Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 9685–9690

Synthesis of glycopeptide dendrimer by a convergent method

Chinatsu Ozawa,[a] Hironobu Hojo,[a,*] Yuko Nakahara,[a] Hidekazu Katayama,[a] Kazuki Nabeshima,[b] Toshiaki Akahane[c] and Yoshiaki Nakahara[a,*]

[a]*Department of Applied Biochemistry, Institute of Glycotechnology, Tokai University, 1117 Kitakaname, Hiratsuka-shi, Kanagawa 259-1292, Japan*
[b]*Department of Pathology, Fukuoka University Hospial 7-45-1, Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan*
[c]*ATTO corporation, 1-25-23, Hongo, Bunkyo-ku, Tokyo 113-8425, Japan*

Received 7 June 2007; revised 9 July 2007; accepted 11 July 2007
Available online 15 July 2007

Abstract—Glycopeptide thioester comprising the sequence of extracellular matrix metalloproteinase inducer (emmprin) (34–58) was prepared and condensed with a dendrimer core having eight amino groups by the thioester method. The desired product, a glycopeptide dendrimer carrying an N-linked core pentasaccharide of about 30 kDa, was successfully isolated by preparative electrophoresis and characterized by mass analysis.

1. Introduction

Glycoprotein–receptor interaction plays essential roles in many biological processes, such as cell adhesion, leukocyte recruitment, and blood coagulation.[1] In addition, the interaction also mediates infection by viruses, parasites, and bacteria. Because of these important roles, glycoprotein mimics and glycoconjugates are attractive candidates for novel therapeutics. However, one of the major obstacles in achieving this goal is the general low-affinity interaction between sugar and its receptor. In nature, this weak interaction is strengthened by the multivalent interaction between the two components, for example, in lectin–sugar binding. Synthetic glycoconjugates can also utilize the effect of multivalency, such as in the form of glycodendrimers.[2] So far, various glycodendrimers have been prepared and used for binding experiments with lectins, to develop inhibitors to bacterial adhesion and mitosis. In contrast to these successful applications of glycodendrimers, glyco*peptide* dendrimers, which are a closer mimic of glycoproteins, have rarely been synthesized.[3] As glycopeptide dendrimers are expected to assume correct conformation around glycosylation sites, they will mimic functions of glycoproteins more precisely. In principle, glycopeptide dendrimers can be prepared by the stepwise elongation of glycopeptide chains on multiple antigenic peptide (MAP) resin.[4] This stepwise elongation usually gives defective products as the number of branches and the length of the peptide chain increase. In glycopeptide dendrimers, the introduction of bulky glycosylated amino acids is particularly incomplete, which makes the MAP method impractical to use. Thus, a convergent approach, in which the glycopeptide portion and branching unit are prepared separately, would be favorable. To couple the two components, several methods have been used, such as segment coupling by protected peptide,[5] oxime formation,[6] thiazolidine formation,[7] and native chemical ligation.[8] In this study, we attempted to synthesize glycopeptide dendrimer by the thioester method as shown in Figure 1, in which partially-protected peptide is condensed by the activation of the thioester linkage by silver ions.[9] Compared with native chemical ligation,[10] this method does not require cysteine residue at the ligation site, which eliminates the presence of multiple free thiol groups within the obtained dendrimer. As the synthesis of glycopeptide dendrimer of this size (~30 kDa) has not yet been achieved, we examined the coupling efficiency of the simultaneous introduction of eight glycopeptide chains to the polyamidoamine (PAMAM) dendrimer core and the purification method of the product.

2. Results and discussion

In this synthesis, a part of the extracellular first Ig domain of emmprin (34–58),[11] an extracellular matrix metalloproteinase (MMP) inducer, was used as an example. The first Ig domain (34–94) in its N-glycosylated form is essential for the stimulation of MMP production. Because multimerization of emmprin seems to be essential for activity, dendrimer

Keywords: Glycopeptide dendrimer; Solid-phase synthesis; Peptide thioester.
* Corresponding authors. Tel./fax: +81 463 50 2075; e-mail addresses: hojo@keyaki.cc.u-tokai.ac.jp; yonak@keyaki.cc.u-tokai.ac.jp

