Liquid-Phase Split-Type Combinatorial Synthesis of Tripeptide Derivatives Encoded by Fluorous Fmoc Reagents

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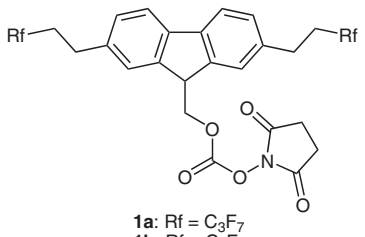
Abstract: A liquid-phase mixture synthesis of 18 tripeptides, some of which are analogues of ACE inhibitors, was effectively conducted by using fluorous Fmoc reagents as encoding tags.

Key words: combinatorial chemistry, liquid-phase synthesis, mixture synthesis, fluorous tag, peptides

Recently, we reported a fluorous mixture synthesis[1] of fluorous-Fmoc reagents (f-Fmoc reagents)[2] **1a**–**c** through a one-pot, double fluorous-tagging strategy (Figure 1).[3] This method provides an efficient way of obtaining various f-Fmoc reagents that are required for fluorous mixture peptide synthesis. Although we had alluded to the possibility of a liquid-phase combinatorial synthesis of various peptides by using f-Fmoc reagents in a past report,[3] until now we have not had the opportunity to attempt it. Here, we would like to describe the first example of a liquid-phase-mixture synthesis of a variety of tripeptide ACE inhibitors encoded by f-Fmoc tags with differing fluorine content.[4] We chose some C-protected tripeptides as the target compounds for the model experiment of a liquid-phase combinatorial synthesis of peptides (Figure 2). The deprotected compounds indicated in bold-type are known ACE inhibitors,[5] and the IC_{50} value of each compound is listed in Figure 2.



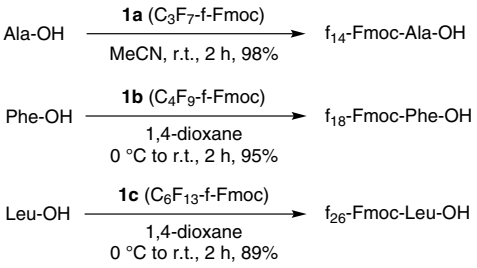
1a: Rf = C_3F_7
1b: Rf = C_4F_9
1c: Rf = C_6F_{13}

Figure 1 Fluorous Fmoc reagents

First, as shown in Scheme 1, each amino acid of L-alanine, L-phenylalanine, and L-leucine was protected with f-Fmoc reagents bearing different fluorine content.[6–8] After mixing these protected amino acids, the mixture was divided into two groups and the condensation reaction for the dipeptide synthesis was accomplished by using the HBTU/HOBt method.[9]

	IC_{50} (mmol/l)		
Ala-Ala-Val-OBn	25	Ala-Ala-Ala-OBn	Phe-Val-Met-OMe
Ala-Ala-Leu-OBn	93	Ala-Val-Ala-OBn	Leu-Ala-Val-OBn
Ala-Val-Leu-OBn	7.1	Phe-Val-Leu-OBn	Leu-Val-Met-OMe
Ala-Val-Met-OMe	8	Phe-Ala-Val-OBn	Leu-Val-Ala-OBn
Phe-Val-Ala-OBn	6	Phe-Ala-Leu-OBn	Leu-Val-Leu-OBn
Leu-Ala-Ala-OBn	13	Phe-Val-Leu-OBn	Leu-Val-Met-OMe

Figure 2 Target tripeptides containing ACE inhibitors



Scheme 1 f-Fmoc protection of starting amino acids

One group was reacted with L-alanine benzyl ester in 95% yield, while the other group was reacted with L-valine benzyl ester in 98% yield (conditions a and b in Scheme 2).[10] Subsequently, after deprotection of the C-terminus by hydrogenolysis[11] of the mixture of the two groups in quantitative yields (conditions c and d in Scheme 2), each mixture was divided into three groups. Each group of the mixture was allowed to react with four different C-protected amino acids (Ala-OBn, Val-OBn, Leu-OBn, and Met-OMe) using the same condensation method (conditions e–j in Scheme 2); the yields were satisfactory, being in the range 86–98%.

We then separated each of the pure C-protected tripeptides from the mixture of each of the six groups (Group A–F). Figure 3 shows the chart of the analytical fluorous-HPLC (FluoroFlash® HPLC column; 4.6 mm i.d.; 150 mm length)[12] of the mixture (group A) of the tripeptide benzyl ester protected f-Fmoc compounds (f_{14}-Fmoc-Ala-Ala-Ala-OBn, f_{18}-Fmoc-Phe-Ala-Ala-OBn, and f_{26}-Fmoc-Leu-Ala-Ala-OBn).

