Fmoc-based Solid-phase Peptide Synthesis using a New t-Alcohol Type 4-(1′,1′-Dimethyl-1′-hydroxypropyl)phenoxyacetyl Handle (DHPP)–Resin (Fmoc = fluoren-9-ylmethoxycarbonyl)

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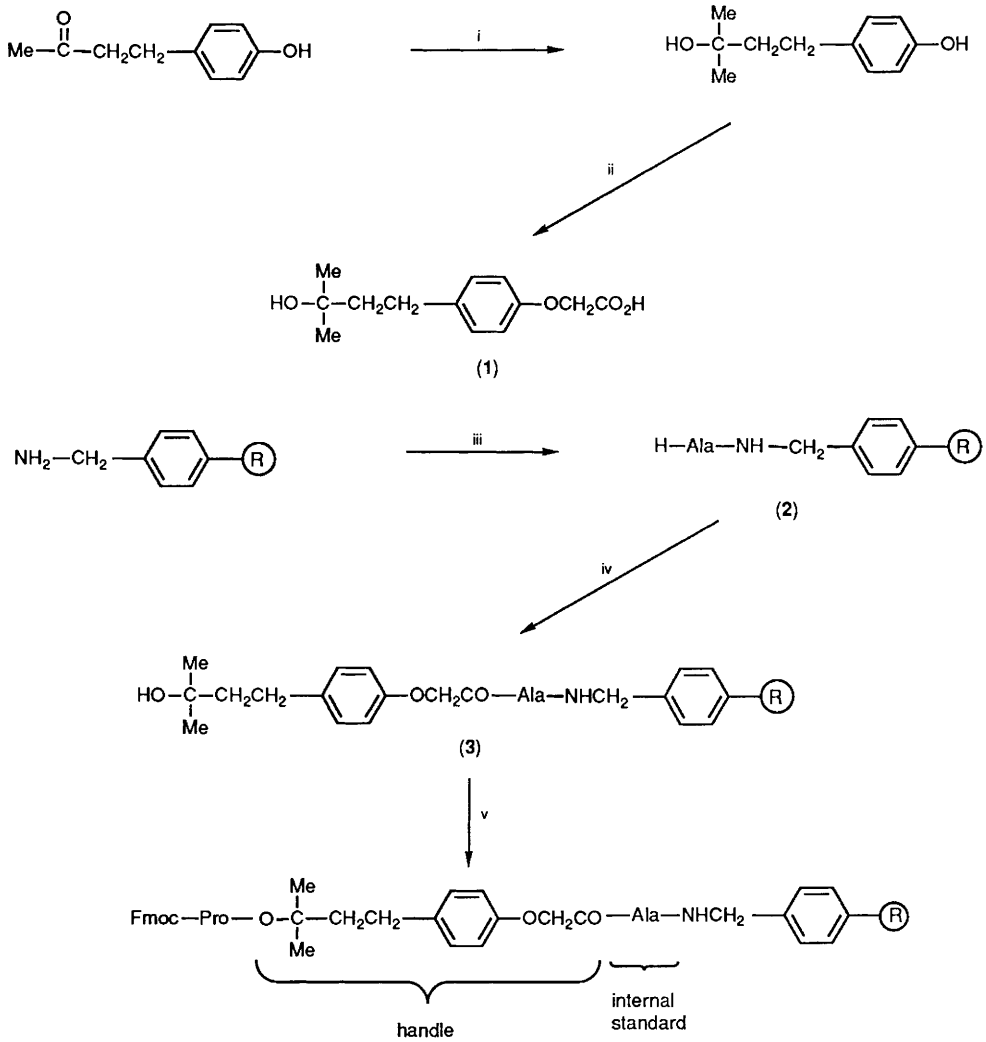
The loss of *C*-terminal dipeptide through diketopiperazine formation during Fmoc-based solid-phase peptide synthesis has been effectively suppressed by anchoring the first Fmoc amino acid chloride to a new 4-(1′,1′-dimethyl-1′-hydroxypropyl)phenoxyacetyl handle (DHPP)–resin; this new t-alcohol type DHPP–resin has been successfully applied to the solid-phase synthesis of bradykinin potentiator B (an eleven-residue peptide) (Fmoc = fluoren-9-ylmethoxycarbonyl).

