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Rapid Microwave-Assisted Solid-Phase Glycopeptide Synthesis

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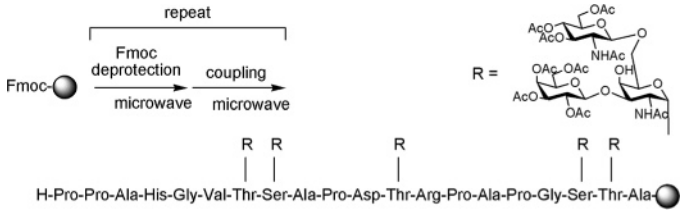
ABSTRACT



Coupling of glycosylated Fmoc-Thr or Fmoc-Ser with N-terminal amino acids on a resin proceeded smoothly under microwave irradiation for 20 min with much higher efficiency (98% yield per coupling) than found in more general conditions. Compared with a conventional protocol, the present method greatly reduces the time required for solid-phase glycopeptide synthesis from 4 days to 7 h, as is the case with the synthesis of Muc-1-related 20-residue glycopeptide carrying five core-2 trisaccharide chains.

The advent of solid-phase peptide synthesis (SPPS) has led to dramatic developments in peptide/protein chemistry and their related fields.[1] Large and diverse peptide libraries have been prepared on solid-phase supports in a combinatorial manner, and these have been used as useful tools for vast areas of many biological investigations. For example, the microarray platform allows for parallel compound generation, high-throughput screening, and data acquisition.[2] Although glycopeptides are attractive candidates for compound libraries because of their various biological functions and consequent therapeutic potential, there has only been limited success in their parallel glycopeptide synthesis and assay.[3] The main difficulty associated with high-throughput glycopeptide synthesis resides in the extremely low coupling efficiency of sterically hindered, sugar-bound amino acid derivatives. At present, solid-phase glycopeptide synthesis (SPGS) must be carried out by employing large excesses of sugar-bound amino acid derivatives, and the time required for achieving a satisfactory yield for each step is generally over 10 h. Since it has been reported that combined chemical and enzymatic synthetic strategies greatly accelerate the efficient construction of complicated glycopeptide libraries,[4] the advent of a novel method for practical solid-phase synthesis of simple glycopeptide intermediates is strongly expected.

Recently, our attention has been directed toward studying the effects of microwave irradiation on accelerating the reaction speed of a variety of chemical reactions.[5] In particular, highly enhanced coupling efficiency in solid-phase

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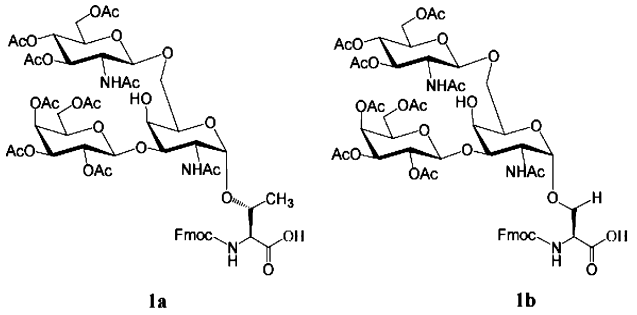


Figure 1. Core-2 class glycosylated amino acid building blocks.

peptide synthesis by microwave irradiation[6] motivated us to apply this procedure to the synthesis of key glycopeptide intermediates. In this communication, we describe a microwave-enhanced, rapid (10−20 min) procedure for the coupling of sterically hindered, glycosylated amino acid building blocks in solid phase.

First, we prepared the Fmoc amino acid building blocks **1a** and **1b**, each having a core-2-type trisaccharide moiety, according to the method reported previously[7] (Figure 1). The reaction of **1a** (1.5 equiv) with model amino acid residues on a solid support was conducted by means of 2-(1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *N*-hydroxybenzotriazole (HOBt), and diisopropylethylamine (DIEA) in DMF under microwave irradiation at 2450 Hz at 50 °C. (We have not optimized conditions such as temperature and pressure for efficiency due to the limitation of the machine used. Further detailed discussion will be reported by using a new generation microwave synthesizer in a subsequent paper as soon as possible.) As shown in Figure 2, a preliminary test indicated the effect of microwave irradiation on the coupling reaction of **1a** with Ala residues immobilized on solid support through Rinkamide linker.[8] It was clearly demonstrated that over 90% yield was observable after 5 min of irradiation in the reaction of **1a** with Ala-resin and that 98% yield of the reaction was realizable after 20 min. In addition, it was suggested that coupling between **1b** with **1a** residues bound on solid **1a**−Ala-resin also proceeded smoothly, and the yield was estimated to be 87%.

