

Synthesis of Glycopeptide Dendrimer by the Thioester Method

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Introduction

Glycoprotein-receptor interactions play various important roles in many biological processes including cell-cell interaction and viral infection. Consequently, glycoprotein mimics and glycoconjugates have been regarded as potential 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. To overcome this problem, glycoconjugates are often prepared multivalently, such as in the form of glycodendrimers. These glycodendrimers have been used for binding experiments with lectins to develop inhibitors of bacterial adhesion and mitosis. In contrast, glycopeptide dendrimers, which are better mimics of glycoproteins, have rarely been synthesized. As glycopeptide dendrimers are expected to assume correct conformation around glycosylation sites, they will mimic functions of glycoproteins more precisely. In this study, we attempted the synthesis of glycopeptide dendrimer by the thioester method [1] using a part of the sequence of the extracellular first Ig domain of emmprin (34-58), an extracellular matrix metalloproteinase (MMP) inducer as a model (Fig. 1).

Results and Discussion

The synthesis of glycopeptide thioester corresponding to the sequence of emmprin (34-58) was achieved as shown in Fig. 2. In this synthesis, Cys⁴¹ of emmprin, which originally forms a disulfide bond with Cys⁸⁷, was substituted with Ala. Because glycosidic linkages are labile to strong acids, such as HF, the peptide was prepared by the modified Fmoc method for thioester preparation as described in our previous report [2]. Fmoc-Gly-SCH₂CH₂-

COOH was coupled to CLEAR-amide resin by the DCC-HOBt method. Then, the Fmoc group was removed with thioester-compatible Fmoc deblocking reagent, Reagent A [3]. The second amino acid was introduced using triisopropylsilyloxycarbonyl (Tsoc)-Lys(Z)-OPfp. The third amino acid was introduced using Fmoc-Leu-F in the presence of a catalytic amount of TBAF. This F⁻ removes the Tsoc group to yield a free amino group, which is immediately acylated by Fmoc-Leu-F with simultaneous regeneration of F⁻. These procedures effectively suppress diketopiperazine formation at the dipeptide stage of the synthesis [4]. Using the resin, the peptide chain was elongated by ABI 433A peptide synthesizer using FastMoc protocol, except that the Fmoc deprotection was performed by the premixed Reagent A. After assembling the sequence of emmprin (45-58), Fmoc-Asn(Man₃GlcNAc₂Bn₁₂) was introduced by HATU in the presence of DIEA at 50 °C. The coupling completed almost quantitatively within an hour. The remaining amino acids were introduced by the synthesizer. After the complete assembly of the peptide chain, the resin was treated with Reagent K [5] to achieve deprotection of the peptide part. The crude peptide obtained by precipitation with ether was treated with low-acidity TFOH [6] to remove Z group of Lys residue next to the C-terminal Gly as well as benzyl groups at the carbohydrate portion. The desired product was obtained without significant decomposition at the carbohydrate portion. After purification by HPLC, the glycosylated peptide thioester 3 was obtained in 2% yield. For subsequent coupling with dendrimer core by the thioester method, amino groups of the product were protected using Boc-OSu in the presence of DIEA. The reaction was completed within 4 h without serious side reactions and the

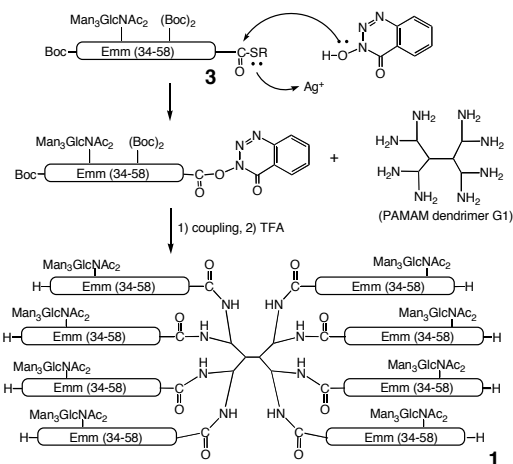


Fig. 1. Preparation of glycopeptide dendrimer 1 by the thioester method.

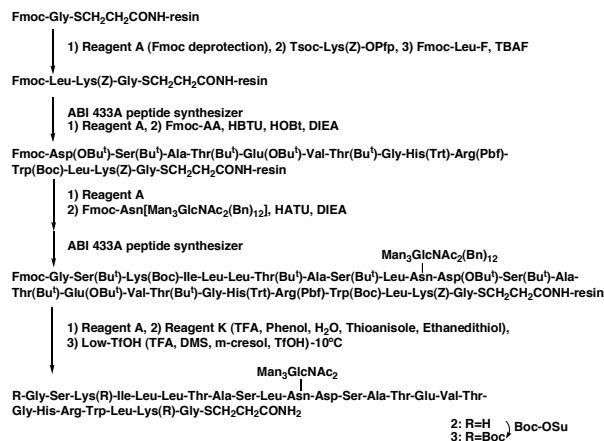


Fig. 2. Synthetic route for glycosylated peptide thioester 3.

desired product **3** was obtained quantitatively by precipitation.

The glycosylated peptide thioester **3** was then used for dendrimer synthesis by the thioester method as shown in Fig. 1. Compound **3** (2 eq to each hand of the dendrimer core) and PAMAM dendrimer G1, which has eight amino groups, were dissolved in DMSO, and the thioester group was activated by AgCl. After overnight reaction, a new peak appeared before the elution position of monomer **3** on GFC, indicating the progress of the reaction. MALDI-TOF mass analysis of the new peak indicated that the desired product was successfully obtained. However, the mass data also showed that defective dendrimers lacking several glycopeptide chains coeluted at the peak. Removal of these side products was not achieved by reverse-phase HPLC, because of the highly adsorptive nature of the product to the column and of the incomplete resolution. We examined another separation method and found that SDS-PAGE retained sufficient resolution. The analysis of the GFC-purified sample by SDS-PAGE showed that the sample contained three major components, which correspond to the desired product **1** and defective dendrimers lacking one or two glycopeptide chains. From the comparison of the density of each band, the content of the product **1** in the GFC-purified sample was roughly estimated to be 34%. Then, the separation of each band was carried out by preparative electrophoresis using Prepforexis-S (ATTO, Tokyo). The eluted samples were separated into 0.8 ml fractions and analyzed again by SDS-PAGE. This separation successfully removed defective dendrimers and the desired glycodendrimer **1** was obtained in high purity. The result of the MALDI-TOF mass analysis (found: m/z 29660 (M+H)⁺ (average); calcd: 29669 (M+H)⁺) as well as amino acid analysis of the acid hydrolysis of the product **1** (Asp_{16,14}Thr_{22,16}Ser_{21,11}Glu_{8,07}Gly_{26,66}Ala₁₆Val_{13,13}Ile_{7,00}Leu_{30,46}Lys_{15,67}His_{7,48}Arg_{7,55}) also supported the success of the synthesis. Thus, we successfully completed the synthesis of highly pure glycopeptide dendrimer of about 30 kDa [7]. The results of this synthesis show that the quantitative introduction of 8 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.

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