The loss of *C*-terminal dipeptide through diketopiperazine formation is a serious unresolved problem in Fmoc-based solid-phase peptide synthesis (Fmoc = fluoren-9-ylmethoxycarbonyl).[1] We report herein that this loss of dipeptide can be suppressed by anchoring the first Fmoc amino acid chloride to a new 4-(1′,1′-dimethyl-1′-hydroxypropyl)phenoxyacetyl handle (DHPP)–resin (**3**).

t-Alcohol type resin was first introduced by Wang and Merrifield to prepare a protected peptide fragment.[2] However, this type of resin was not used widely since its preparation was laborious and no satisfactory procedure for esterification of the first amino acid could be found. We considered that the t-alcohol type resin would be effective to suppress the loss of dipeptide through diketopiperazine formation due to the steric hindrance and introduced two procedures to overcome the above problems.

Firstly, we employed a new handle reagent, 4-(1′,1′-dimethyl-1′-hydroxypropyl)phenoxyacetic acid (**1**), to prepare the chemically well-defined resin. The handle reagent (**1**) [m.p. 101—102 °C, MS, *m/z* 238(M^+), satisfactory elemental



Scheme 1. Preparation of the t-alcohol type DHPP–resin and incorporation of Fmoc-Pro to the resin. *Reagents and conditions*: i, MeLi; ii, $ClCH_2CO_2H$/NaH; iii, Fmoc-Ala-OH/DIPCDI–HOBt, then 20% piperidine; iv, (**1**)/DIPCDI–HOBt; v, Fmoc-Pro-Cl. R = resin.

analyses ($C_{13}H_{18}O_4$) and 200 MHz ^{1}H NMR data] was prepared easily by two reactions starting from 4-(4′-hydroxyphenyl)butan-2-one, *i.e.*, methylation with MeLi followed by alkylation of the phenolic alcohol with chloroacetic acid (Scheme 1, overall yield 60%). The resulting handle reagent (**1**) was loaded on the H-Ala-aminomethyl polystyrene–1% divinylbenzene (**2**) (Ala content 0.61 mmol g^{-1} resin) by condensation with di-isopropylcarbodiimide (DIPCDI)[3] in the presence of *N*-hydroxybenzotriazole (HOBT)[4] until the resin became negative to the Kaiser test,[5] giving DHPP–resin (**3**). An inserted amino acid (Ala in this case) served as an internal standard to monitor the progress of reactions and the loss of dipeptide through diketopiperazine formation, after acid hydrolysis.

Secondly, we employed Fmoc amino acid chloride[6] to esterify the first amino acid to the resin bound t-alcohol function. As the first amino acid, we focused on Pro in this study since the loss of dipeptide through cyclization occurs most seriously when the dipeptide has Pro at its *C*-terminal position.[7] Fmoc-Pro-Cl (5 equiv.) in 40% pyridine in CH_2Cl_2† was esterified to the DHPP–resin (**3**) quantitatively after 10 h reaction at 25 °C, which was confirmed by comparing the recovery of Pro and Ala on an amino acid analyser. The racemization during the esterification was less than 1.5%‡ when examined by the GITC (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate) method.[10] This esterification procedure can be generally applicable since a variety of Fmoc amino acid chlorides have been prepared and fully charac-

Table 1. Amount of remaining dipeptide on each resin after 20% piperidine/DMF[a] treatment (25 °C, 20 min).

	Wang resin[b]/%	DHPP resin[c]/%
Pro-Pro	2.7	93.7
D-Val-Pro	3.3	95.5
Tyr(Bu)-Pro	6.5	96.7

[a] DMF = dimethylformamide. [b] Wang resin = *p*-benzyloxybenzyl alcohol resin. [c] DHPP resin = 4-(1′,1′-dimethyl-1′-hydroxypropyl)-phenoxyacetyl resin.

† The stability of the Fmoc group toward pyridine was well established by Carpino and Han.[8] In fact, the Fmoc amino acid remained intact in pyridine even after 48 h at 25 °C (examined by TLC).

‡ This result agreed well with the literature data[9] which showed racemization-free acylations using several N^{α}-protected amino acid (Phe, Ala, or Val) chlorides at 23—70 °C for ~12 h.

terized by Carpino *et al.*[6] The esterification of the first Pro residue with DIPCDI in the presence of dimethylaminopyridine (DMAP)[11] was not successful and only 2% esterification was observed even after 20 h reaction.