These exciting results motivated us to examine the effects of microwave irradiation on the practical solid-phase synthesis of glycopeptides. We selected the O-linked glycopeptide named MUC1 for study. MUC1 has an antigenic structure that consists of heavily glycosylated tandem-

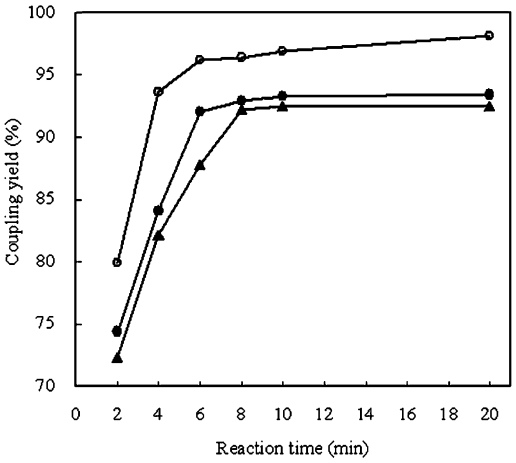
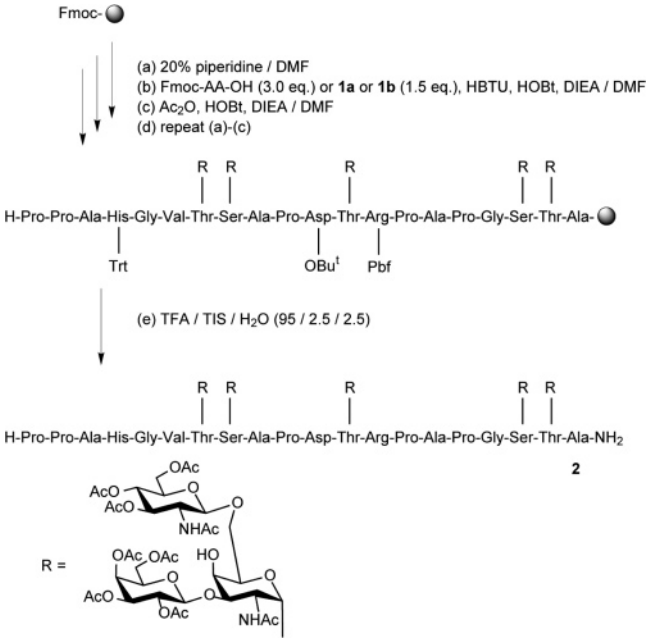


Figure 2. Coupling reaction of **1a** with Ala-resin at 50 °C under microwave irradiation (○), at 50 °C without microwave irradiation (●), and at room temperature without microwave irradiation (▲). The yield herein means Fmoc-based yield.

repeating glycopeptide units of mucin-type glycoprotein, which is expressed on the surfaces of epithelial cells in a variety of tissues.[9] The tandem-repeating unit of MUC1 is composed of 20 amino acid residues bearing core-2 class *O*-glycans, and all serine and threonine residues are found in sequence. Scheme 1 shows the synthetic route to a MUC1-related glycopeptide bearing five trisaccharide branches (**2**) using building blocks **1a** and **1b**.

Scheme 1. Synthetic Route to Compound **2**



Solid-phase synthesis was performed on a TentaGel resin functionalized with Rinkamide linker (0.25 mmol/g). Fmoc-

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Table 1. Microwave Irradiation for Solid-Phase Synthesis of Glycopeptide **2**

	entry		
SPGS condition	1	2	3
microwave	+	−	−
temp (°C)	50	50	room temp
coupling for Fmoc-AA-OH (3.0 equiv)	10 min	10 min	2 h
coupling for **1a** and **1b** (1.5 equiv)	20 min	20 min	12 h
Fmoc deprotection	3 min	3 min	20 min
total operating time (h)[a]	7.0	7.0	98.8
overall yield (%)[b]	43.9	14.7	41.0

[a] Sum total time of iteration procedures of coupling, Fmoc-deprotection, and acetyl capping (5 min, rt). [b] Determined by Fmoc photometric test.

