Glycopeptide Synthesis

Molecular Transporter Between Polymer Platforms: Highly Efficient Chemoenzymatic Glycopeptide Synthesis by the Combined Use of Solid-Phase and Water-Soluble Polymer Supports**

*Masataka Fumoto, Hiroshi Hinou, Takahiko Matsushita, Masaki Kurogochi, Takashi Ohta, Takaomi Ito, Kuriko Yamada, Akio Takimoto, Hirosato Kondo, Toshiyuki Inazu, and Shin-Ichiro Nishimura**

Polymer-supported synthesis is a practical and convenient method because it simplifies purification of the final products and makes combinatorial processes feasible. Chemical synthesis on solid-phase polymers made the automated synthesis of nucleotides (DNA/RNA) and peptides (proteins) possible,[1,2] and they are now indispensable devices for the investigation of the functional roles of genomes and proteins as well as the development of a variety of therapeutic reagents. Chemical synthesis of glycoconjugates, however, is a much more difficult task than the synthesis of nucleic acids or polypeptides because complex structures of glycoconjugates require extremely time-consuming and tedious procedures of regioselective protection and stereoselective glycosylation reactions. Although progress in solid-phase chemical synthesis meanwhile allowed the construction of a variety of oligosaccharides,[3] these methods still entail limitation of the target structures and technical difficulty for general biochemists or medical scientists. Enzymatic synthesis is a potential alternative to the chemical synthesis of complex oligosaccharides because of the specificities of both the stereochemistry and regioselectivity in the glycosylation reactions.[4] However, glycosyl acceptor substrates immobilized on solid supports are not suited for these enzymatic reactions in terms of efficiency and versatility in practical synthesis.

In the course of our studies of enzymatic synthesis, based on the cluster effect[5] of sugar-attached water-soluble polymers as multivalent acceptor substrates,[6] our interest has been focused on the efficient synthesis of glycopeptides as important signal molecules in cellular recognition.[7] To achieve a concerted and efficient glycopeptide synthesis based on a combined chemical and enzymatic strategy, we thought that the advent of an appropriate methodology to combine solid-phase peptide synthesis and liquid-phase carbohydrate synthesis was highly desirable.[4] Herein, we report a novel strategy of rapid and efficient synthesis of glycopeptides by using a convenient "molecular transporter" that interfaces two different polymer supports.

Our synthetic strategy is summarized in Figure 1 as follows: a) solid-phase synthesis of the photosensitive *O*-GlcNAc-peptides terminated by the molecular transporter **1**, b) deprotection and release of the transporter from the resin, c) chemoselective blotting of the molecular transporter that carries glycopeptide primers using a water-soluble polymer with alkoxyamino functional groups,[8] d) one-pot sugar elongation with glycosyltransferases, and e) release of full-length glycopeptides from the transporter on the polymer platform with a photoselective cleavage reaction.

The molecular transporter **1** was synthesized from L-proline, which was to be used as the N-terminal residue of the target peptide, by modification with a reactive ketone group with a photolabile linker moiety, 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy]butyric acid[9] (see the Supporting Information).[10] This heterobifunctional linker acts as a transporter between the two different polymer platforms and allows both chemoselective blotting and photoselective cleavage (catch and release) of glycopeptides (Figure 1, steps c and e).

We demonstrated the feasibility of our method by constructing a dodecapeptide with a sialyl Lewis^X tetrasaccharide residue (**7**) as a model compound. Scheme 1 shows the solid-phase synthesis of a transporter molecule carrying an intermediate glycopeptide (**3**) on a Fmoc-Arg(Pbf)-NovaSynTGA resin using Fmoc-protected amino acids (Fmoc-AA) and Fmoc-Ser(Ac$_3$GlcNAcβ)-OH.[11] Next, the intermediate on the transporter (**3**) was blotted onto the water-soluble polyacrylamide derivative **4**[8] by chemoselective ligation of the ketone group of **3** with the alkoxyamino group of **4** without any purification process. We suggest that this blotting step using polymer **4**, which we proved is completed in three hours at room temperature under mild conditions (pH 5.0–5.5) by monitoring with reversed-phase (RP) HPLC (Figure 2), is crucial. As anticipated, polymer **4**