After 20% piperidine treatment of the resulting resin, the cleavage of Pro from the DHPP–resin was examined using an amino acid analyser. Quantitative cleavage of Pro was achieved within 120 min with trifluoroacetic acid (TFA)–thioanisole[12] at 25 °C or within 30 min with 1 M HBF_4–thioanisole–TFA[13] at 4 °C. The latter reagent was developed by us recently and was shown to be a more effective deprotecting reagent than TFA–thioanisole in Fmoc-based solid-phase peptide synthesis.

Next, the loss of a peptide during the removal of the Fmoc group with 20% piperidine (20 min, 25 °C) was studied using three model N^{α}-Fmoc-dipeptides attached to the resin, *i.e.*, Fmoc-Pro-Pro, Fmoc-D-Val-Pro, and Fmoc-Tyr(Bu^t)-Pro. These sequences are known to be very prone to cyclization.[7] As a resin support, the DHPP–resin and conventional *p*-benzyloxybenzylalcohol resin[14] were selected and the amount of retained peptide on each resin after piperidine treatment was compared using an amino acid analyser (Table 1). These peptides were retained nearly quantitatively on the DHPP–resin (93.7 ~ 96.7%), whereas almost complete loss of each peptide from *p*-benzyloxybenzyl alcohol resin was observed under identical conditions.

In order to demonstrate the usefulness of this new DHPP–resin for Fmoc-based solid-phase peptide synthesis, we have synthesized bradykinin potentiator B (H-Pyr-Gly-Leu-Pro-Pro-Arg-Pro-Lys-Ile-Pro-Pro-OH).[15] This peptide contains a Pro-Pro sequence at the *C*-terminal position. The protected peptide resin [H-Pyr-Gly-Leu-Pro-Pro-Arg(Mtr)-Pro-Lys(Boc)-Ile-Pro-Pro-$OCMe_2CH_2CH_2C_6H_4OCH_2CO$-Ala-$NHCH_2C_6H_4$–Polymer (Mtr = 4-methoxy-2,3,6-trimethylphenylsulphonyl, Boc = t-butoxycarbonyl)] was constructed manually using Fmoc-Pro-DHPP–resin (Ala content, 0.61 mmol g^{-1} resin) according to the procedure proposed by Sheppard *et al.*[16] Each condensation reaction proceeded smoothly and satisfactory increase of the resin weight was obtained on completion of the synthesis. Quantitative coupling of each amino acid was further confirmed by amino acid analysis of the fully protected peptide resin.§ The final deprotection and purification were carried out as follows: (i) treatment with TFA–thioanisole (25 °C, 3 h)[12] or 1 M HBF_4–thioanisole–TFA (4 °C, 1 h)[13] in the presence of *m*-cresol; (ii) precipitation with dry diethyl ether; (iii) dissolving the residue in AcOH (1 M) and removal of the resin by filtration; (iv) purification by fast protein liquid chromatography (FPLC, Pharmacia) on a column packed with YMC gel ODS-AQ 120A S-50, using a gradient of aq. 60% acetonitrile (0—100%) in aq. 0.1% TFA. The homogeneous peptide¶ was obtained in 66% (TFA method) or 68% (HBF_4 method) yield (based on the internal standard Ala in the starting DHPP-resin) and showed the same elution pattern on HPLC‖ as that of the commercial sample (Peptide Institute, Osaka, Japan).

Thus, the use of chemically well-defined t-alcohol type DHPP–resin has been shown to be efficient for the Fmoc-based solid-phase peptide synthesis.

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§ Amino acid ratios after acid hydrolysis with 12 M HCl–phenol–AcOH, 2:1:1: Glu 0.93, Pro 4.79, Gly 0.92, Ile 0.92, Leu 0.93, Lys 0.92, Arg 0.91, Ala 1.00 (internal standard).

¶ Single spot on TLC, R_f 0.39 (Bu^nOH:AcOH:Pyridine:H_2O, 4:1:1:2); $[\alpha]_D^{20}$ −164° (*c* 0.5, H_2O); amino acid ratio in 6 M HCl hydrolysate: Glu 0.97, Pro 5.08, Gly 1.02, Ile 1.00, Leu 1.05, Lys 0.99, Arg 1.01.

‖ HPLC [YMC AM302, 4.6 × 150 mm, MeCN (10—60%, 25 min) in aq. 0.1 M NaCl, 1 ml min^{-1}], the multiple peaks were obtained on both synthetic and authentic samples due to *cis–trans* isomerization at the X–Pro bonds.