibody titer is defined as the highest dilution with 0.1 optical absorption or with an absorbance greater than that of negative control sera.[9c]

In conclusion, by using CuBr and thioanisole as a catalyst system, we successfully and efficiently synthesized a heptavalent MUC1 glycopeptide dendrimer based on β-CD, with high purity. To our limited knowledge, this is the first glycopeptide dendrimer based on β-CD. By using this heptavalent glycopeptide dendrimer, we observed that antibody avidity to a multivalent epitope was about five times higher than that to a monovalent epitope. This difference in antibody avidity indicated a multivalent effect in antibody and epitope interactions, which might have applications in antibody detection, serum analysis, and disease diagnosis.

Funding Information

This work is funded by the Major State Basic Research Development Program of China (2013CB910700) and the National Natural Science Foundation of China (21332006 and 21672126)

Acknowledgment

We thank Professor Hua Fu for helpful suggestions and discussions.

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1590796.

References and Notes

(1) (a) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754. (b) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555. (c) Fasting, C.; Schalley, C. A.; Weber, M.; Seitz, O.; Hecht, S.; Koksch, B.; Dernedde, J.; Graf, C.; Knapp, E.-W.; Haag, R. *Angew. Chem. Int. Ed.* **2012**, *51*, 10472.

(2) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 696.

(3) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Angew. Chem. Int. Ed.* **2006**, *45*, 2348.

(4) Darbre, T.; Reymond, J.-L. *Acc. Chem. Res.* **2006**, *39*, 925.

(5) Martínez, A.; Ortiz Mellet, C.; García Fernández, J. M. *Chem. Soc. Rev.* **2013**, *42*, 4746.

(6) Bernardi, A.; Jiménez-Barbero, J.; Casnati, A.; De Castro, C.; Darbre, T.; Fieschi, F.; Finne, J.; Funken, H.; Jaeger, K.-E.; Lahmann, M.; Lindhorst, T. K.; Marradi, M.; Messner, P.; Molinaro, A.; Murphy, P. V.; Nativi, C.; Oscarson, S.; Penadés, S.; Peri, F.; Pieters, R. J.; Renaudet, O.; Reymond, J.-L.; Richichi, B.; Rojo, J.; Sansone, F.; Schäffer, C.; Turnbull, W. B.; Velasco-Torrijos, T.; Vidal, S.; Vincent, S.; Wennekes, T.; Zuilhof, H.; Imberty, A. *Chem. Soc. Rev.* **2013**, *42*, 4709.

(7) (a) Niederhafner, P.; Šebestík, J.; Ježek, J. *J. Pept. Sci.* **2008**, *14*, 2. (b) Niederhafner, P.; Šebestík, J.; Ježek, J. *J. Pept. Sci.* **2008**, *14*, 44. (c) Niederhafner, P.; Reiniš, M.; Šebestík, J.; Ježek, J. *J. Pept. Sci.* **2008**, *14*, 556. (d) Chabre, Y. M.; Roy, R. *Curr. Top. Med. Chem. (Sharjah, United Arab Emirates)* **2008**, *8*, 1237. (e) Tam, J. P.; Lu, Y. A. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 9084.

(8) (a) Kadam, R. U.; Bergmann, M.; Hurley, M.; Garg, D.; Cacciarini, M.; Swiderska, M. A.; Nativi, C.; Sattler, M.; Smyth, A. R.; Williams, P.; Cámara, M.; Stocker, A.; Darbre, T.; Reymond, J.-L. *Angew. Chem. Int. Ed.* **2011**, *50*, 10631. (b) Reymond, J.-L.; Bergmann, M.; Darbre, T. *Chem. Soc. Rev.* **2013**, *42*, 4814.

(9) (a) Engelmann, K.; Baldus, S. E.; Hanisch, F. G. *J. Biol. Chem.* **2001**, *276*, 27764. 21. (b) Burchell, J.; Taylor-Papadimitrmiou, J.; Boshell, M.; Gendler, S.; Duhig, T. *Int. J. Cancer* **1989**, *44*, 691. (c) Ingale, S.; Wolfert, M. A.; Gaekwad, J.; Buskas, T.; Boons, G.-J. *Nat. Chem. Biol.* **2007**, *3*, 663. (d) Lakshminarayanan, V.; Thompson, P.; Wolfert, M. A.; Buskas, T.; Bradley, J. M.; Pathangey, L. B.; Madsen, C. S.; Cohen, P. A.; Gendler, S. J.; Boons, G.-J. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 261. (e) Gaidzik, N.; Westerlind, U.; Kunz, H. *Chem. Soc. Rev.* **2013**, *42*, 4421.