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.07.023

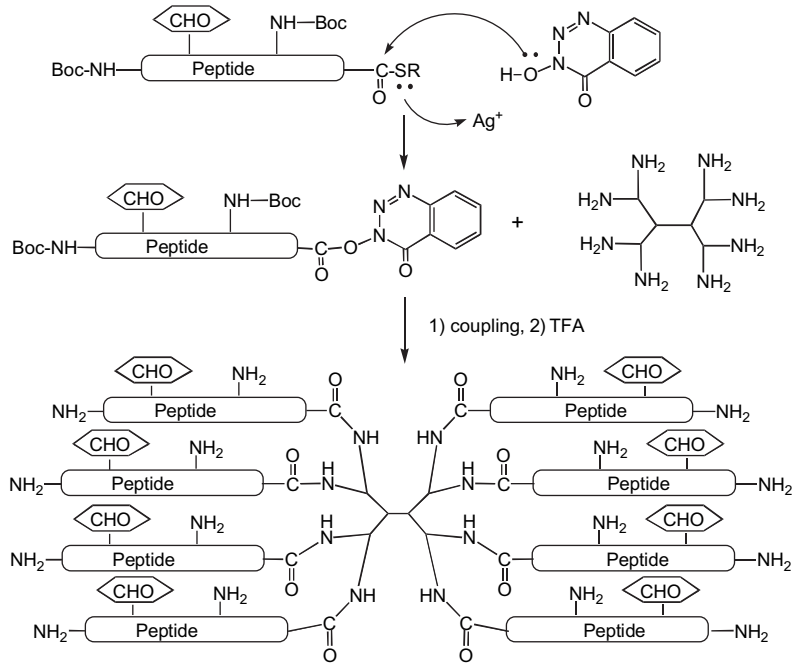

Figure 1. A scheme for the preparation of glycopeptide dendrimer by the thioester method.

composed of emmprin (34–58) carrying an N-linked core pentasaccharide at Asn[44] was prepared to analyze the multivalent effect on MMP stimulation activity. Cys[41], which originally forms a disulfide bond with Cys[87], was substituted with Ala in this synthesis.

The synthesis of the glycopeptide thioester was achieved as shown in Figure 2. Because of the acid-sensitivity of glycosidic linkages, especially the β-mannoside bond, the peptide was prepared by the modified Fmoc method for thioester preparation as described in our previous report.[11c] Fmoc–Gly–SCH_2CH_2COOH was condensed to CLEAR-amide resin by the *N*,*N*′-dicyclohexylcarbodiimide (DCC)-1-hydroxybenzotriazole (HOBt) method. The resin was treated with thioester-compatible Fmoc deblocking reagent, Reagent A[12] to remove the Fmoc group. Then, the second amino acid was introduced using triisopropylsilyloxycarbonyl (Tsoc)–Lys(Z)–OPfp, which was prepared from Boc–Lys(Z)–OPfp by Ohfune's conditions.[13] The third amino acid was introduced using Fmoc–Leu–F in the presence of

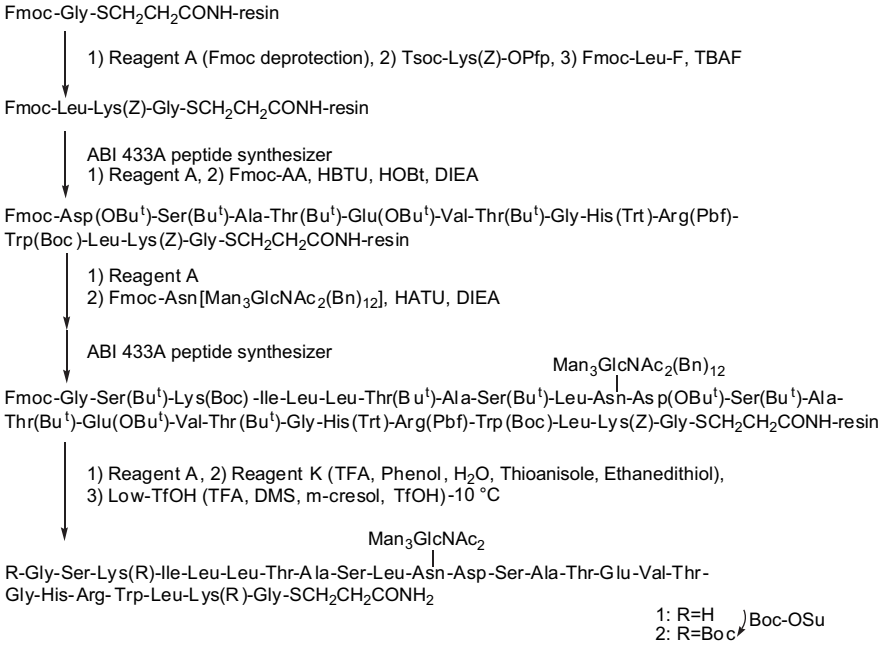

Figure 2. Synthetic route for glycosylated peptide thioester **2**.