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[*] Dr. H. Hinou, Dr. T. Ohta, Prof. S.-I. Nishimura
Glycochemosynthesis Team, Research Center for Glycoscience
National Institute of Advanced Industrial Science and Technology (AIST)
Sapporo 062-8517 (Japan)
Fax: (+81) 11-857-8441
E-mail: tiger.nishimura@aist.go.jp

T. Matsushita, Dr. M. Kurogochi, Prof. S.-I. Nishimura
Division of Biological Sciences, Graduate School of Science
Hokkaido University, Sapporo 001-0021, Japan
E-mail: shin@glyco.sci.hokudai.ac.jp

M. Fumoto, Dr. K. Yamada
Research Association for Biotechnology
Minato-ku, Tokyo 105-0003 (Japan)

M. Fumoto, T. Ito, Dr. A. Takimoto, Dr. H. Kondo
Shionogi & Co. Ltd.
Chuo-ku, Osaka 541-0045 (Japan)

Dr. K. Yamada
Hitachi High-Technologies Co.
Minato-ku, Tokyo 105-8717 (Japan)

Dr. T. Inazu
Department of Applied Chemistry, School of Engineering, and Institute of Glycotechnology
Tokai University, Kanagawa 259-1292 (Japan)

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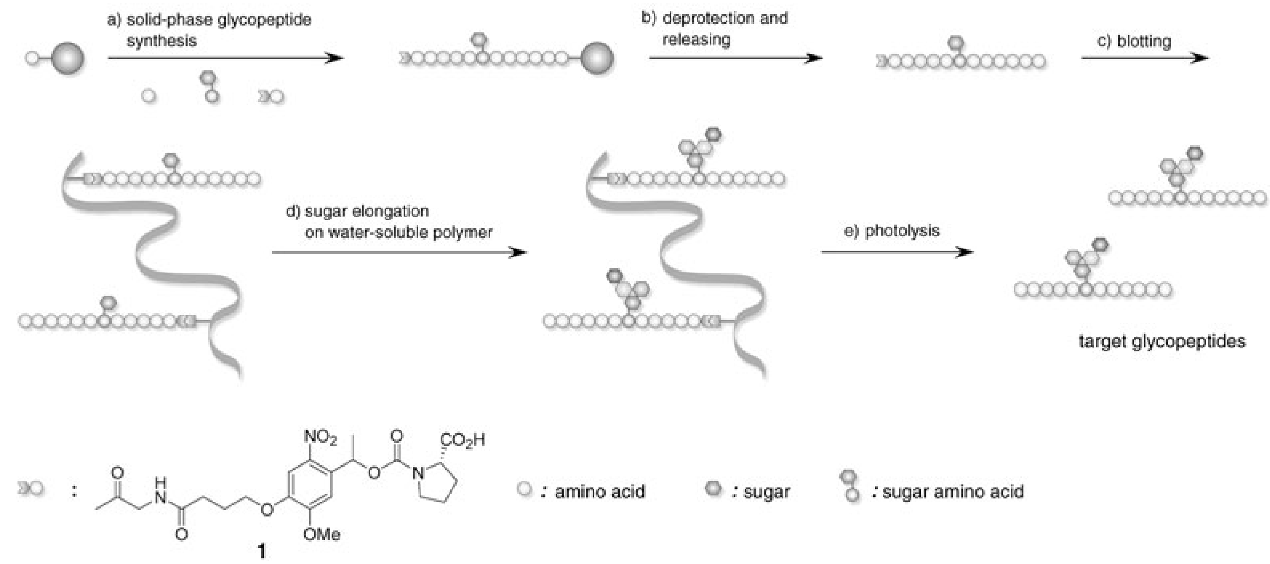