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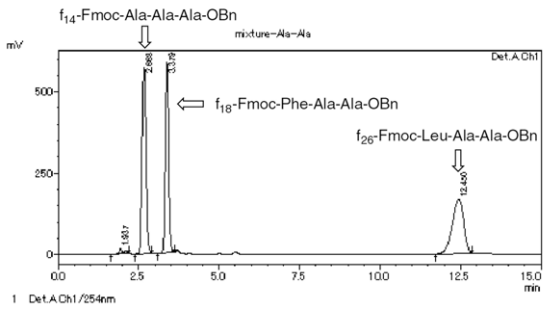
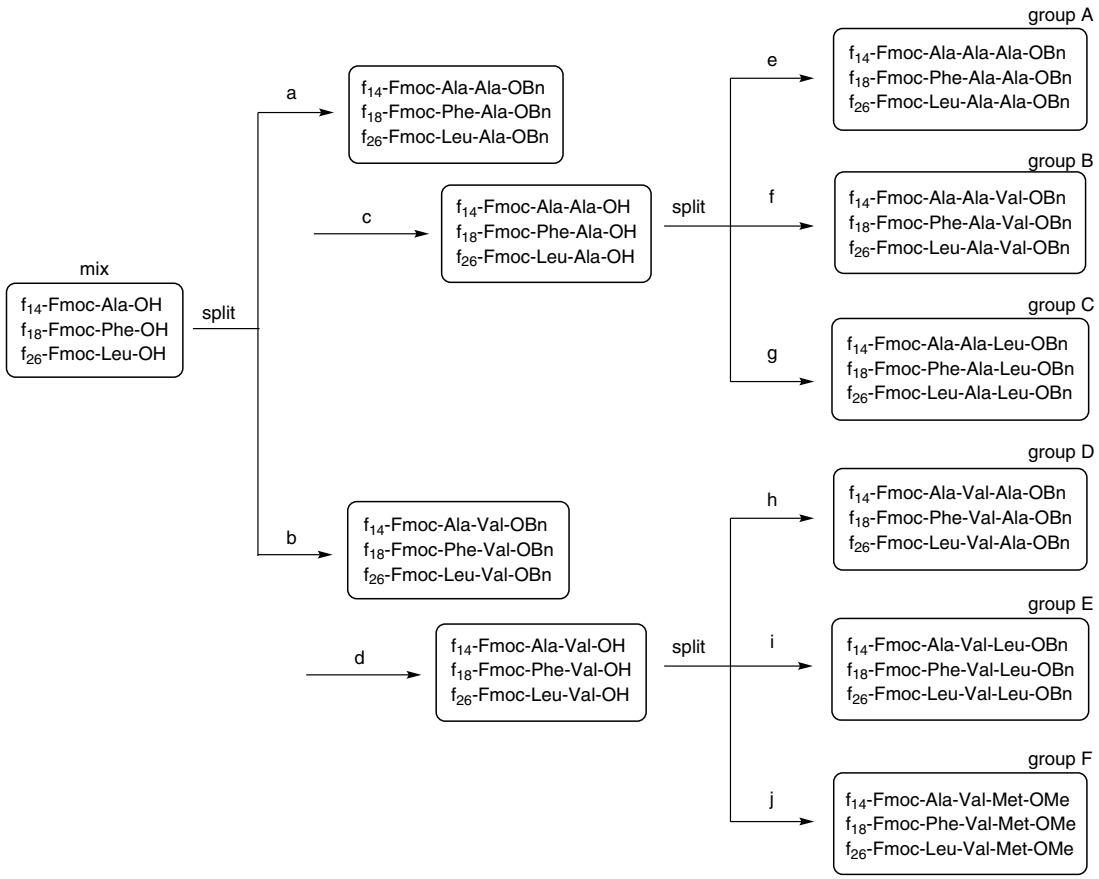


Figure 3 Analytical fluorous HPLC chart of a mixture of f_{14}-Fmoc-Ala-Ala-Ala-OBn, f_{18}-Fmoc-Phe-Ala-Ala-OBn, and f_{26}-Fmoc-Leu-Ala-Ala-OBn. *Reaction conditions*: FluoroFlash® HPLC column; 254nm; flow rate: 1.0 mL/min; 0 to 30 min: 80% MeCN–H_2O to 100% MeCN; over 30 min: 100% MeCN.