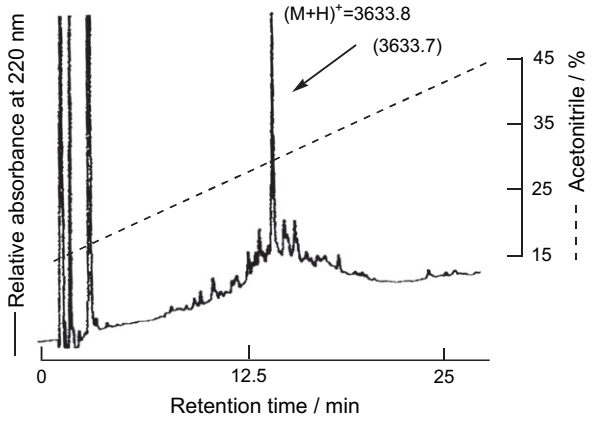

Figure 3. HPLC profile of the crude glycopeptide thioester **1**. Elution conditions: column, Mightysil RP-18 GP (4.6×150 mm, Kanto, Japan) at a flow rate of 1 ml/min; eluant, A, 0.1% TFA, B, acetonitrile containing 0.1% TFA.

a catalytic amount of *n*-tetrabutylammonium fluoride (TBAF). This F^- removes the Tsoc group to yield a free amino group, which is then immediately acylated by Fmoc–Leu–F with simultaneous regeneration of F^-. These procedures effectively suppress diketopiperazine formation at the dipeptide stage of the synthesis.[14] The resin was subjected to the automated synthesis by ABI 433A peptide synthesizer using FastMoc protocol, which uses *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) for the coupling of amino acids. Fmoc deprotection was performed by the premixed Reagent A. After assembling the emmprin (45–58), Fmoc–Asn($Man_3GlcNAc_2Bn_{12}$) was introduced by *O*-(7-azabenzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) at 50 °C. The coupling completed almost quantitatively within an hour. The remaining amino acids were again introduced by the synthesizer. After the complete assembly of the peptide chain, the resin was treated with Reagent K[15] to achieve deprotection of the peptide part. The crude peptide obtained by precipitation was treated with low-acidity TfOH[11c,16] to remove Z group of Lys residue next to the C-terminal Gly as well as benzyl groups at the carbohydrate portion. As shown in Figure 3, the desired product was obtained without significant decomposition at the carbohydrate portion. After purification by HPLC, the glycosylated peptide thioester **1** was obtained in 2% yield. This yield corresponds well with that of the previous synthesis.[11c] For subsequent coupling with dendrimer core by the thioester method, amino groups of

