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A novel peptide synthesis using fluorous chemistry

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Three new fluorous supports for peptide synthesis, *i.e.*, the trialkoxybenzhydryl-type (6), the Wang-type (7) and the *tert*-butyl-type support (8), were prepared. A bioactive peptide TRH was easily synthesized by an Fmoc strategy using the benzhydryl-type fluorous support with fluorous chemistry.

Usually, peptides are easily prepared by solid-phase synthesis. Solid-phase synthesis allows for very simple product isolation by filtration, however, the solid-phase method suffers from some serious disadvantages, such as reduced reactivity and the inability to monitor the reaction by TLC, NMR, and mass spectrometry.

Recently, fluorous chemistry has been studied in several fields such as catalytic chemistry, combinatorial chemistry, parallel synthesis.[1] A fluorous (highly fluorinated) solvent is immiscible in an organic solution, and a fluorous compound partitions out of an organic phase and into a fluorous phase. Therefore a fluorous compound is readily separated from nonfluorinated compounds by a simple "fluorous/organic" extraction. 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 also common 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 in the liquid-phase synthesis (Fig. 1). Recently, we reported oligosaccharide synthesis using fluorous synthesis.[2]

In this study, we synthesized several fluorous supports suitable for peptide synthesis in fluorous chemistry, such as the trialkoxybenzhydryl-type **6**, Wang-type **7** and *tert*-butyl-type fluorous support **8**. We also prepared a bioactive peptide TRH (thyrotropin-releasing hormone)[3] using fluorous chemistry.

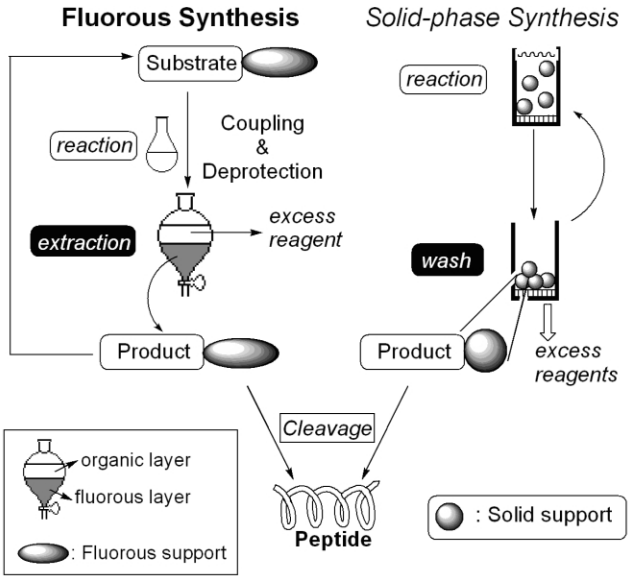
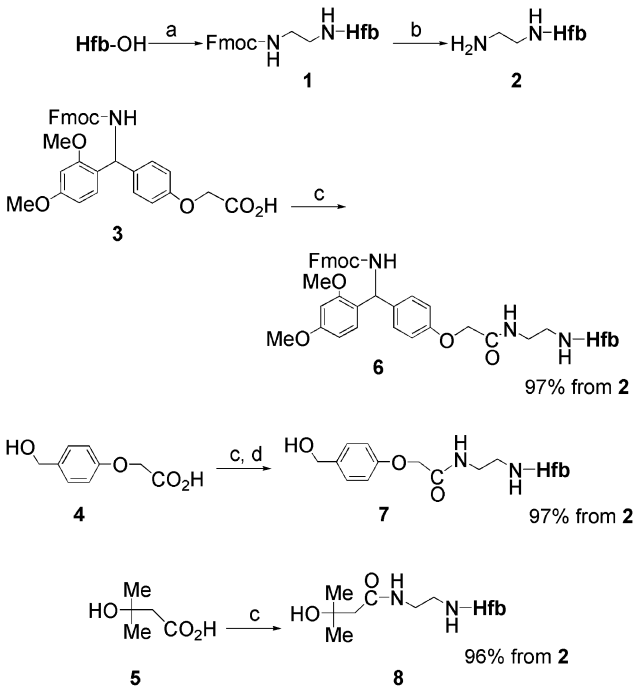


Fig. 1 Concept of peptide synthesis using fluorous chemistry.