(10) (a) Becker, T.; Kaiser, A.; Kunz, H. *Synthesis* **2009**, 1113. (b) Keil, S.; Kaiser, A.; Syed, F.; Kunz, H. *Synthesis* **2009**, 1355. (c) Glaffig, M.; Palitzsch, B.; Hartmann, S.; Schüll, C.; Nuhn, L.; Gerlitzki, B.; Schmitt, E.; Frey, H.; Kunz, H. *Chem. Eur. J.* **2014**, *20*, 4232. (d) Glaffig, M.; Palitzsch, B.; Stergiou, N.; Schüll, C.; Straßburger, D.; Schmitt, E.; Frey, H.; Kunz, H. *Org. Biomol. Chem.* **2015**, *13*, 10150.

(11) (a) Ozawa, C.; Hojo, H.; Nakahara, Y.; Katayama, H.; Nabeshima, K.; Akahane, T.; Nakahara, Y. *Tetrahedron* **2007**, *63*, 9685. (b) Ozawa, C.; Katayama, H.; Hojo, H.; Nakahara, Y.; Nakahara, Y. *Org. Lett.* **2008**, *10*, 3531.

(12) (a) Cai, H.; Huang, Z.-H.; Shi, L.; Zhao, Y.-F.; Kunz, H.; Li, Y.-M. *Chem. Eur. J.* **2011**, *17*, 6396. (b) Cai, H.; Sun, Z.-Y.; Chen, M.-S.; Zhao, Y-. F.; Kunz, H.; Li, Y.-M. *Angew. Chem. Int. Ed.* **2014**, *53*, 1699.

(13) Nuhn, L.; Hartmann, S.; Palitzsch, B.; Gerlitzki, B.; Schmitt, E.; Zentel, R.; Kunz, H. *Angew. Chem. Int. Ed.* **2013**, *52*, 10652.

(14) Chun, C. K. Y.; Payne, R. J. *Aust. J. Chem.* **2009**, *62*, 1339.

(15) Kakwere, H.; Chun, C. K. Y.; Jolliffe, K. A.; Payne, R. J.; Perrier, S. *Chem. Commun.* **2010**, *46*, 2188.

(16) Skwarczynski, M.; Zaman, M.; Urbani, C. N.; Lin, I-C.; Jia, Z.; Batzloff, M. R.; Good, M. F.; Monteiro, M. J.; Toth, I. *Angew. Chem. Int. Ed.* **2010**, *49*, 5742.

(17) (a) Lee, D. J.; Yang, S.-H.; Williams, G. M.; Brimble, M. A. *J. Org. Chem.* **2012**, *77*, 7564. (b) Galan, M. C.; Dumy, P.; Renaudet, O. *Chem. Soc. Rev.* **2013**, *42*, 4599. (c) Cremer, G.-A.; Bureaud, N.; Piller, V.; Kunz, H.; Piller, F.; Delmas, A. F. *ChemMedChem* **2006**, *1*, 965.

(18) Geraci, C.; Consoli, G. M. L.; Granata, G.; Galante, E.; Palmigiano, A.; Pappalardo, M.; Di Puma, S. D.; Spadaro, A. *Bioconjugate Chem.* **2013**, *24*, 1710.

(19) (a) Faugeras, P.-A.; Boëns, B.; Elchinger, P.-H.; Brouillette, F.; Montplaisir, D.; Zerrouki, R.; Lucas, R. *Eur. J. Org. Chem.* **2012**, 4087. (b) Bellia, F.; La Mendola, D.; Pedone, C.; Rizzarelli, E.; Saviano, M.; Vecchio, G. *Chem. Soc. Rev.* **2009**, *38*, 2756.

(20) (a) Schaschke, N.; Fiori, S.; Weyher, E.; Escrieut, C.; Fourmy, D.; Müller, G.; Moroder, L. *J. Am. Chem. Soc.* **1998**, *120*, 7030. (b) Joshi, A.; Kate, S.; Poon, V.; Mondal, D.; Boggara, M. B.; Saraph, A.; Martin, J. T.; McAlpine, R.; Day, R.; Garcia, A. E.; Mogridge, J.; Kane, R. S. *Biomacromolecules* **2011**, *12*, 791. (c) Hjørringgaard, C. U.; Vad, B. S.; Matchkov, V. V.; Nielsen, S. B.; Vosegaard, T.; Nielsen, N. C.; Otzen, D. E.; Skrydstrup, T. *J. Phys. Chem. B* **2012**, *116*, 7652.