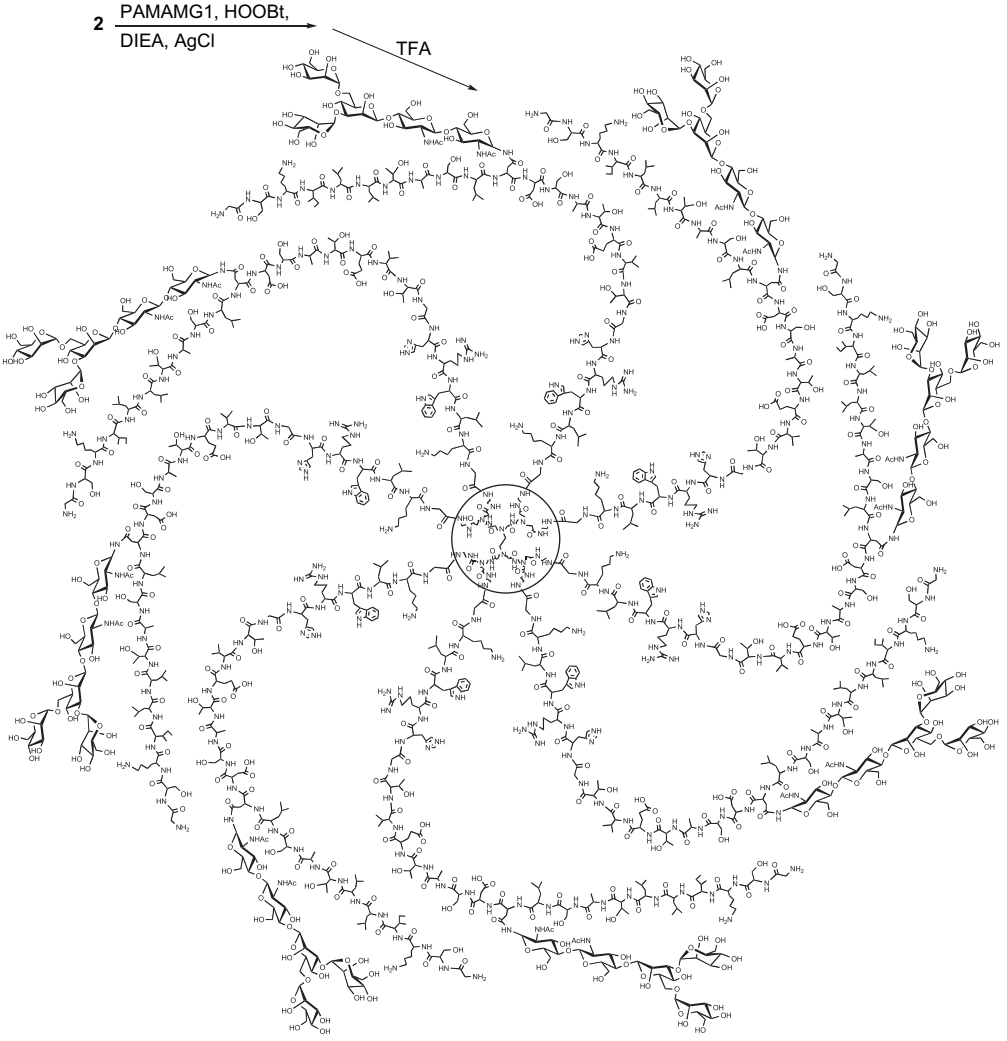

Figure 4. Synthetic route for glycopeptide dendrimer **3**.

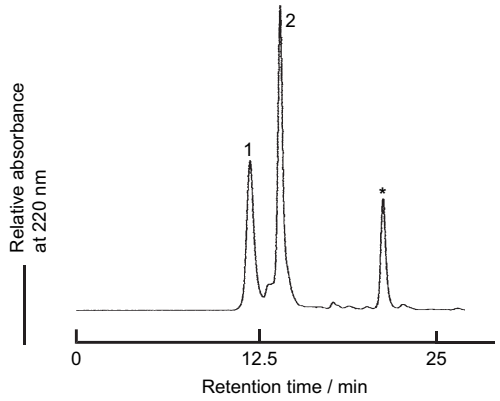

Figure 5. GFC profile of crude glycopeptide dendrimer **3**. Elution conditions: column, G3000SW$_{XL}$ (7.5×300 mm, TOSOH, Japan) at a flow rate of 0.5 ml/min; eluant 50% aq acetonitrile containing 0.1% TFA. The asterisked fraction is non-peptidic components.

the product were protected using *N*-(*tert*-butoxycarbonyloxy)-succinimide (Boc–OSu) in the presence of *N*,*N*-diisopropylethylamine (DIEA). The reaction completed within 4 h without serious side reactions and the desired product **2** was obtained quantitatively by precipitation.

The glycosylated peptide thioester **2** was then used for dendrimer synthesis by the thioester method (Fig. 4). Compound **2** (2 equiv to each hand of the dendrimer core) and PAMAM dendrimer G1 were dissolved in DMSO, and the thioester group was activated by AgCl. After overnight reaction, a new peak (peak 1) appeared before the elution position of monomer **2** (peak 2) on gel filtration chromatography (GFC) as shown in Figure 5, indicating the progress of the reaction. MALDI-TOF mass analysis of peak 1 (Fig. 6a) indicated that the desired product was successfully obtained. However, the mass data also showed that defective dendrimers lacking several glycopeptide chains coeluted at this position. Attempts to remove these side products by reverse-phase HPLC failed because of the highly adsorptive nature of the product to the column. Thus, we examined

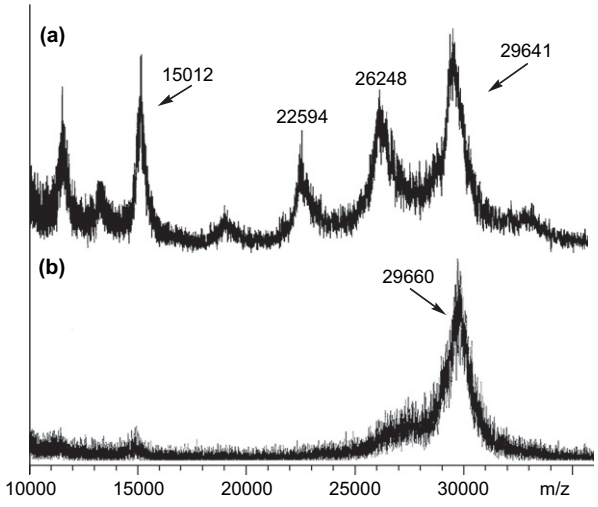

Figure 6. MALDI-TOF mass analysis of dendrimer **3**: (a) GFC-purified dendrimer **3**, (b) after preparative SDS-PAGE purification. Mass numbers indicated are observed values.