The synthetic route of the three fluorous supports for peptide synthesis **6–8** is shown in Scheme 1. The highly fluorinated carboxylic acid, Hfb-OH[4] (Fig. 2), was coupled with mono-Fmoc ethylenediamine using PyBOP as the coupling reagent in the mixed solvent, C_4F_9OEt[5] and CH_2Cl_2. The reaction mixture was partitioned with a fluorous solvent FC-72[6] and MeCN (or MeOH). Excess reagents were extracted with the organic layer. From the FC-72 layer, the fluorous compound **1** was obtained. The Fmoc group of **1** was cleaved by 5% piperidine/FC-72–DMF solution or 10% Et_2NH/FC72–DMF solution. In the partition step with FC-72 and MeCN (or MeOH), a trace amount of these secondary amines was partitioned into the FC-72 layer. However, Et_2NH is removed during the evaporation of FC-72, because the boiling point of Et_2NH (55 °C) is almost same as that of FC-72 (56 °C). When using piperidine, the fluorous layer was washed with aqueous citric acid to remove the piperidine.



Scheme 1 Synthesis of fluorous supports for peptide synthesis. (a) Fmoc-$NH(CH_2)_2NH_2$ HCl salt, PyBOP, $(i\text{-}Pr)_2NEt/C_4F_9OEt–CH_2Cl_2$, RT, 1 h; (b) 5% piperidine or 10% Et_2NH/DMF–FC-72, RT, 30 min; (c) **2**, PyBOP, $(i\text{-}Pr)_2NEt/C_4F_9OEt–CH_2Cl_2$, RT, 1 h; (d) cat. NaOMe/MeOH, RT, 30 min.

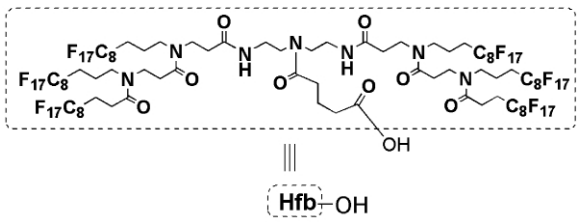


Fig. 2 Structure of Hfb-OH.

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From the FC-72 layer, the fluorous compound **2** was obtained. The coupling of compound **2** with a linker reagent **3–5** gave the trialkoxybenzhydryl-type **6**,[7] Wang-type **7**[8] and *tert*-butyl-type fluorous supports **8**,[9] respectively, in excellent yield by only one purification as the final step.

Using the fluorous support **6**, the tripeptide TRH was prepared by the Fmoc strategy (Scheme 2). The Fmoc group was cleaved by 5% piperidine/FC-72–DMF solution, and the coupling was carried out using PyBOP as the coupling reagent in the mixed solvent, C_4F_9OEt[5] and CH_2Cl_2. The partition step was performed as described above. A 4-fold excess of the amino acid derivative was used in each coupling reaction. The peptide with fluorous support **6** was treated with TFA containing 2.5% H_2O and 2.5% 1,4-butanedithiol to cleave the peptide from the fluorous support and remove the side-chain protecting group. After partitioning between FC-72, water, and toluene, the desired peptide was extracted into the water layer. The derivative of the fluorous support and other reagents were extracted with the FC-72 layer and toluene layer, respectively. The HPLC chart of the crude peptide is shown in Fig. 3. After purification of the water layer by RP-HPLC, the TRH was obtained in 62% yield in 7 reaction steps by only one purification as the final step.[10]

In conclusion, a peptide was very easily prepared using fluorous chemistry. Each synthetic intermediate was able to be easily purified by simple FC-72/organic solvent extraction and monitored by NMR, mass spectrometry and TLC. Fluorous chemistry has become an excellent strategic alternative to solid-phase synthesis.