(21) (a) Cai, H.; Huang, Z.-H.; Shi, L.; Sun, Z.-Y.; Zhao, Y.-F.; Kunz, H.; Li, Y.-M. *Angew. Chem. Int. Ed.* **2012**, *51*, 1719. (b) Huang, Z.-H.; Shi, L.; Ma, J.-W.; Sun, Z.-Y.; Cai, H.; Chen, Y.-X.; Zhao, Y.-F.; Li, Y.-M. *J. Am. Chem. Soc.* **2012**, *134*, 8730.

(22) Dokurno, P.; Bates, P. A.; Band, H. A.; Stewart, L. M. D.; Lally, J. M.; Burchell, J. M.; Taylor-Papadimitriou, J.; Snary, D.; Sternberg, M. J. E.; Freemont, P. S. *J. Mol. Biol.* **1998**, *284*, 713.
(23) Zemplén, G.; Pascu, E. *Ber. Dtsch. Chem. Ges.* **1929**, *62*, 1613.
(24) Srinivasachari, S.; Fichter, K. M.; Reineke, T. M. *J. Am. Chem. Soc.* **2008**, *130*, 4618.
(25) Wang, F.; Fu, H.; Jiang, Y.; Zhao, Y. *Green Chem.* **2008**, *10*, 452.
(26) **Compound 1; Typical Procedure**
To a solution of thioanisole (20 µL 166 mol) in DMF (200 µL) was added CuBr (2.1mg, 15.0 µmol) to form a dark-green solution when the CuBr was fully dissolved. A solution of peptide **3** (13 mg; 15.2 µmol) in DMF (0.8 mL) was added to a solution of **per-6-azido-β-cyclodextrin** (2 mg; 1.5 µmol) in DMF (0.5 mL), and the soln was deoxygenated with N_2 gas. The dark-green catalyst solution was then carefully added to the peptide solution and the mixture was stirred at 65 °C for 2 h. The DMF was removed in vacuo, and the crude product was dissolved in 1:1 H_2O–MeCN (3 mL), purified by RP-HPLC (C18 column), and lyophilized to give a white powder; yield: 7.9 mg (1.1 µmol, 73%).
HRMS: *m/z* $[M + H]^+$ calcd for $C_{301}H_{476}N_{84}O_{126}$: 7284.3422; found (MALDI-TOF) 7285.5317; (ESI) $[M + 4H]^{4+}$ 1823.0, $[M + 5H]^{5+}$ 1458.4, $[M + 6H]^{6+}$ 1215.7, $[M + 7H]^{7+}$ 1042.1.
(27) **Compound 2**
Glycopeptide dendrimer **2** was obtained by a similar procedure to that of peptide dendrimer **1**, except that the reaction time was 10 h: yield: 9.4 mg (1.1 µmol, 59%).
HRMS: *m/z* $[M + H]^+$calcd for $C_{357}H_{567}N_{91}O_{161}$: 8705.8978; found: (MALDI-TOF): 8709.4877; (ESI): $[M + 5H]^{5+}$ 1743.5, $[M + 6H]^{6+}$ 1453.1, $[M + 7H]^{7+}$ 1245.5.
(28) (a) Dziadek, S.; Hobel, A.; Schmitt, E.; Kunz, H. *Angew. Chem. Int. Ed.* **2005**, *44*, 7630. (b) Cai, H.; Chen, M.-S.; Sun, Z.-Y.; Zhao, Y.-F.; Kunz, H.; Li, Y.-M. *Angew. Chem. Int. Ed.* **2013**, *52*, 6106.
(29) (a) Gao, Y.; Sun, Z.-Y.; Huang, Z.-H.; Chen, P.-G.; Chen, Y.-X.; Zhao, Y.-F.; Li, Y.-M. *Chem. Eur. J.* **2014**, *20*, 13541. (b) Liu, Y.-F.; Sun, Z.-Y.; Chen, P.-G.; Huang, Z.-H.; Gao, Y.; Shi, L.; Zhao, Y.-F.; Chen, Y.-X.; Li, Y.-M. *Bioconjugate Chem.* **2015**, *26*, 1439. (c) Sun, Z.-Y.; Chen, P.-G.; Liu, Y.-F.; Zhang, B.-D.; Wu, J.-J.; Chen, Y.-X.; Zhao, Y.-F.; Li, Y.-M. *Chem. Commun.* **2016**, *52*, 7572.