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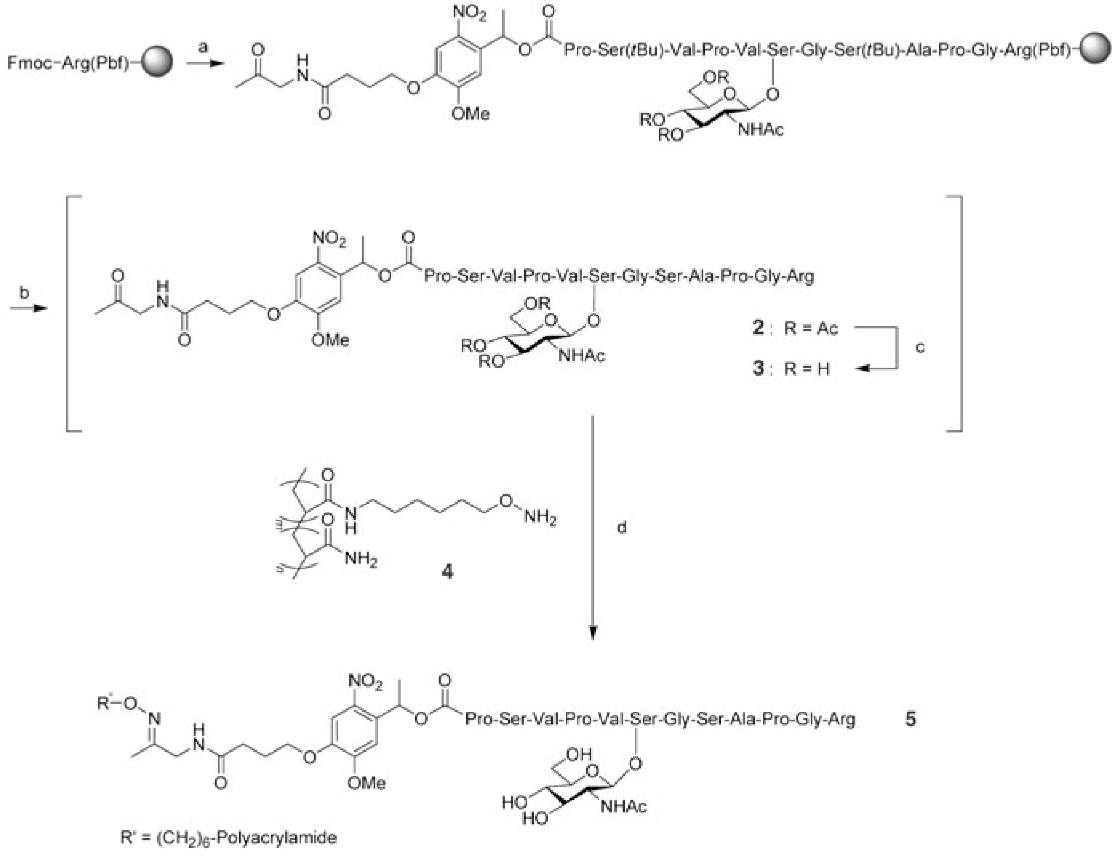
Figure 1. Glycopeptide synthesis using molecular transporter **1**.



Scheme 1. a) 1. Piperidine, DMF; 2. Fmoc-AA or Fmoc-Ser(Ac$_3$GlcNAcβ)-OH or **1**, HBTU, HOBt, ethyldiisopropylamine, *N*-methylpyrrolidone/DMF; 3. Ac$_2$O, HOBt, ethyldiisopropylamine, DMF; b) 90% trifluoroacetic acid in water; c) NaOH, MeOH; d) AcOH/AcONa buffer (pH 5.0–5.5). DMF = dimethylformamide; HBTU = *O*-(benzotriazol-l-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate; HOBt = 1-hydroxy-l*H*-benzotriazole. Pbf = 2,2,5,7,8-pentamethyldihydrobenzofurane-5-sulfonyl; Fmoc = 9-fluorenylmethoxycarbonyl; R' = (CH$_2$)$_6$–polyacrylamide.

captured only the full-length peptide **3**, since free amino groups of the incomplete peptides were capped by acetylation prior to the removal of the Fmoc groups. After one-pot sugar elongation reactions by recombinant glycosyltransferases in the presence of sugar nucleotides as glycosyl donor substrates, selective cleavage reaction by photoirradiation at 365 nm proceeded smoothly and the target product **7** was obtained in 12% overall yield from the initial solid-phase synthesis (Scheme 2). As shown in Figure 3, the purity of the crude compound **7** released from the transporter was satisfactory

