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Peptide synthesis on fluorous support

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Abstract—New fluorous supports were synthesized and used to prepare a peptide having a C-terminal COOH based on fluorous chemistry. The hexakisfluorous chain-type support was suitable for the synthesis of a pentapeptide or a peptide derivative on a fluorous support whose fluorine content is over 40 w/w%. A bioactive peptide, Leu-enkephalin, was easily synthesized using an Fmoc-strategy based on fluorous chemistry.
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Recently, fluorous chemistry has been studied in several fields such as catalytic chemistry, combinatorial chemistry,[1] and oligosaccharide synthesis.[2] A fluorous (highly fluorinated) solvent is immiscible in an organic solution, and a fluorous compound partitions out of the organic phase and into the fluorous phase. Therefore, a fluorous compound is readily separated from nonfluorinated compounds by a simple 'fluorous/organic' extraction. Similar to a solid-phase synthesis, fluorous synthesis does not resort to chromatography. Therefore, the strategy of 'fluorous synthesis' is designed to combine the advantages of solid-phase synthesis (facile purification) with those of traditional organic synthesis in the liquid-phase synthesis (purification of intermediate and reaction monitoring; large scale reaction).

The C-terminal of almost all natural peptides is an amide type or a carboxyl type. In a fluorous peptide synthesis, a suitable linker corresponding to the C-terminal form is introduced to a fluorous support the same as in a solid-phase method. Previously, we described the synthesis of the C-terminal amide peptide using a Rink™-type fluorous support in fluorous chemistry.[3] In this study, we synthesized new fluorous supports for the synthesis of a C-terminal carboxyl peptide, and a bioactive peptide, Leu-enkephalin, was easily synthesized by an Fmoc-strategy based on fluorous chemistry.

As a fluorous support, a hexakisfluorous chain-type amine **1** (Hfa) was used in this study. A 4-hydroxymethylphenoxyacetyl (HMPA)-type fluorous support **2**[4,5] was prepared for the synthesis of a C-terminal COOH-type peptide. The loading of the amino acid on the fluorous support **2** was investigated using Fmoc-Ala-OH. Although 1.5 equiv of Fmoc-Ala-OH did not provide a sufficient esterification yield using either DCC-DMAP and PyBop-DMAP, 3.0 equiv of Fmoc-Ala-OH produced a high yield. (Fig. 1, Table 1).

The cleavage of the amino acid from the HMPA-type fluorous support was examined. Fmoc-Ala-HMPA-Hfa was treated with 95% aqueous TFA for 2 h at room temperature. After the partition with an organic solvent (MeCN) and a fluorous solvent (FC-72),[6] the Fmoc-Ala-OH was obtained in 46% yield from the organic layer. The HMPA-Hfa and a fluorous compound having a fluorene moiety were recovered from the FC-72 layer. It appeared that the fluorene moiety was not the Fmoc group based on the ^{1}H NMR spectra. We postulated that the Fmoc group was damaged under the cleavage conditions. To study whether or not this side reaction

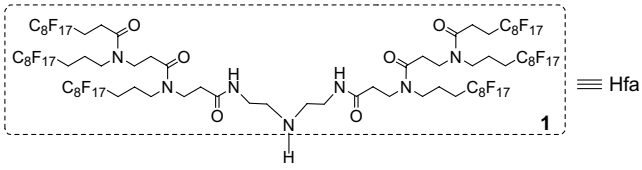

Figure 1. Hexakisfluorous chain-type amine (Hfa) **1**.

Keywords: Fluorous support; Leu-enkephalin; Fluorous chemistry; Peptide synthesis.
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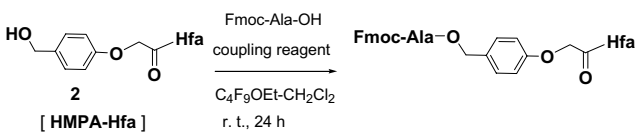
Table 1. Loading of amino acid on the HMPA-type fluorous support **2**



Fmoc-Ala-OH	Coupling reagent[a]	
	DCC (1.1 equiv) DMAP (0.1 equiv)	PyBOP (1.1 equiv) DIEA (1.2 equiv) DMAP (0.1 equiv)
1.5 equiv	46%	74%
3.0 equiv	95%	95%

