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Combination of silyl carbamate and amino acid fluoride for solid-phase peptide synthesis†

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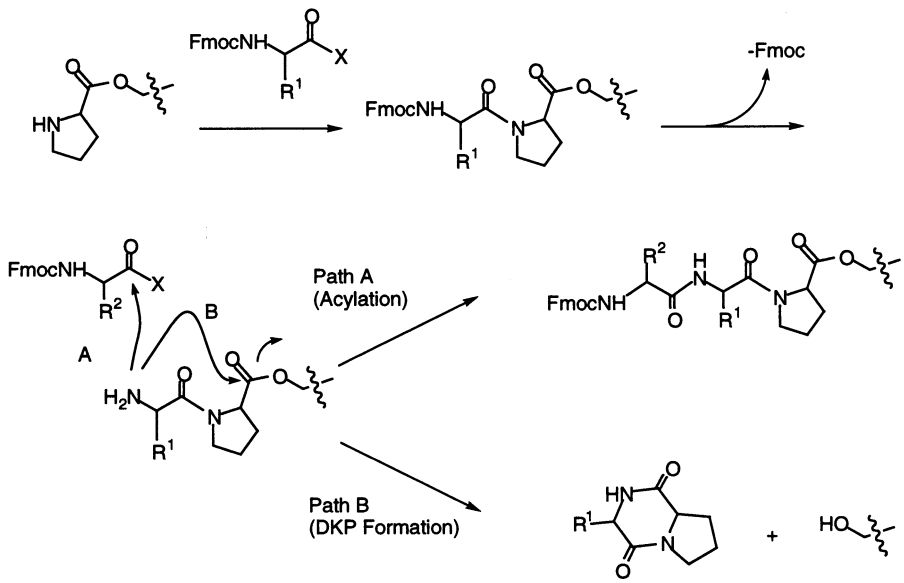
Abstract—Diketopiperadine (DKP) formation is an often encountered side reaction in the synthesis of peptide having C-terminal proline ester. A novel strategy for the avoidance of this side reaction was developed, which utilizes Pfp ester of Tsoc-amino acid and Fmoc-amino acid fluoride as the second and the third amino acid, respectively. This strategy was applied to the synthesis of 37–53 fragment of the β-chain of human chorionic gonadotropin (hCG). © 2002 Elsevier Science Ltd. All rights reserved.

For the synthesis of long chain peptides, a building block approach is generally more advantageous than a stepwise one.[1] In order to eliminate the potential risk of epimerization in the course of the block coupling, fragments having C-terminal glycine or proline are usually used as the carboxylic acid components, which in turn are prepared by solid-phase peptide synthesis (SPPS) performed in a stepwise manner. In that case, a proper choice of the linker is critical. Peptide fragments should be cleavable from the resin as a free acid, and the cleavage conditions are required to be mild enough to preserve all of the side chain protecting groups. To fulfill these requirements, allyl ester-type linkers have been developed; Pd(0) can achieve removal of which in a facile manner.[2]



Scheme 1. Competition between acylation and DKP formation.