The f_{14}-Fmoc-Ala-Ala-Ala-OBn, f_{18}-Fmoc-Phe-Ala-Ala-OBn, and f_{26}-Fmoc-Leu-Ala-Ala-OBn appeared at 2.7, 3.4, and 12.5 min, respectively. Thus, a liquid-phase mixture synthesis of various peptides by using the f-Fmoc encoding method is possible.[13] Since we have already confirmed that these protected peptides were stable under aqueous conditions, we used the same conditions used for analysis also for the preparative f-HPLC (FluoroFlash® HPLC column; 20 mm i.d.; 250 mm length).[2] A 200 mg-scale fluorous-HPLC purification of the f-Fmoc-protected peptides f_{14}-Fmoc-Ala-Ala-Ala-OBn, f_{18}-Fmoc-Phe-Ala-Ala-OBn, and f_{26}-Fmoc-Leu-Ala-Ala-OBn led to their isolation in 43, 63, and 86 mg, respectively, with minimal loss.[14–16]

Similarly, preparative f-HPLC of the other five groups (groups B–F) were conducted, and each pure tripeptide compound was effectively separated with almost quantitative recovery.[17] In this range of the numbers of fluoride, the difference in the numbers of fluorine in the Fmoc groups did not significantly affect the characteristics of the amino acids or peptides. Therefore, regular organic synthetic procedures could be used. Finally, deprotection of the f-Fmoc was conducted. Scheme 3 shows the yield of each deprotection reaction of f-Fmoc group by using excess diethylamine in acetonitrile under room temperature conditions.[18]



Scheme 2 Fluorous mixture synthesis of C-protected tripeptides. *Reagents and conditions*: (a) Ala-OBn (1.2 equiv), HOBt (1.2 equiv), HBTU (1.2 equiv), DIPEA (2.4 equiv), DMF, r.t., 24 h, 95%; (b) Val-OBn (1.2 equiv), HOBt (1.2 equiv), HBTU (1.2 equiv), DIPEA (2.4 equiv), DMF, r.t., 24 h, 98%; (c) Pd/C (5 mol%), 1 atm H_2, MeOH–THF, r.t., 3 h, quant.; (d) Pd/C (5 mol%), 1 atm H_2, MeOH/THF, r.t., 3 h, quant.; (e) Ala-OBn (1.2 equiv), HOBt (1.2 equiv), HBTU (1.2 equiv), DIPEA (2.4 equiv), DMF, r.t., 24 h, 97%; (f) Val-OBn (1.2 equiv), HOBt (1.2 equiv), HBTU (1.2 equiv), DIPEA (2.4 equiv), DMF, r.t., 24 h, 98%; (g) Leu-OBn (1.2 equiv), HOBt (1.2 equiv), HBTU (1.2 equiv), DIPEA (2.4 equiv), DMF, r.t., 24 h, 92%; (h) Ala-OBn (1.2 equiv), HOBt (1.2 equiv), HBTU (1.2 equiv), DIPEA (2.4 equiv), DMF, r.t., 24 h, 95%; (i) Leu-OBn (1.2 equiv), HOBt (1.2 equiv), HBTU (1.2 equiv), DIPEA (2.4 equiv), DMF, r.t., 24 h, 98%; (j) Met-OMe (1.2 equiv), HOBt (1.2 equiv), HBTU (1.2 equiv), DIPEA (2.4 equiv), DMF, r.t., 24 h, 86%; HOBt=1-hydroxybenzotriazole, HBTU= *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluorophosphate, DIPEA= *N*,*N*-diisopropylethylamine.

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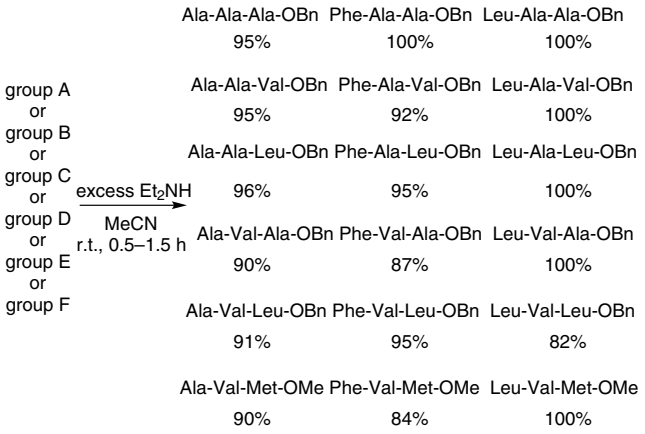

Scheme 3 Yields for the deprotection of the f-Fmoc group

In summary, we have demonstrated a liquid-phase split-type combinatorial synthesis of a large variety of tripeptides, some of which are ACE inhibitors, by using fluorous Fmoc reagents as an encoding tag. If the tripeptides were synthesized individually by the same linear synthetic route as outlined in this work, 90 synthetic steps would have been required; however, we carried out the syntheses in a mere 31 steps, which includes f-Fmoc protections. We believe that the f-Fmoc encoding strategy will be one of the most useful methods for divergent polypeptide synthesis.