[a] Equivalent of a coupling reagent corresponds to Fmoc-Ala-OH.

damaged not only the Fmoc group but also the cleavage of the amino acid from the fluorous support, the naphtylacetyl group, which is stable under the deprotection condition of the Fmoc group, was used for the amino group protection instead of Fmoc group. The naphtylacetyl-Ala-HMPA-Hfa was then treated under the same conditions, and the naphtylacetyl-Ala-OH was obtained in 94% yield from the MeCN layer. From these results, it was found that this side reaction had no influence on the cleavage of the amino acid from HMPA-Hfa, and the HMPA-Hfa can be used for the synthesis of a peptide containing no Fmoc group (Fig. 2).

Furthermore, the properties of the 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyl (HMPB)-type fluorous support **3**[7] regarding the loading and the cleavage were examined using Fmoc-Ala-OH. The synthesis of HMPB-Hfa **3** was performed using 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid via the same method of preparing **2**. Although the esterification yield was slightly lower than the HMPA-type, the cleavage of Fmoc-Ala-OH completely occurred (Scheme 1).

Additionally, we investigated the property of the Hfa-type support for the peptide elongation. Two peptide syntheses were demonstrated using Rink™-Hfa (Fig. 3).[9] For test peptide 1, although the hexapeptide derivative (F content = 40 w/w%) was easily prepared, the heptapeptide derivative (F content = 39 w/w%) precipitated in the F-C72 layer. For test peptide 2, although the pentapeptide derivative (F content = 43 w/w%) was obtained without any trouble, the hexapeptide derivative (F content = 40 w/w%) produced an organic layer emulsion in the partition step. Based on these results, a pentapeptide or a peptide derivative on a fluorous support whose fluorine content is over 40 w/w% can be synthesized by the Hfa-type support (Scheme 2).

Based on these results, the synthesis of Leu-enkephalin (F content of protected Leu-enkephalin **6** is 47 w/w%) was demonstrated. HMPA-Hfa was used as a fluorous support to investigate the possibility that the HMPA-type support can be used for peptide synthesis. Fmoc-Leu-OH was esterified onto HMPA-Hfa in 94% yield. The Fmoc group was cleaved by 40% Et_2NH/MeCN–FC-72 (1:1) solution, and the coupling was carried out using PyBOP as the coupling reagent in the mixed solvent, C_4F_9OEt[8]–CH_2Cl_2–DMF (5:4:1). A 4-fold excess of the amino acid derivative was used in each coupling reaction. During these reaction steps, the reaction mixture was partitioned with a fluorous solvent FC-72 and MeCN. The peptide having a fluorous support **6** was

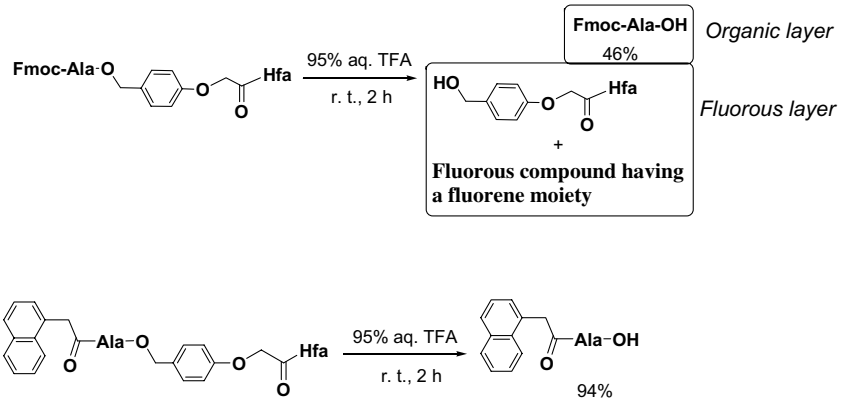
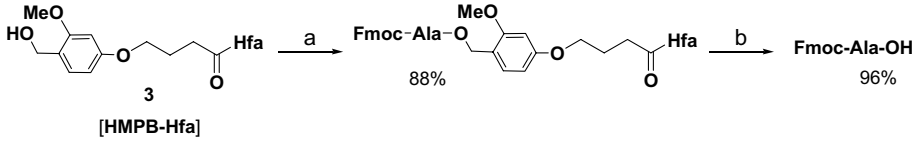


Figure 2. Cleavage of amino acid from HMPA-Hfa.