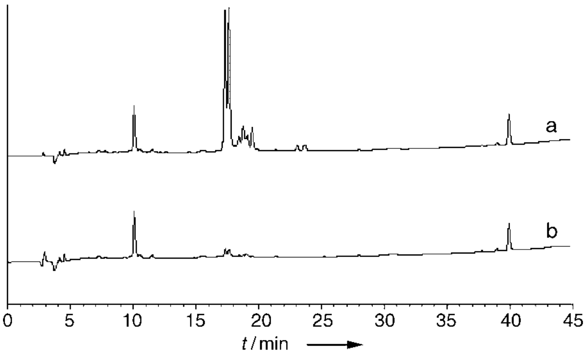
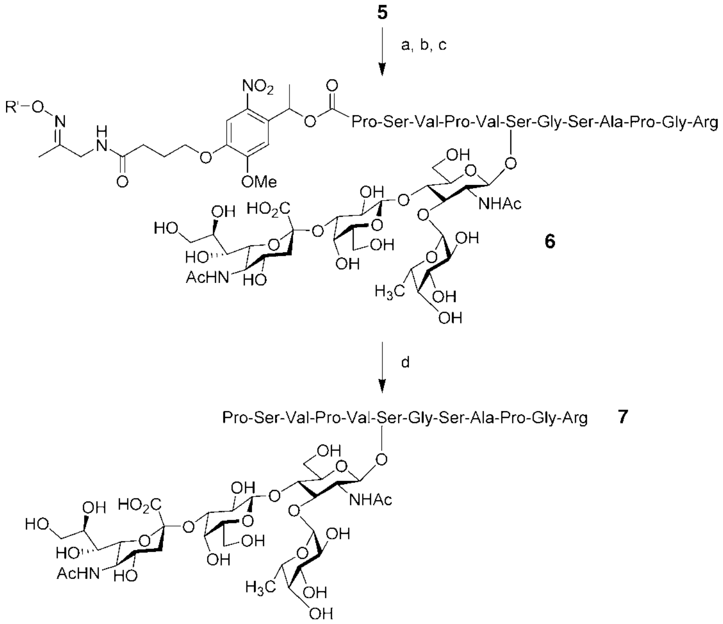


Figure 2. RP-HPLC analysis of the chemoselective ligation of the ketone group of compound **3** with the alkoxyamino group of polymer **4**. a) Chromatogram of the crude mixture of **3** before blotting; b) chromatogram after blotting for 3 h.



Scheme 2. a) UDP-Gal (5 mM), β-1,4-galactosyltransferase (100 mU mL^{-1}), MnCl$_2$ (10 mM), 0.1 % BSA, HEPES buffer (50 mM, pH 7.0), 100 %; b) CMP-NANA (5 mM), α-2,3-sialyltransferase (37 mU mL^{-1}), 0.1 % BSA, HEPES buffer (50 mM, pH 7.0), 100 %; c) GDP-Fuc (5–7 mM), α-1,3-fucosyltransferase (20–30 mU mL^{-1}), MnCl$_2$ (10 mM), 0.1 % BSA, HEPES buffer (50 mM, pH 7.0), 100 %; d) UV irradiation (365 nm). UDP = uracil diphosphate; BSA = bovine serum albumin; HEPES = *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; CMP-NANA = cytidine-5'-monophospho-*N*-acetylneuraminic acid sodium salt; GDP = guanosine diphosphate.

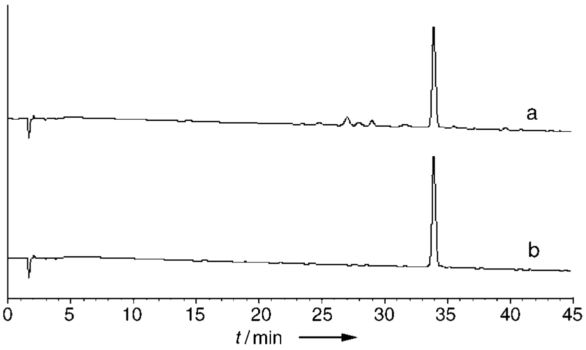


Figure 3. RP-HPLC analysis of glycopeptide **7**. a) Crude product **7**; b) product **7** purified by preparative-scale RP-HPLC.

enough for further biological assay, which suggests that blotting and one-pot glycosylation reactions on the water-soluble polymer mediated by molecular transporter **1** will become an efficient and versatile strategy for the high-throughput synthesis of a glycopeptide library.