amino acid derivatives (3.0 equiv) and compounds **1a** and **1b** (1.5 equiv) were incorporated with HBTU, HOBt, and DIEA in DMF. Side-chain functional groups of His, Asp, and Arg were protected with trityl, *tert*-butyl, and 2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl (Pbf) groups, respectively. After treatment with Ac_2O, HOBt, and DIEA in DMF to cap the unreacted amino terminal group, N^{α}-Fmoc groups were deprotected by treatment with 20% piperidine in DMF. The reaction conditions examined in this study and the corresponding overall yields are summarized in Table 1. As expected, it was demonstrated that the overall yield in the synthesis of target glycopeptide **2** was greatly enhanced by employing microwave irradiation at 50 °C (Table 1, entry 1, 43.9%), while the yield obtained without irradiation was found to be 14.7% at the same temperature (entry 2). To achieve a similar level of yield for the synthesis of **2** by means of classical solid-phase synthesis, we had to employ a much longer reaction time, as indicated in entry 3 (41.0%, 98.8 h), compared with the time required for entry 1 (43.9%, 7 h).

Compound **2** released from resin under general conditions [TFA/triisopropylsilane/dichloromethane (95/2.5/2.5), 2 h at room temperature] was characterized by reversed-phase HPLC and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

Figure 3(A) shows our HPLC analysis of the crude glycopeptide **2** as prepared by the conditions listed in Table 1 (entries 1−3). The peaks eluting at 25.8 min (indicated by arrows) were identified as compound **2** by means of MALDI-TOF MS ($M + H^+$: *m*/*z* 6197.429) (Figure 3B), and other peaks were byproducts. This suggested that microwave irradiation significantly enhanced the efficiency of all coupling reactions even in the case of the coupling of two glycosylated amino acid residues. In fact, the HPLC profile of the crude product clearly shows much improved yield and quality of the target compound in entry 1. It is noteworthy that the current synthetic protocol can be easily scaled up to a more practical scale, for example, 10−100 mg scale synthesis, and these isolated glycopeptides are now being used as key intermediates for subsequent enzymatic modifications to afford biologically interesting materials.

Structural characterization of compound **2** and other byproducts (Supporting Information) was carried out by using MALDI-TOF/TOF MS in the presence of 2,5-dihydroxy-

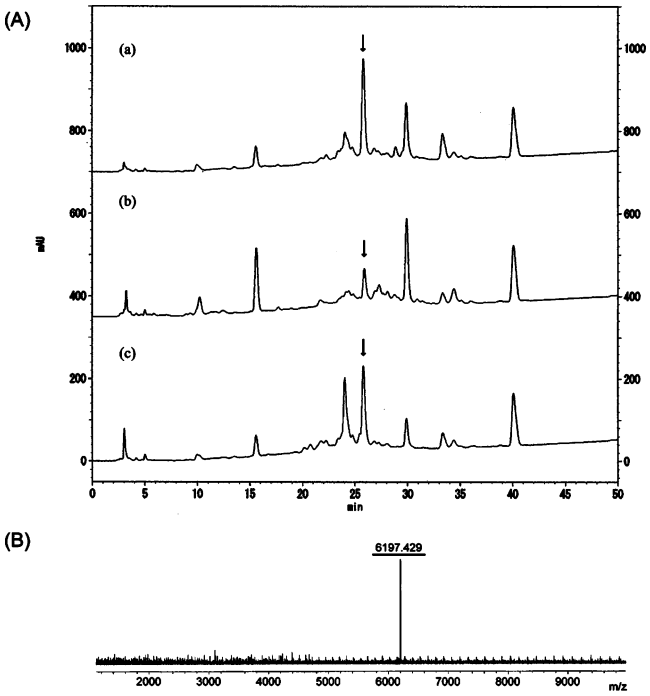

Figure 3. (A) Analytical RP-HPLC profiles of a crude glycopeptide **2** under the synthetic conditions of entry 1 (a), entry 2 (b), and entry 3 (c). Arrows indicate the peak of desired glycopeptide **2**. (B) MALDI-TOFMS of the product. Calcd for $C_{260}H_{379}N_{36}O_{137}$ (M + H), 6197.380; found, 6197.429.

benzoic acid (DHB) as a matrix.[10] As shown in Figure 4, fragmentation by LIFT-TOF/TOF occurred successfully in the peptide bond and gave meaningful b- and y-series product ion peaks without serious cleavage at *O*-glycoside linkages. Fragment ion alignment and identification were performed by means of Biotool 2.2 software (Bruker Daltonik GmbH). The results suggested that important fragment ion peaks such as b6, b8, b11, b12, b17, b18, b19, y1, y2, y3, y8, y9, y12, y13, and y14 are due to the target 20-residue glycopeptide having five acetylated-core2 trisaccharide residues attached to the positions at T7, S8, T12, S18, and T19.