Acknowledgment

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(6) **Analytical Data for f_{14}-Fmoc-Ala-OH:** Pale-yellow solid; mp 95.7–96.5 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.49 (d, *J* = 7.0 Hz, 3 H), 2.31–2.51 (m, 4 H), 2.96–3.02 (m, 4 H), 4.16–4.21 (m, 1 H), 4.39–4.48 (m, 3 H), 5.28 (d, *J* = 7.0 Hz, 1 H), 7.25 (d, *J* = 7.3 Hz, 2 H), 7.42 (s, 2 H), 7.68 (d, *J* = 8.1 Hz, 2 H). ^{19}F NMR (466 MHz, $CDCl_3$): δ = –80.4 (6F), –115.4 (4F), –127.6 (4F); HRMS [FAB+]: *m*/*z* calcd for $C_{28}H_{23}NO_4F_{14}$: 704.1482; found: 704.1456.

(7) **Analytical Data for f_{18}-Fmoc-Phe-OH:** White solid; mp 122.2–123.1 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.33–2.50 (m, 4 H), 2.94–3.00 (m, 4 H), 3.09–3.25 (m, 2 H), 4.13–4.17 (m, 1 H), 4.33–4.47 (m, 2 H), 4.66–4.73 (m, 1 H), 5.21 (d, *J* = 7.8 Hz, 1 H), 7.12 (d, *J* = 6.5 Hz, 2 H), 7.23–7.26 (m, 5 H), 7.39 (d, *J* = 3.8 Hz, 2 H), 7.67 (d, *J* = 8.6 Hz, 2 H). ^{19}F NMR (466 MHz, $CDCl_3$): δ = –80.8 (6F), –114.7 (4F), –124.2 (4F), –125.9 (4F). HRMS [FAB+]: *m*/*z* calcd for $C_{36}H_{27}NO_4F_{18}$: 880.1731; found: 880.1685.

(8) **Analytical Data for f_{26}-Fmoc-Leu-OH:** White solid; mp 134.5–135.4 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.97 (d, *J* = 5.4 Hz, 6 H), 1.59–1.76 (m, 3 H), 2.32–2.45 (m, 4 H), 2.96–3.02 (m, 4 H), 4.17–4.22 (m, 1 H), 4.39–4.44 (m, 3 H), 5.11 (d, *J* = 8.3 Hz, 1 H), 7.24 (d, *J* = 7.3 Hz, 2 H), 7.43 (s, 2 H), 7.67 (d, *J* = 8.1 Hz, 2 H). ^{19}F NMR (466 MHz, $CDCl_3$): δ = –80.6 (6F), –114.4 (4F), –121.7 (4F), –122.7 (4F), –123.3 (4F), –125.9 (4F). HRMS [FAB+]: *m*/*z* calcd for $C_{37}H_{29}NO_4F_{26}$: 1046.1760; found: 1046.1747.

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(10) **Mixture Synthesis for Three-Component Mixture of f_{14}-Fmoc-Ala-Ala-OBn, f_{18}-Fmoc-Phe-Ala-OBn, and f_{26}-Fmoc-Leu-Ala-OBn; Typical Procedure:** f_{14}-Fmoc-Ala-OH (560.0 mg, 0.79 mmol), f_{18}-Fmoc-Phe-OH (714.4 mg, 0.79 mmol), and f_{26}-Fmoc-Leu-OH (832.0 mg, 0.79 mmol) were mixed and dissolved in DMF (20 mL). To the solution were added HOBt·H_2O (438.8 mg, 2.86 mmol) and HBTU (1086 mg, 2.86 mmol), separately. After stirring for 5 min, Ala-OBn (1.01 g, 2.86 mmol) and DIPEA (974 μL, 5.72 mmol) were added to the above reaction mixture separately. The reaction mixture was stirred for 24 h at room temperature. After the addition of aq 1.0 M HCl and then dilution with ethyl acetate, the organic layer was washed with H_2O, sat. aq $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The crude residue was purified by silica gel chromatography ($CHCl_3$–MeOH, 20:1) to give the title compound (2.4 g, 95% based on the average molecular weight of the mixture). .

