Fluorous Glycopeptide Synthesis without Protection of Sugar Hydroxy Groups

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The synthesis of a glycopeptide was easily achieved by an Fmoc-strategy based on fluorous chemistry. In this study, hCG(β12-16) having an N-acetylglucosamine was synthesized in good yield using Fmoc-Asn(GlcNAc)-OH without protection of the sugar hydroxy groups by the dimethylphosphinothioic mixed anhydride (Mpt-MA) method. Each synthetic intermediate was able to be easily purified by simple fluorous-organic solvent extraction and monitored by TLC, NMR, and MS.

Glycoproteins play an important role in biological processes, such as cell recognition, cell adhesion, immunogenic recognition, and so on. Additionally, the carbohydrate moieties of the glycoprotein contribute to the solubility and thermal stability of proteins and to protection against proteolysis.[1] In order to study these mechanisms, the preparation of glycopeptides is required. Normally, glycopeptides are prepared by a solid-phase synthesis. However, the usual solid-phase method suffers from some serious disadvantages, such as the difficulty of large-scale synthesis, reduced reactivity, and the inability to monitor the reaction by TLC, NMR spectroscopic analysis, or mass spectrometry. Although the Kiser test, which is a color test for the detection of free terminal amino groups, is usually used to monitor a solid-phase glycopeptide synthesis, the negative result of the Kiser test is observed in spite of the incomplete reaction in some cases.[2]

A fluorous (highly fluorinated) solvent, such as perfluorohexane, is immiscible in almost all organic solvents and water. A fluorous compound exhibits a high solubility for fluorous solvents and is readily separated from nonfluorinated compounds by the simple fluorous-organic solvent partition. Similar to solid-phase synthesis, fluorous synthesis does not resort to chromatography. Since a fluorous compound is also soluble in not only fluorous solvents but some organic solvents, the fluorous reaction can be carried out in common organic solvents. Therefore, the strategy of "fluorous synthesis" is designed to combine the advantages of solid-phase synthesis with those of traditional organic synthesis using liquid-phase synthesis.[3–6]

Previously, we described that peptides can be easily prepared by fluorous chemistry.[7,8] In this study, we synthesized a glycopeptide without protection of the sugar hydroxy groups using the dimethylphosphinothioic mixed anhydride (Mpt-MA) method[9,10] based on fluorous chemistry.

In this experiment, the hexakisfluorous chain-type fluorous support **1** (Figure 1) was used.[11] Each reaction was monitored by TLC, and the intermediates were checked by MALDI TOF mass spectroscopy.

4-Hydroxymethylphenoxyacetic acid (HMPA) was coupled with **1** using PyBOP™ (Benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate) as the coupling reagent in the mixed solvent, Novec HFE-7200™ (C_4F_9OEt), NMP and CH_2Cl_2 (homogeneous solvent). The reaction mixture was partitioned with a fluorous solvent FC72™ and MeCN.[12] Excess reagents were extracted into the MeCN layer. From the FC72 lay-

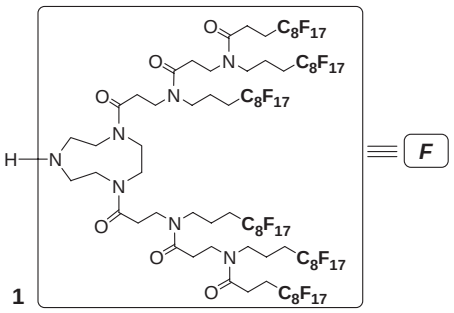
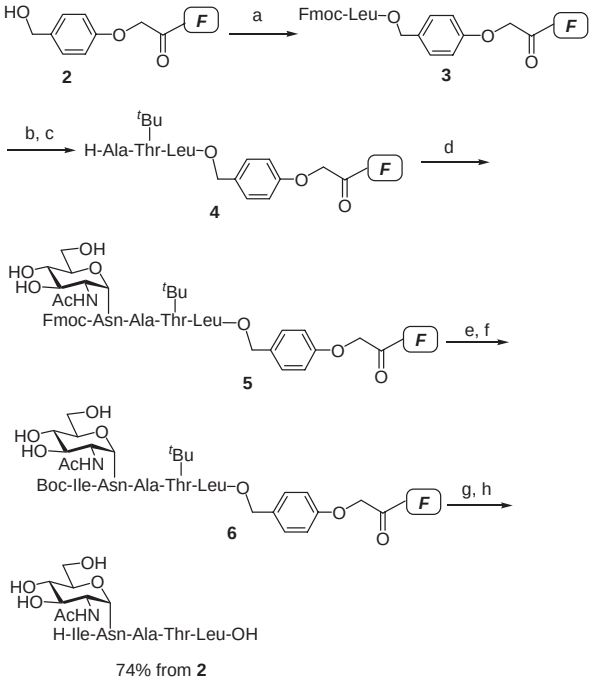


Figure 1. The hexakisfluorous chain-type fluorous support.



Scheme 1. Synthesis of [Asn(GlcNAc)[13]]-hCG(β12-16) by a fluorous synthesis. *Condition*: (*a*) Fmoc-Leu-OH (5.0 equiv.), PyBOP, DIEA, DMAP, C_4F_9OEt-CH_2Cl_2, r.t., 18 h × 2; (*b*) 10% piperidine/DMF-FC72 (1:1), r.t., 20 min; (*c*) Fmoc-AA-OH (4.0 equiv.), PyBOP, DIEA, C_4F_9OEt-CH_2Cl_2, r.t., 1 h; (*d*) Fmoc-Asn(GlcNAc)-OMpt (1.5 equiv.), DIEA, r.t., 1 h; (*e*) 5% piperidine/DMF-FC72 (1:1), r.t., 20 min; (*f*) Boc-Ile-OMpt (4.0 equiv.), DIEA, C_4F_9OEt-DMF, r.t., 1 h × 2; (*g*) 95% aq.TFA, r.t., 2 h; (*h*) RP-HPLC.

