

## A novel peptide synthesis using fluororous chemistry

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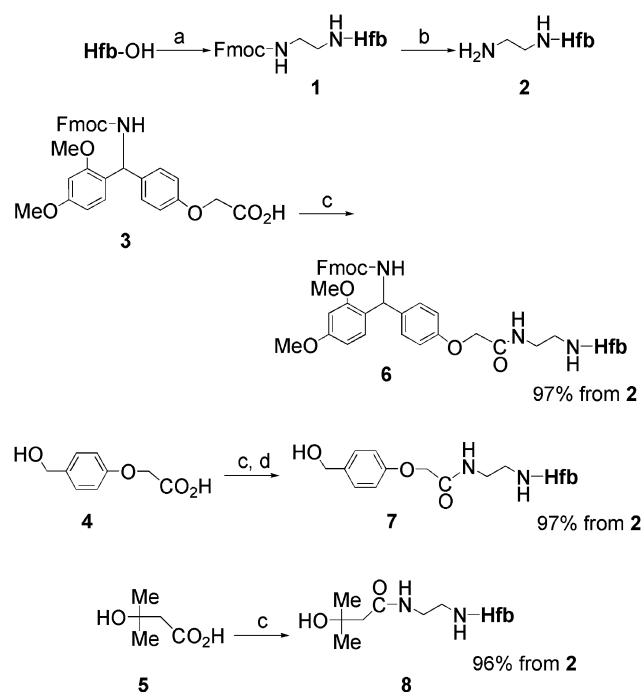
Three new fluororous supports for peptide synthesis, *i.e.*, the trialkoxybenzhydryl-type (6), the Wang-type (7) and the *tert*-butyl-type support (8), were prepared. A bioactive peptide TRH was easily synthesized by an Fmoc strategy using the benzhydryl-type fluororous support with fluororous chemistry.

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.

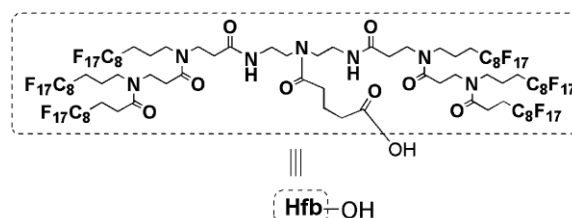
Recently, fluororous chemistry has been studied in several fields such as catalytic chemistry, combinatorial chemistry, parallel synthesis.<sup>1</sup> A fluororous (highly fluorinated) solvent is immiscible in an organic solution, and a fluororous compound partitions out of an organic phase and into a fluororous phase. Therefore a fluororous compound is readily separated from nonfluorinated compounds by a simple "fluororous/organic" extraction. Similar to solid-phase synthesis, fluororous synthesis does not resort to chromatography. Since a fluororous compound is also soluble in not only fluororous solvents but also common organic solvents, the fluororous reaction can be carried out in common organic solvents. Therefore, the strategy of "fluororous 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 fluororous synthesis.<sup>2</sup>

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

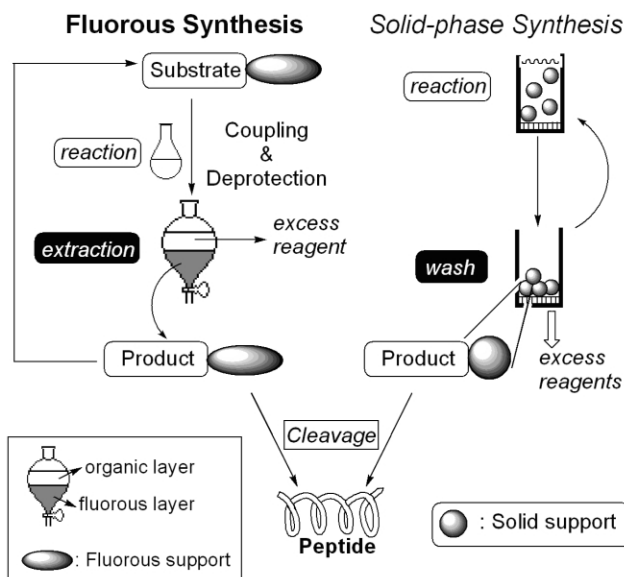
The synthetic route of the three fluororous supports for peptide synthesis 6–8 is shown in Scheme 1. The highly fluorinated carboxylic acid, Hfb-OH<sup>4</sup> (Fig. 2), was coupled with mono-Fmoc ethylenediamine using PyBOP as the coupling reagent in the mixed solvent, C<sub>4</sub>F<sub>9</sub>OEt<sup>5</sup> and CH<sub>2</sub>Cl<sub>2</sub>. The reaction mixture was partitioned with a fluororous solvent FC-72<sup>6</sup> and MeCN (or MeOH). Excess reagents were extracted with the organic layer. From the FC-72 layer, the fluororous compound 1 was obtained. The Fmoc group of 1 was cleaved by 5% piperidine/FC-72–DMF solution or 10% Et<sub>2</sub>NH/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<sub>2</sub>NH is removed during the evaporation of FC-72, because the boiling point of Et<sub>2</sub>NH (55 °C) is almost same as that of FC-72 (56 °C). When using piperidine, the fluororous layer was washed with aqueous citric acid to remove the piperidine.