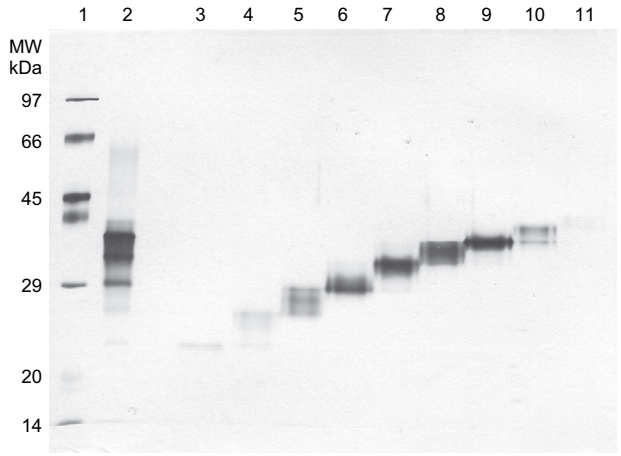

Figure 7. SDS-PAGE of the synthetic glycodendrimer. Lane 1: m.w. standard, lane 2: dendrimer **3** after GFC purification, lane 3: frac. 5 of prep. PAGE, lane 4: frac. 6, lane 5: frac. 7, lane 6: frac. 8, lane 7: frac. 9, lane 8: frac. 10, lane 9: frac. 11, lane 10: frac. 12, lane 11: frac.13.

another method for separation and found that SDS-PAGE retained sufficient resolution to remove these impurities. As shown in Figure 7, lane 2, GFC-purified sample (peak 1) gave three major bands, which correspond to the desired product **3** and defective dendrimers lacking one or two glycopeptide chains. From the comparison of the density of each band, the content of the product **3** in the GFC-purified sample was roughly estimated to be 34%. Then, the separation of each band was carried out by preparative electrophoresis using Prepforesis S (ATTO, Tokyo). The eluted samples were separated into 0.8 ml fractions and analyzed again by SDS-PAGE as shown in Figure 7, lane 3–11 (fractions 5 through 13). From mass analysis of these fractions, it was shown that fractions 5 (lane 3), 8 (lane 6), 9 (lane 7), and 11 (lane 9) contained highly pure dendrimers having 4, 6, 7, and 8 glycopeptide chains, respectively. Other fractions were composed of mixtures of two defective dendrimers. The result of the mass analysis of fraction 11 (lane 9) is shown in Figure 6b, indicating the successful purification of the desired glycodendrimer **3**. Amino acid analysis of the acid hydrolysis of the product **3** (see Section 4) also supported the success of the synthesis. Thus, we successfully completed the synthesis of highly pure glycopeptide dendrimer of about 30 kDa.

3. Conclusion

In conclusion, glycosylated peptide thioester was prepared by the solid-phase method and used to synthesize glycopeptide dendrimer having eight glycopeptide chains by the thioester method. Although the yield was not quantitative, the desired glycodendrimer with a molecular weight of about 30 kDa was successfully isolated and characterized. The result in this synthesis shows that the quantitative introduction of eight glycopeptide chains to the dendrimer core is difficult. Thus, a novel method, in which dendrimers with several glycopeptide chains can be cross-linked together at a specific position, has to be developed to achieve a more efficient preparation of larger glycopeptide dendrimers. Further studies are being preformed to this end.

4. Experimental

4.1. General

Reagent A (*N*-methylpyrrolidine–hexamethyleneimine–HOBt–NMP/DMSO, 25:2:2:71) was prepared according to Ref. 12. Amino acid composition was determined with a Lachrom amino acid analyzer (Hitachi, Tokyo) after hydrolysis with 6 M HCl at 150 °C for 2 h in an evacuated sealed tube. The amount of the peptide was calculated based on the data of the amino acid analysis. MALDI-TOF mass measurement was performed by Voyager DE-Pro (Applied Biosystems, CA) or Autoflex (Bruker Daltonics, MA). PAMAM dendrimer generation 1 (ethylenediamine core) was purchased from Sigma–Aldrich (MO).

4.2. [Ala^{41}, Asn(Man$_3$GlcNAc$_2$)^{44}]–emmprin (34–58)–SCH$_2$CH$_2$CONH$_2$ 1