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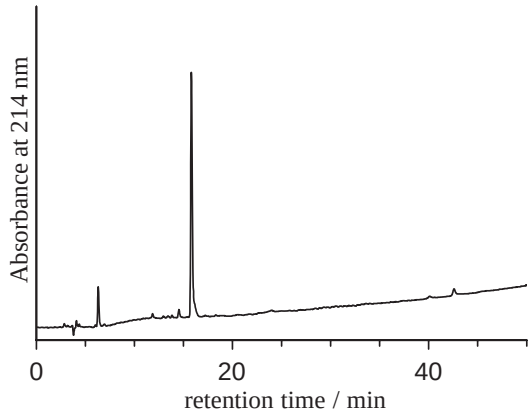

Figure 2. HPLC profile of crude of [Asn(GlcNAc)[13]]-hCG(β12-16) produced by fluorous synthesis. Elution conditions of crude glycopeptide: column, GL Sciences Inertsil ODS-3 (4.6 × 250 mm); eluent, 10–70% MeCN/H_2O-0.1% TFA (v/v/v), 50 min; Flow rate, 1.0 mL/min.

er, the HMPA-type fluorous support **2** was obtained. The loading of Fmoc-Leu-OH on the fluorous support **2** was carried out using PyBOP-DMAP. After the partition with FC72-MeCN, compound **3** was obtained from the FC72 layer. From **3** to **4**, the deprotection of the Fmoc group was carried out with a bisphase solution of 10% piperidine-DMF solution and FC72 (1:1), and a 4-fold excess of the amino acid derivative was used in each coupling reaction. The Asn(GlcNAc) residue was introduced by the Mpt-MA method using a 1.5-fold excess of Fmoc-Asn(GlcNAc)-OH. Because of the low solubility of the fluorous compound **5** in FC72, the cleavage of the Fmoc group of **5** was performed in a 5% piperidine-DMF-C_4F_9OEt mixed solution (homogeneous solution). Although Boc-Ile-OH (4.0 equiv.) was also coupled using the Mpt-MA method for 1 h, an incomplete reaction was observed on TLC. Previously, we described that the reactivity of the Asn(GlcNAc) residue is not very high, but repeated coupling overcomes this problem.[13] Based on this result, the double coupling of Boc-Ile-OH was carried out and the complete introduction of the Ile residue was observed on TLC. Starting from **2**, the crude product **6** was obtained in 91% yield in 9 steps (Scheme 1).

The fluorous glycopeptide **6** was treated with 95% aqueous TFA to cleave the glycopeptide from the fluorous support and remove the side-chain protecting group. After concentration, the residue was partitioned with FC72 −30% aqueous MeCN, the aqueous MeCN layer was washed with Et_2O, and then the crude glycopeptide was extracted into the aqueous MeCN layer. The derivative of the fluorous support and other reagents were extracted by the FC72 layer and Et_2O layer, respectively. The TLC analysis of the FC72 layer showed that the HMPA-type fluorous support **2** was partially decomposed during the TFA treatment. The crude fluorous compound that was partitioned into the FC72 layer was purified by silica-gel chromatography (CH_3Cl: MeOH = 9:1), and **2** was recovered in 68% yield.

The HPLC chart of the crude peptide is shown in Figure 2. After purification of the aqueous MeCN layer by RP-HPLC, the [Asn(GlcNAc)[13]]-hCG(β12-16) was obtained in 74% yield in 10 reaction steps with only one purification during the final step.[14]

In conclusion, a glycopeptide was very easily prepared using fluorous chemistry. The Mpt-MA method is useful for fluorous glycopeptide synthesis without protection of the sugar hydroxy groups during liquid-phase synthesis. Each synthetic intermediate was able to be easily purified by a simple FC72/organic solvent extraction and monitored by NMR, mass spectroscopy and TLC. Fluorous chemistry has become an excellent strategic alternative to solid-phase synthesis.

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11 Compound **1**: amorphous solid, ^1H NMR (400 MHz, $CDCl_3$) δ = 1.84–1.94 (m, 8H), 2.02–2.15 (m, 8H), 2.46–3.00 (m, 20H), 3.25–3.36 (m, 4H), 3.39–3.51 (m, 9H), 3.54–3.81 (m, 12H). MALDI-TOF MS. Found: m/z $[M + H]^+$ 3204.22, Calcd for $C_{84}H_{62}F_{102}N_7O_6$ $[M + H]^+$: 3203.27.
12 FC72 consists of perfluorohexane (C_6F_{14}) isomers.
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14 Amino acid analysis of [Asn(GlcNAc)[13]]-hCG(β12-16) (6M HCl, 110 °C, 24 h) $Asp_{0.92}$, $Thr_{0.93}$, $Ala_{0.95}$, $Ile_{1.00}$, $Leu_{1.08}$. MALDI-TOF MS. Found: m/z $[M + H]^+$ 732.53, Calcd for $C_{31}H_{53}N_7O_{13}$ $[M + H]^+$ 732.38.

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