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(12) Analytical and preparative f-HPLC were conducted by using FluoroFlash® columns purchased from Fluorous Technologies Inc. The corporation is no longer trading; however, fluorous column (Wakopak® Fluofix-II 120E) with almost the same separation ability is available from Wako Pure Chemical Industries, Ltd.

(13) The f-HPLC separation could also be applied to a tripeptide that was derived from hydrophilic amino acids such as glycine. The f_{14}-Fmoc-Gly-Gly-Gly-OMe, f_{18}-Fmoc-Gly-

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Gly-Gly-OMe and f_{26}-Fmoc-Gly-Gly-Gly-OMe appeared at 2.4, 3.0, and 6.4 min, respectively, in the analytical f-HPLC trace under the same conditions.

(14) **Analytical Data for f_{14}-Fmoc-Ala-Ala-Ala-OBn:** White solid; mp 153.8–154.6 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.36–1.42 (m, 9 H), 2.31–2.44 (m, 4 H), 2.96–3.02 (m, 4 H), 4.16–4.19 (m, 1 H), 4.22–4.28 (m, 1 H), 4.39–4.50 (m, 3 H), 4.56–4.61 (m, 1 H), 5.13 (d, *J* = 11.9 Hz, 1 H), 5.19 (d, *J* = 12.7 Hz, 1 H), 5.44 (d, *J* = 7.3 Hz, 1 H), 6.55–6.62 (m, 2 H), 7.24 (d, *J* = 9.9 Hz, 2 H), 7.31–7.36 (m, 5 H), 7.42 (s, 2 H), 7.68 (d, *J* = 8.1 Hz, 2 H). ^{19}F NMR (466 MHz, $CDCl_3$): δ = –80.3 (6F), –115.4 (4F), –127.6 (4F). HRMS [FAB+]: *m*/*z* calcd for $C_{41}H_{40}N_3O_6F_{14}$: 936.2694; found: 936.2704.

(15) **Analytical Data for f_{18}-Fmoc-Phe-Ala-Ala-OBn:** White solid; mp 171.5–172.4 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.30 (d, *J* = 6.8 Hz, 3 H), 1.40 (d, *J* = 7.3 Hz, 3 H), 2.34–2.44 (m, 4 H), 2.95–3.02 (m, 4 H), 3.07–3.11 (m, 2 H), 4.10–4.15 (m, 1 H), 4.30–4.46 (m, 4 H), 4.52–4.58 (m, 1 H), 5.13 (d, *J* = 11.9 Hz, 1 H), 5.19 (d, *J* = 12.7 Hz, 1 H), 5.35 (d, *J* = 6.8 Hz, 1 H), 6.38 (d, *J* = 5.9 Hz, 1 H), 6.45 (d, *J* = 7.0 Hz, 1 H), 7.13–7.16 (m, 2 H), 7.23–7.40 (m, 12 H), 7.68 (d, *J* = 7.8 Hz, 2 H). ^{19}F NMR (466 MHz, $CDCl_3$): δ = –80.6 (6F), –114.5 (4F), –124.1 (4F), –125.8 (4F); HRMS [FAB+]: *m*/*z* calcd for $C_{52}H_{45}N_3O_5F_{18}$: 1112.2943; found: 1112.2969.

(16) **Analytical Data for f_{26}-Fmoc-Leu-Ala-Ala-OBn:** White solid; mp 179.9–181.0 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.94 (d, *J* = 5.4 Hz, 6 H), 1.36–1.42 (m, 6 H), 1.50–1.70 (m, 3 H), 2.35–2.48 (m, 4 H), 2.96–3.02 (m, 4 H), 4.15–4.20 (m, 2 H), 4.36–4.59 (m, 3 H), 4.45–4.59 (m, 1 H), 5.16–5.22 (m, 3 H), 6.48 (d, *J* = 6.5 Hz, 2 H), 7.32–7.43 (m, 9 H), 7.68 (d, *J* = 7.2 Hz, 2 H). ^{19}F NMR (466 MHz, $CDCl_3$): δ = –80.6 (6F), –114.4 (4F), –121.6 (4F), –122.6 (4F), –123.2 (4F), –125.9 (4F). HRMS [FAB+]: *m*/*z* calcd for $C_{52}H_{46}N_3O_6F_{26}$: 1278.2971; found: 1278.3009.

(17) Analytical data for these tripeptides are provided in the Supporting Information

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