Fmoc-CLEAR-amide resin (0.29 mmol, 640 mg, 0.46 mmol/g) was treated with 20% piperidine for 5 and 15 min. After washing with 1-methyl-2-pyrrolidinone (NMP), Fmoc–Gly–SCH$_2$CH$_2$COOBt (0.6 mmol), which was prepared by mixing Fmoc–Gly–SCH$_2$CH$_2$COOH (230 mg, 0.6 mmol), 1 M DCC/NMP (0.6 ml), and 1 M HOBt/NMP (0.6 ml) for 30 min at room temperature, was added and the reaction mixture was vortexed for 1 h. The resin was washed with NMP and treated with Reagent A for 2 and 5 min. After NMP washing, the resin was reacted with Tsoc–Lys(Z)–OPfp (280 mg, 0.43 mmol) in THF for 15 min. After washing with THF, the reaction was repeated using the same amount of Tsoc–Lys(Z)–OPfp. The resin was washed with CH_2Cl_2. Fmoc–Leu–F (0.53 mmol), prepared by mixing Fmoc–Leu (190 mg, 0.53 mmol), fluoro-*N*,*N*,*N*′,*N*′-tetramethylformadinium hexafluorophosphate (140 mg, 0.53 mmol), and DIEA (0.19 ml, 1.1 mmol) in CH_2Cl_2, was then added. The reaction was initiated by adding 1 M TBAF in THF (22 μl) to the reaction mixture. After vortexing for 1 h, the resin was washed with CH_2Cl_2 and NMP. The resin was then subjected to automated synthesis by ABI 433A peptide synthesizer using FastMoc protocol. The Fmoc deprotection protocol was modified so that the premixed Reagent A was introduced to the reaction vessel without dilution by NMP. After the synthesis of emmprin (45–58) was completed, part of the resin was taken and the remaining resin was stored. The resin (11 μmol) was treated with Reagent A for 5 and 15 min. After washing with NMP, Fmoc–Asn(Man$_3$GlcNAc$_2$Bn$_{12}$) (50 mg, 22 μmol), HATU (8.2 mg, 22 μmol), and DIEA (4.9 μl, 28 μmol) were added to the resin. The reaction mixture was vortexed for 1 h at 50 °C. The remaining amino acids were introduced by the peptide synthesizer. After completion of the chain assembly, Gly-Ser(^{t}Bu)-Lys(Boc)-Ile-Leu-Leu-Thr(^{t}Bu)-Ala-Ser(^{t}Bu)-Leu-Asn(Man$_3$GlcNAc$_2$Bn$_{12}$)-Asp(O^{t}Bu)-Ser(^{t}Bu)-Ala-Thr(^{t}Bu)-Glu(O^{t}Bu)-Val-Thr(^{t}Bu)-Gly-His(Trt)-Arg(Pbf)-Trp(Boc)-Leu-Lys(Boc)-Gly-SCH$_2$CH$_2$CONH-resin (72 mg) was obtained. The resin (72 mg) was treated with Reagent K (1.0 ml) for 1 h at room temperature. TFA was removed under nitrogen stream and the peptide was precipitated with ether and washed twice with ether. The precipitate was further treated with low-acidity TfOH (0.7 ml) for 2 h at −10 °C. The peptide was precipitated with ether, washed successively with ether containing 1% pyridine and ether, and dried in vacuo. The crude peptide was purified by reverse-phase HPLC using aq acetonitrile containing 0.1% TFA to obtain glycopeptide thioester **1** (0.73 mg, 0.2 μmol, 1.8% yield). MALDI-TOF mass, found: *m*/*z* 3633.8 (M+H)$^+$, calcd: 3633.7 (M+H)$^+$. Amino acid analysis: Asp$_{2.09}$Thr$_{2.90}$Ser$_{2.68}$-Glu$_{1.06}$Gly$_3$Ala$_{2.06}$Val$_{1.52}$Ile$_{0.87}$ Leu$_{4.00}$Lys$_{1.97}$His$_{0.99}$Arg$_{0.97}$.

4.3. Boc–[Ala^{41}, Asn(Man$_3$GlcNAc$_2$)^{44}, Lys(Boc)^{36,57}]–emmprin (34–58)–SCH$_2$CH$_2$CONH$_2$ 2

Glycopeptide thioester **1** (0.73 mg, 0.2 μmol) was dissolved in DMSO (10 μl), and Boc–OSu (0.54 mg, 2.5 μmol) and DIEA (0.4 μl, 2.3 μmol) were added. After the reaction mixture had stood for 4 h at room temperature, the product was precipitated by adding ethyl acetate to the reaction mixture, washed thrice with ethyl acetate, and lyophilized from dioxane suspension to give **2** (0.79 mg, 0.2 μmol). MALDI-TOF mass, found: *m*/*z* 3933.5 (M+H)$^+$, calcd: 3933.9 (M+H)$^+$. Amino acid analysis: Asp$_{2.13}$Thr$_{2.90}$Ser$_{2.72}$Glu$_{1.09}$Gly$_3$Ala$_{2.06}$-Val$_{1.54}$Ile$_{0.87}$Leu$_{4.00}$Lys$_{2.05}$His$_{0.99}$Arg$_{0.96}$.