Interestingly, it was also demonstrated that irradiation with a MALDI-TOF MS N$_2$ gas laser efficiently cleaved the transporter at its photosensitive position in the presence of 2,5-dihydroxybenzoic acid as matrix. The product-ion peaks can be used for monitoring and characterizing glycosylation reactions on the polymer support by subsequent precise structural analysis in the TOF/TOF mode.[12,13] As indicated in Figure 4, precise structural characterization of compound **7** was carried out by matrix-dependent selective fragmentation (MDSF)[12b] in the MALDI-TOF/TOF MS mode.

In conclusion, we have established a simple and efficient method for rapid glycopeptide synthesis by using a hetero-

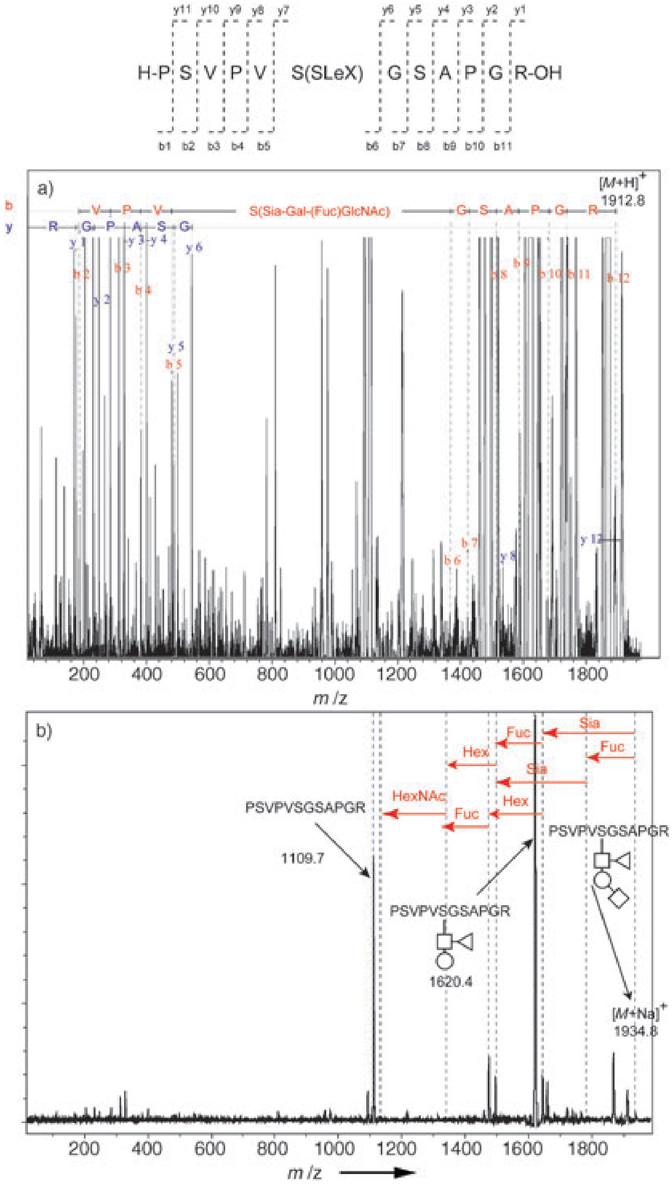


Figure 4. MALDI-TOF/TOF MS analysis of compound **7** by the MDSF method in the presence of a) 2,5-dihydroxybenzoic acid (DHB) or b) α-cyano-4-hydroxycinnamic acid as the matrix.

bifunctional molecular transporter, which interfaces two types of polymer platforms for chemical and enzymatic synthesis. The merit of our strategy is evident: chemical blotting based on a terminal ketone group of the molecular transporter permits selective and quantitative transportation of a variety of products synthesized by general solid-phase synthesis. Moreover, the photoselective cleavage reaction of water-soluble polymers was suitable for an efficient and mild procedure to yield complex and unstable target compounds safely. The present two-dimensional synthetic scheme using a versatile molecular transporter will greatly accelerate both practical enzymatic synthesis with immobilized glycosyltransferases[14] and functional identification of biologically important glycopeptides.

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