In conclusion, we have demonstrated a rapid and efficient method for solid-phase glycopeptide synthesis using microwave technology. Coupling of sterically hindered glycosylated amino acid building blocks was greatly accelerated by microwave irradiation in terms of the "speed and yield" of the synthesis. Indeed, *our synthesis of the MUC1-related 20-residue glycopeptide **2** was accomplished in only 7 h using microwave heating*, while a conventional protocol generally required more than 4 days to synthesize this same compound. The microwave-assisted protocol for the synthesis of glycopeptide intermediates should greatly contribute to the high-speed parallel syntheses of glycopeptide libraries in combination with enzyme-assisted glycosylation strategy. These glycopeptide libraries related to MUC1 may become nice

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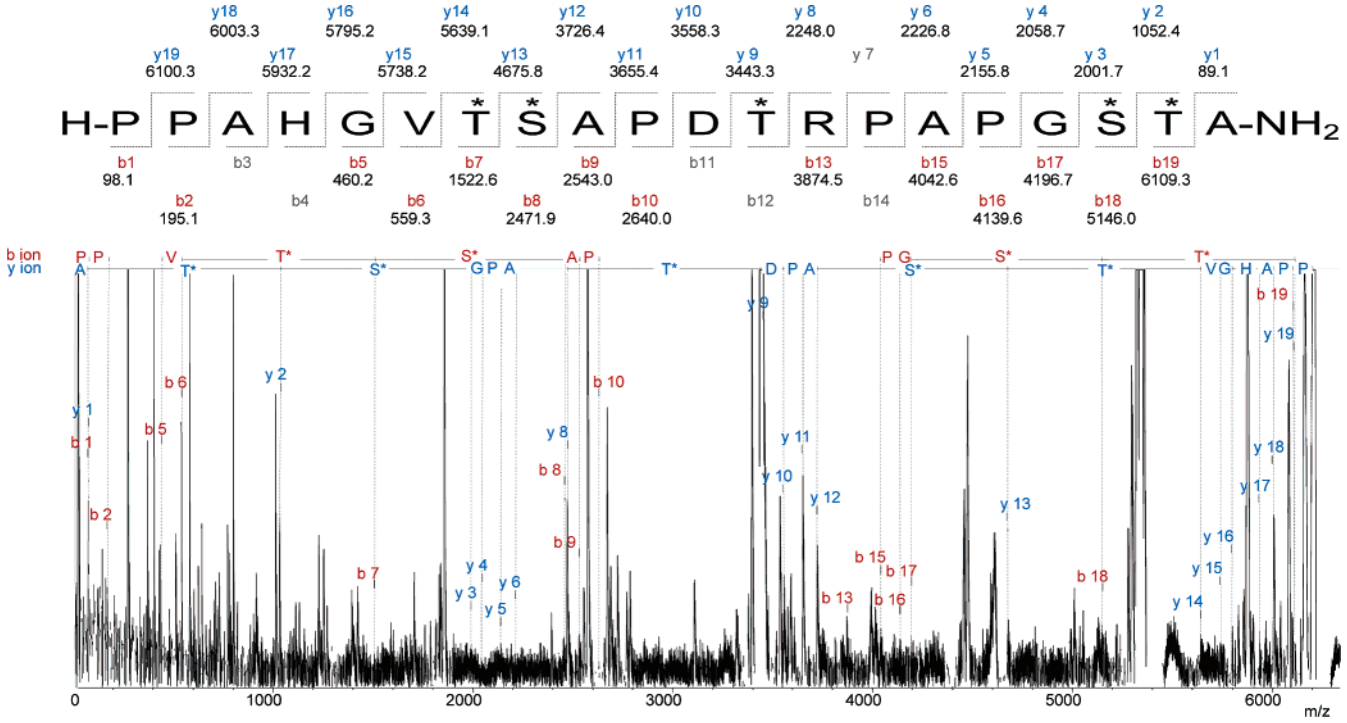

Figure 4. Structural analysis of glycopeptide **2** by MALDI-LIFT-TOF/TOF MS. S* and T* indicate serine and threonine carrying acetylated core-2 trisaccharide, respectively.

tools for further biochemical and immunological studies, and the results will be reported as soon as possible.

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Note Added after ASAP Publication. There were two errors in the body of Table 1 in the version published ASAP February 1, 2005. The corrected version was published February 1, 2005.

Supporting Information Available: Characterization data of glycosylated amino acid building blocks and solid-phase glycopeptide syntheses. This material is available free of charge via the Internet at http://pubs.acs.org.

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