Scheme 1. Loading and cleavage to HMPB-Hfa. Reagents and conditions: (a) Fmoc-Ala-OH (3.0 equiv), PyBOP (3.3 equiv), DIEA (3.6 equiv), DMAP (0.3 equiv)/C_4F_9OEt–CH_2Cl_2, rt, 24 h; (b) 95% aq TFA, rt, 2 h.

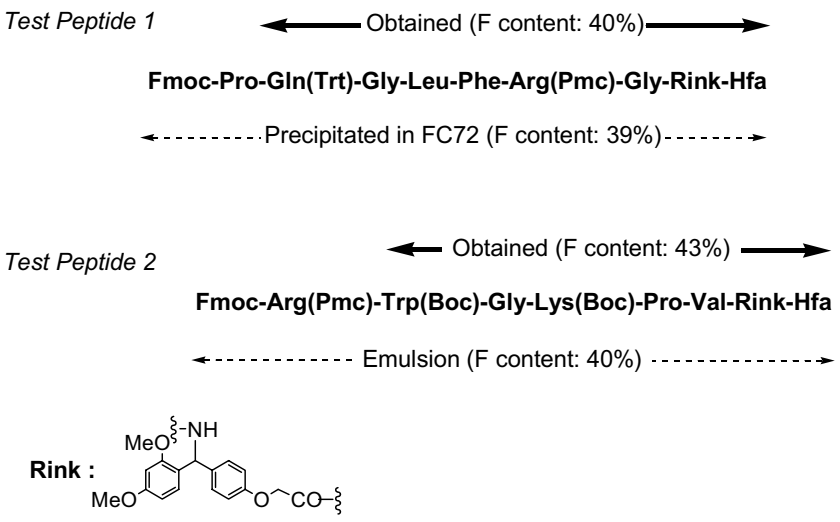
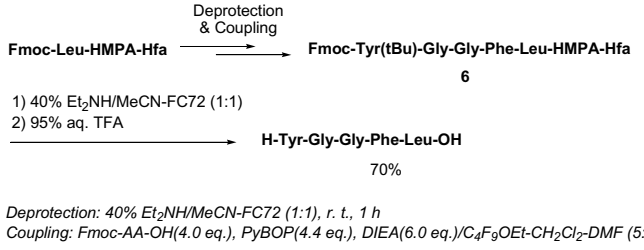


Figure 3. Ability of fluorous support for peptide synthesis.



Scheme 2. Synthesis of Leu-enkephalin on the Hfa-type fluorous support. Reagents and conditions: Deprotection: 40% Et_2NH/MeCN-FC72 (1:1) rt, 1 h. Coupling: Fmocc-AA-OH (4.0 equiv), PyBOP (4.4 equiv), DIEA (6.0 equiv)/C_4F_9OEt–CH_2Cl_2–DMF (5:4:1), rt, 30 min.

treated with TFA containing 5% H_2O to cleave the peptide from the fluorous support and remove the side-chain protecting group. After partition between C_4F_9OEt–EtOAc (2:1) and water, the desired peptide was extracted into the water layer. The HPLC chart of the crude peptide is shown in Figure 4. After purification of the water layer by RP-HPLC, the Leu-enkephalin was provided in 70% yield in nine reaction steps with only one purification as the final step.[10]

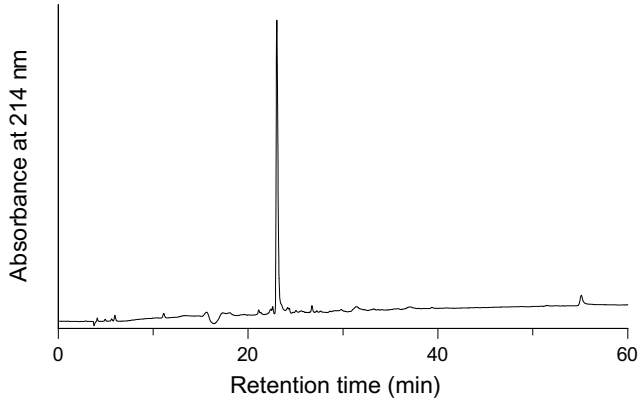


Figure 4. HPLC profile of crude Leu-enkephalin synthesized by fluorous synthesis.[11]