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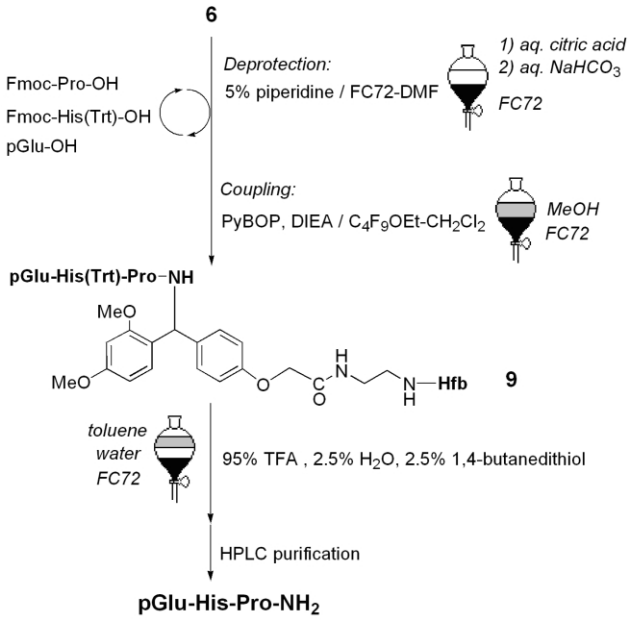

Scheme 2 Synthesis of TRH using fluorous chemistry.

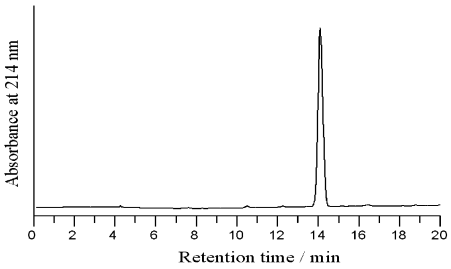

Fig. 3 HPLC profile of crude TRH synthesized by fluorous synthesis.[11]

Notes and references

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5 C_4F_9OEt is a commercially available fluorocarbon solvent (3M, Tokyo), which is called Novec™ HFE-7200.

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 **6**: amorphous solid, ^{1}H NMR (600 MHz, $CDCl_3$) δ = 1.81–1.90 (m, 10H), 2.06–2.17 (m, 15H), 2.32–2.78 (m, 17H), 3.37–3.44 (m, 20H), 3.57–3.67 (m, 8H), 3.74 (s, 3H), 3.80 (s, 3H), 4.23 (br, 1H), 4.41 (d, 1H, 6.9 Hz), 4.46 (s, 2H), 5.91 (m, 1H), 6.03 (m, 1H), 6.47 (m, 2H), 6.84 (m, 2H), 7.09 (m, 1H), 7.16 (m, 3H), 7.30 (m, 2H), 7.39 (m, 3H), 7.58 (d, 2H, J = 6.9 Hz), 7.76 (d, 2H, J = 6.9 Hz). MALDI-TOF MASS: Calcd. for $C_{121}H_{98}F_{102}N_{10}NaO_{14}$ [M + Na^+]: 3876.96, found: 3875.05.

8 Compound **7**: amorphous solid, ^{1}H NMR (600 MHz, $CDCl_3$) δ = 1.85–1.91 (m, 10H), 2.08–2.16 (m, 11H), 2.33–2.69 (m, 19H), 3.34–3.67 (m, 31H), 4.49 (s, 2H), 4.61 (s, 2H), 6.91–6.92 (m, 2H), 7.30–7.32 (m, 2H). MALDI-TOF MASS: Calcd. for $C_{98}H_{79}F_{102}N_9NaO_{11}$ [M + Na^+]: 3519.55, found: 3516.94.

9 Compound **8**: amorphous solid, ^{1}H NMR (600 MHz, $CDCl_3$) δ = 1.27 (s, 6H), 1.82–1.92 (m, 11H), 2.04–2.23 (m, 11H), 2.36–2.82 (m, 21H), 3.41–3.46 (m, 21H), 3.59–3.68 (m, 9H). MALDI-TOF MASS: Calcd. for $C_{94}H_{79}F_{102}N_9NaO_{10}$ [M + Na^+]: 3455.51, found: 3455.07.

10 Amino acid analysis. Glu 1.02 (1), Pro 1.00 (1), His 0.98 (1). MALDI-TOF MASS: Calcd for $C_{16}H_{23}N_6O_4$ [M + H^+]: 363.39, found: 363.41.

11 Elution conditions of crude peptide: column, GL Sciences Inertsil ODS-3 (4.6 × 250 mm); eluent, 2–20% MeCN/H_2O–0.1% TFA (v/v/v), 20 min; flow rate, 1.0 mL min^{-1}.