**Scheme 1** Synthesis of fluororous supports for peptide synthesis. (a) Fmoc-NH(CH<sub>2</sub>)<sub>2</sub>NH<sub>2</sub> HCl salt, PyBOP, (*i*-Pr)<sub>2</sub>NEt/C<sub>4</sub>F<sub>9</sub>OEt–CH<sub>2</sub>Cl<sub>2</sub>, RT, 1 h; (b) 5% piperidine or 10% Et<sub>2</sub>NH/DMF–FC-72, RT, 30 min; (c) 2, PyBOP, (*i*-Pr)<sub>2</sub>NEt/C<sub>4</sub>F<sub>9</sub>OEt–CH<sub>2</sub>Cl<sub>2</sub>, RT, 1 h; (d) cat. NaOMe/MeOH, RT, 30 min.



**Fig. 2** Structure of Hfb-OH.



**Fig. 1** Concept of peptide synthesis using fluororous chemistry.

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

Using the fluororous 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<sub>4</sub>F<sub>9</sub>OEt<sup>5</sup> and CH<sub>2</sub>Cl<sub>2</sub>. 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 fluororous support **6** was treated with TFA containing 2.5% H<sub>2</sub>O and 2.5% 1,4-butanedithiol to cleave the peptide from the fluororous 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 fluororous 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.<sup>10</sup>

In conclusion, a peptide was very easily prepared using fluororous 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. Fluororous chemistry has become an excellent strategic alternative to solid-phase synthesis.

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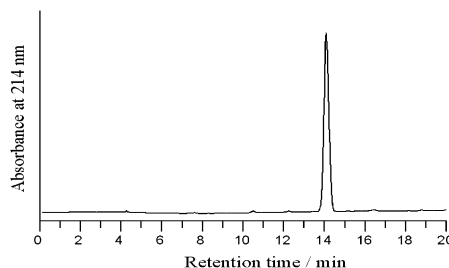
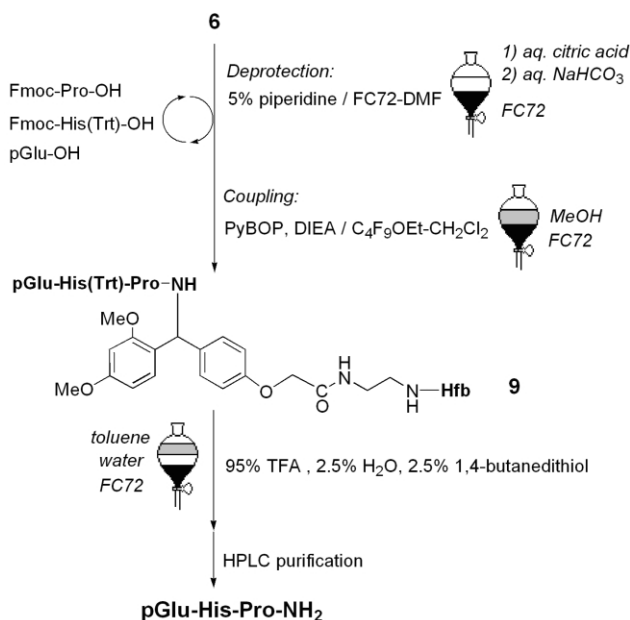


Fig. 3 HPLC profile of crude TRH synthesized by fluororous synthesis.<sup>11</sup>

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- C<sub>4</sub>F<sub>9</sub>OEt is a commercially available fluorocarbon solvent (3M, Tokyo), which is called Novec™ HFE-7200.
- FC-72 is a commercially available fluorocarbon solvent (3M, Tokyo), which consists of perfluorohexane (C<sub>6</sub>F<sub>14</sub>) isomers and is called Fluorinert™ FC-72.
- Compound **6**: amorphous solid, <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) 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<sub>121</sub>H<sub>98</sub>F<sub>102</sub>N<sub>10</sub>NaO<sub>14</sub> [M + Na<sup>+</sup>]: 3876.96, found: 3875.05.
- Compound **7**: amorphous solid, <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) 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<sub>98</sub>H<sub>79</sub>F<sub>102</sub>N<sub>9</sub>NaO<sub>11</sub> [M + Na<sup>+</sup>]: 3519.55, found: 3516.94.
- Compound **8**: amorphous solid, <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) 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<sub>94</sub>H<sub>79</sub>F<sub>102</sub>N<sub>9</sub>NaO<sub>10</sub> [M + Na<sup>+</sup>]: 3455.51, found: 3455.07.
- Amino acid analysis. Glu 1.02 (1), Pro 1.00 (1), His 0.98 (1). MALDI-TOF MASS: Calcd for C<sub>16</sub>H<sub>23</sub>N<sub>6</sub>O<sub>4</sub> [M + H<sup>+</sup>]: 363.39, found: 363.41.
- Elution conditions of crude peptide: column, GL Sciences Inertsil ODS-3 (4.6 × 250 mm); eluent, 2–20% MeCN/H<sub>2</sub>O–0.1% TFA (v/v/v), 20 min; flow rate, 1.0 mL min<sup>−1</sup>.



Scheme 2 Synthesis of TRH using fluororous chemistry.