In conclusion, the C-terminal COOH-type peptide was very easily prepared using fluorous chemistry. Each synthetic intermediate was able to be easily purified by a simple FC-72/organic solvent extraction and monitored by NMR, mass spectroscopy and TLC. The HMPA and HMPB-type fluorous supports are useful to prepare a peptide having the C-terminal COOH based on the fluorous chemistry. A hexakisfluorous chain-type support was suitable for the synthesis of a pentapeptide or a peptide derivative on a fluorous support whose fluorine content is over 40 w/w%.

Acknowledgements

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4. Synthesis of **2**. A hexakisfluorous chain-type amine **1** (1.62 g, 0.50 mmol) and 4-hydroxymethylphenoxyacetic acid (0.14 g, 0.75 mmol) were dissolved in a mixed solution of $EtOC_4F_9$ (15 mL), CH_2Cl_2 (15 mL) and DMF (7 mL). To the solution, PyBOP (0.39 g, 0.75 mmol) and DIEA (0.26 mL, 1.50 mmol) were added and stirred at room temperature for 1 h. The reaction mixture was concentrated to approximately 1/3 volume, and MeCN (50 mL) was then added. The mixture was extracted with FC-72 (3×50 mL) and concentrated. The residue was dissolved in a mixed solution of $EtOC_4F_9$ (15 mL) and MeOH (15 mL). To the solution, a catalytic amount of NaOMe was added and stirred at room temperature for 1 h. After concentration, the MeCN (50 mL) was added to the residue and extracted with FC-72 (3×50 mL). The FC-72 layer was concentrated in vacuo. The residue was purified by silica-gel chromatography [$CHCl_3$–MeOH–H_2O, 90:10:0.6] to give compound **2** in 97% from **1**.
5. Compound **2**: amorphous solid, ^1H NMR (600 MHz, $CDCl_3$) $\delta = 1.72$–1.95 (m, 8H), 1.98–2.16 (m, 8H), 2.29–2.77 (m, 16H), 3.31–3.69 (m, 24H), 4.55–4.72 (s, 2H), 4.61 (m, 4H), 6.76–6.90 (m, 2H), 7.27–7.30 (m, 2H). MALDI-TOF MASS: Calcd for $C_{91}H_{67}F_{102}N_7NaO_9[M+Na]^+$: 3363.37, found: 3363.63.
6. FC-72 is a commercially available fluorocarbon solvent (3M, Tokyo), which consists of perfluorohexane (C_6F_{14}) isomers and is called Fluorinert™ FC-72.
7. Compound **3**: amorphous solid, ^1H NMR (600 MHz, $CDCl_3$) $\delta = 1.77$–1.93 (m, 8H), 1.99–2.15 (m, 10H), 2.35–2.80 (m, 18H), 3.30–3.72 (m, 24H), 3.79–3.85 (m, 3H), 3.95–4.04 (m, 2H), 4.55–4.62 (m, 2H), 6.34–6.49 (m, 2H), 7.10–7.16 (m, 2H). MALDI-TOF MASS: Calcd for $C_{94}H_{73}F_{102}N_7NaO_{10}[M+Na]^+$: 3421.45, found: 3420.66.
8. C_4F_9OEt is a commercially available fluorocarbon solvent (3M, Tokyo), which is called Novec™ HFE-7200.
9. To avoid a diketopiperadine formation, the C-terminal amide type linker (Rink™) was used in Figure 3.
10. Amino acid analysis of Leu-enkephalin: Gly 2.00 (2), Leu 1.08 (1), Tyr 0.87 (1), Phe 0.68 (1). MALDI-TOF MASS: Calcd for $C_{28}H_{38}N_5O_7[M+H]^+$: 556.28, found: 556.31.
11. Elution conditions of crude peptide: column, GL Sciences Inertsil ODS-3 (4.6×250 mm); eluent, 10–80% MeCN/H_2O–0.1%TFA (v/v/v), 60 min; flow rate, 1.0 mL/min.