4.4. {[Ala^{41}, Asn(Man$_3$GlcNAc$_2$)^{44}]–emmprin (34–58)}$_8$–PAMAM dendrimer 3

Glycosylated peptide thioester **2** (2.0 mg, 0.5 μmol) and PAMAM dendrimer generation 1 in methanol (0.2 μl, 30 nmol) were dissolved in DMSO (35 μl), and 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HOOBt) (2.3 mg, 14 μmol), DIEA (1.7 μl, 9.5 μmol), and AgCl (0.3 mg, 2.4 μmol) were added. The reaction mixture was stirred overnight at room temperature in the dark. AgCl was removed by centrifugation and the product was precipitated by ether. The residue was washed with ether three times and dried in vacuo. The residue was dissolved in TFA containing 5% EDT (100 μl) and stood at room temperature for 10 min. TFA was removed under nitrogen stream and the product was precipitated with ether, washed thrice with ether and dried in vacuo. The crude dendrimer was dissolved in 50% aq acetonitrile and purified by GFC using G3000PW$_{XL}$ (7.5× 300 mm) to give partly purified product **3** (0.9 mg, 30 nmol). Part of the concentrate (150 μg) was then subjected to non-reduced preparative SDS-PAGE in a 10% gel using Prepforesis S (ATTO, Tokyo). The eluted samples were separated into 48 fractions (0.8 ml each). The fractions were then desalted by GFC using 50% aq acetonitirile containing 0.1% TFA and lyophilized. The yield of the purified product obtained from fraction 11 (Fig. 7) was 10 μg. MALDI-TOF mass, found: *m*/*z* 29,660 (M+H)$^+$ (average), calcd: 29,669 (M+H)$^+$. Amino acid analysis: Asp$_{16.14}$Thr$_{22.16}$Ser$_{21.11}$Glu$_{8.07}$-Gly$_{26.66}$Ala$_{16}$Val$_{13.13}$Ile$_{7.00}$ Leu$_{30.46}$Lys$_{15.67}$His$_{7.48}$Arg$_{7.55}$.

Acknowledgements

We thank Dr. Emi Ito, Dr. Yusuke Suzuki, Prof. Minoru Suzuki, and Prof. Akemi Suzuki at RIKEN for mass analysis. This work was partly supported by Grant-Aid for Scientific Research from the Ministry of Education, Culture, Sport, Sciences and Technology of Japan. We thank Tokai University for a grant-in-aid for high-technology research. We also thank the Japan Society for the Promotion of Science for a Grant-in-Aid for Creative Scientific Research (No. 17GS0420). This work was also supported by the Program

for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN). We thank Mr. Junji Nakagawa for discussions about preparative SDS-PAGE.

References and notes

1. (a) Varki, A. *Glycobiology* **1993**, *3*, 97–130; (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720.
2. (a) Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391–5393; (b) Grandjean, C.; Rommens, C.; Gras-Masse, H.; Melnyk, O. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2967–2975; (c) Dimick, S. M.; Powell, S. C.; McMahon, S. A.; Moothoo, D. N.; Naismith, J. H.; Toone, E. J. *J. Am. Chem. Soc.* **1999**, *121*, 10286–10296; (d) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669–672; (e) Merritt, E. A.; Zhang, Z.; Pickens, J. C.; Ahn, M.; Hol, W. G. J.; Fan, E. *J. Am. Chem. Soc.* **2002**, *124*, 8818–8824; (f) Benito, J. M.; Gomez-Garcia, M.; Mellet, C. O.; Baussanne, I.; Defaye, J.; Garcia Fernandez, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 10355–10363; (g) Kalovidouris, S. A.; Blixt, O.; Nelson, A.; Vidal, S.; Turnbull, W. B.; Paulson, J. C.; Stoddart, J. F. *J. Org. Chem.* **2003**, *68*, 8485–8493; (h) Kensinger, R. D.; Yowler, B. C.; Benesi, A. J.; Schengrund, C.-L. *Bioconjugate Chem.* **2004**, *15*, 349–358; (i) Lee, N.-Y.; Jang, W.-J.; Yu, S.-H.; Im, J.; Chung, S.-K. *Tetrahedron Lett.* **2005**, *46*, 6063–6066; (j) Appeldoorn, C. C. M.; Joosten, J. A. F.; el Maate, F. A.; Dobrindt, U.; Hacker, J.; Liskamp, R. M. J.; Khan, A. S.; Pieters, R. J. *Tetrahedron: Asymmetry* **2005**, *16*, 361–372; (k) Srinivas, O.; Radhika, S.; Bandaru, N. M.; Nadimpalli, S. K.; Jayaraman, N. *Org. Biomol. Chem.* **2005**, *3*, 4252–4257; (l) Rele, S. M.; Cui, W.; Wang, L.; Hou, S.; Barr-Zarse, G.; Tatton, D.; Gnanou, Y.; Esko, J. D.; Chaikof, E. L. *J. Am. Chem. Soc.* **2005**, *127*, 10132–10133; (m) Wolfenden, M. L.; Cloninger, M. J. *J. Am. Chem. Soc.* **2005**, *127*, 12168–12169; (n) Dubber, M.; Sperling, O.; Lindhorst, T. K. *Org. Biomol. Chem.* **2006**, *4*, 3901–3912; (o) Han, S.; Baigude, H.; Hattori, K.; Yoshida, T.; Uryu, T. *Carbohydr. Polym.* **2007**, *68*, 26–34. Also see following reviews on glycodendrimer: (p) Turnbull, W. B.; Stoddart, J. F. *J. Biotechnol.* **2002**, *90*, 231–255; (q) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578; (r) Bezouska, K. *J. Biotechnol.* **2002**, *90*, 269–290; (s) Roy, R.; Baek, M.-G. *J. Biotechnol.* **2002**, *90*, 291–309; (t) Roy, R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 291–310; (u) Schengrund, C.-L. *Biochem. Pharmacol.* **2003**, *65*, 699–707; (v) Pieters, R. J. *Trends Glycosci. Glycotechnol.* **2004**, *16*, 243–254.
3. (a) Jezek, J.; Velek, J.; Veprek, P.; Velkova, V.; Trnka, T.; Pecka, J.; Ledvina, M.; Vondrasek, J.; Pisacka, M. *J. Pept. Sci.* **1999**, *5*, 46–55; (b) Zhang, Z.; Liu, J.; Verlinde, C. L. M. J.; Hol, W. G. J.; Fan, E. *J. Org. Chem.* **2004**, *69*, 7737–7740; (c) Lagnoux, D.; Darbre, T.; Schmitz, M. L.; Reymond, J.-L. *Chem.—Eur. J.* **2005**, *11*, 3941–3950; (d) Renaudet, O.; Dumy, P. *Org. Biomol. Chem.* **2006**, *4*, 2628–2636; (e) Veprek, P.; Hajduch, M.; Dzubak, P.; Kuklik, R.; Polakova, J.; Bezouska, K. *J. Med. Chem.* **2006**, *49*, 6400–6407.
4. (a) Tam, J. P. *Proc. Natl. Acad. Sci. U.S.A* **1988**, *85*, 5409–5413; (b) Sadler, K.; Tam, J. P. *J. Biotechnol.* **2002**, *90*, 195–229.
5. McLean, G. W.; Cross, A. M.; Munns, M. S.; Marsden, H. S. *J. Immunol. Methods* **1992**, *155*, 113–120.
6. Mitchell, J. P.; Roberts, K. D.; Langley, J.; Koentgen, F.; Lambert, J. N. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2785–2788.
7. Chaves, F.; Calvo, J. C.; Carvajal, C.; Rivera, Z.; Ramirez, L.; Pinto, M.; Trujillo, M.; Guzman, F.; Patarroyo, M. E. *J. Peptide Res.* **2001**, *58*, 307–316.
8. Dirksen, A.; Meijer, E. W.; Adriaens, W.; Hackeng, T. M. *Chem. Commun.* **2006**, 1667–1669.
9. (a) Hojo, H.; Aimoto, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 111–117; (b) Aimoto, S. *Biopolymer (Pept. Sci.)* **1999**, *51*, 247–265.
10. (a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776–779; (b) Dawson, P. E.; Kent, S. B. H. *Annu. Rev. Biochem.* **2000**, *69*, 923–960.
11. (a) Nabeshima, K.; Iwasaki, H.; Koga, K.; Hojo, H.; Suzumiya, J.; Kikuchi, M. *Pathol. Int.* **2006**, *56*, 359–367; (b) Hojo, H.; Watabe, J.; Nakahara, Y.; Nakahara, Y.; Ito, Y.; Nabeshima, K.; Toole, B. P. *Tetrahedron Lett.* **2001**, *42*, 3001–3004; (c) Hojo, H.; Haginoya, E.; Matsumoto, Y.; Nakahara, Y.; Nabeshima, K.; Toole, B. P.; Watanabe, Y. *Tetrahedron Lett.* **2003**, *44*, 2961–2964.
12. Li, X.; Kawakami, T.; Aimoto, S. *Tetrahedron Lett.* **1998**, *39*, 8669–8672.
13. Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870–876.
14. Sakamoto, K.; Nakahara, Y.; Ito, Y. *Tetrahedron Lett.* **2002**, *43*, 1515–1518.
15. King, D. S.; Fields, C. G.; Fields, G. B. *Int. J. Pept. Protein Res.* **1990**, *36*, 255–266.
16. Tam, J. P.; Heath, W. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1986**, *108*, 5242–5251.