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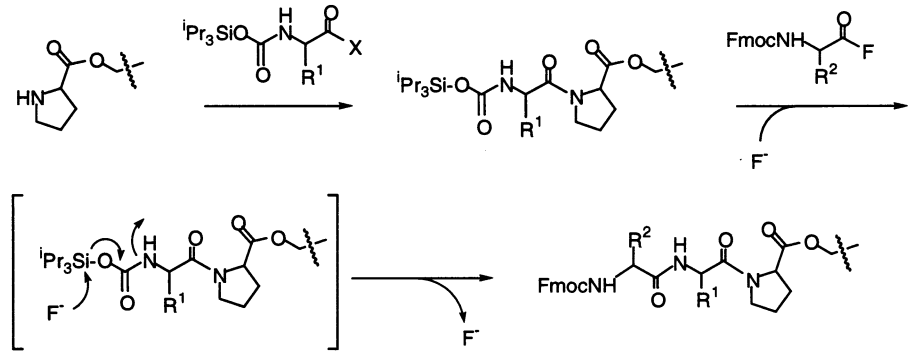
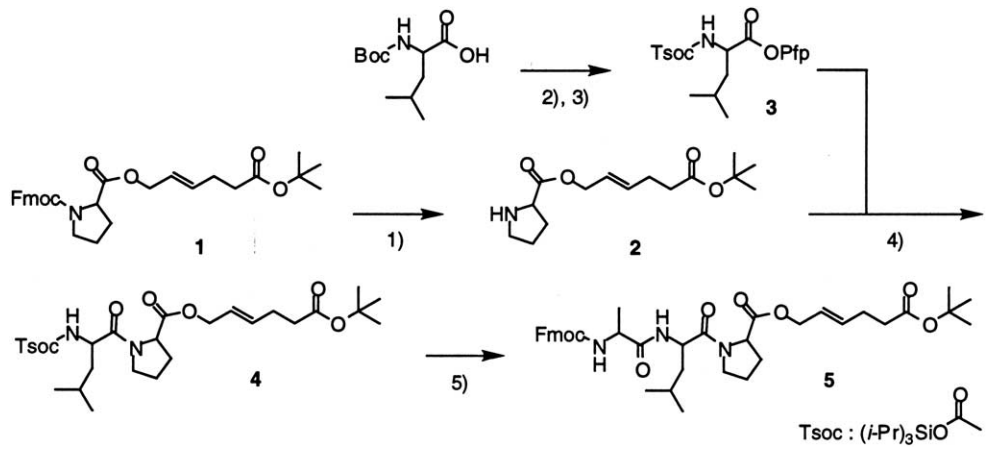
However, in Fmoc-based SPPS, when the C-terminal amino acid (AA) is linked via a non-bulky ester group (e.g. benzyl or allyl ester linker), the formation of diketopiperazine (DKP, path B) often accompanies the deprotection of the second AA residue and subsequent acylation (path A), and results in the formation of peptides lacking C-terminal two AA residues.[3] This problem is particularly serious in the case of peptides having C-terminal proline (Scheme 1). In our synthetic studies on the β-subunit 37–53 fragment of human chorionic gonadotropin (hCG)[4] using allyl ester linker, we encountered this particular problem (vide infra). Herein, we report a new strategy effective for the suppression of DKP formation, which utilizes triisopropylsilyloxycarbonyl (Tsoc)[5] protected AA pentafluorophenyl (Pfp) ester and AA fluoride, as the second and third AA residue, respectively.

Since DKP formation derives from the nucleophilic attack of liberated amine to the C-terminal ester, its extent would most likely depend upon the duration of Fmoc deprotection. Therefore, it was inferred that, if amine is liberated transiently and captured instantaneously by an acylating agent, DKP formation could be avoided. To create such a circumstance, we turned our attention to the combination of silyl carbamate and acid fluoride[6] (Scheme 2). When treated with F^-, this system was expected to give peptide with avoidance of DKP formation, because (1) *N*-deprotection and acylation steps are combined, (2) amine generally reacts rapidly with acid fluoride[7] and (3) only a catalytic amount of F^- should be sufficient to keep the reaction media near-neutral.

This conception was initially tested in solution phase using allyl linker esterified Fmoc-Pro **1**[2b,c] (Scheme 3). At first, Fmoc removal with *t*-dodecanethiol (10 equiv.)[8] and DBU (0.1 equiv.) in THF gave **2**. On the other hand, Tsoc-Leu-OPfp **3** was prepared from Boc-Leu-OH in two steps, including esterification and carbamate exchange reaction. Namely, Boc-Leu-OH was treated with Pfp-OH and DCC in AcOEt to afford Boc-Leu-OPfp, which was subsequently converted to the silylcarbamate **3** under modified Ohfune's conditions[9] [(*i*-Pr)$_3$SiOTf, 2,6-di-*t*-butyl-4-methylpyridine/CH_2Cl_2, rt, 1.5 h, 88% over two steps]. Compound **3** (1.5 equiv.) was then coupled with **2** (THF, rt, 30 min) to give **4** (84%). Subsequent reaction with Fmoc-Ala-F (1.5 equiv.) was effected with a catalytic amount (0.1 equiv.) of Bu_4NF (CH_2Cl_2, rt, 45 min).



Scheme 2. Peptide bond formation from silylcarbamate and acid fluoride.



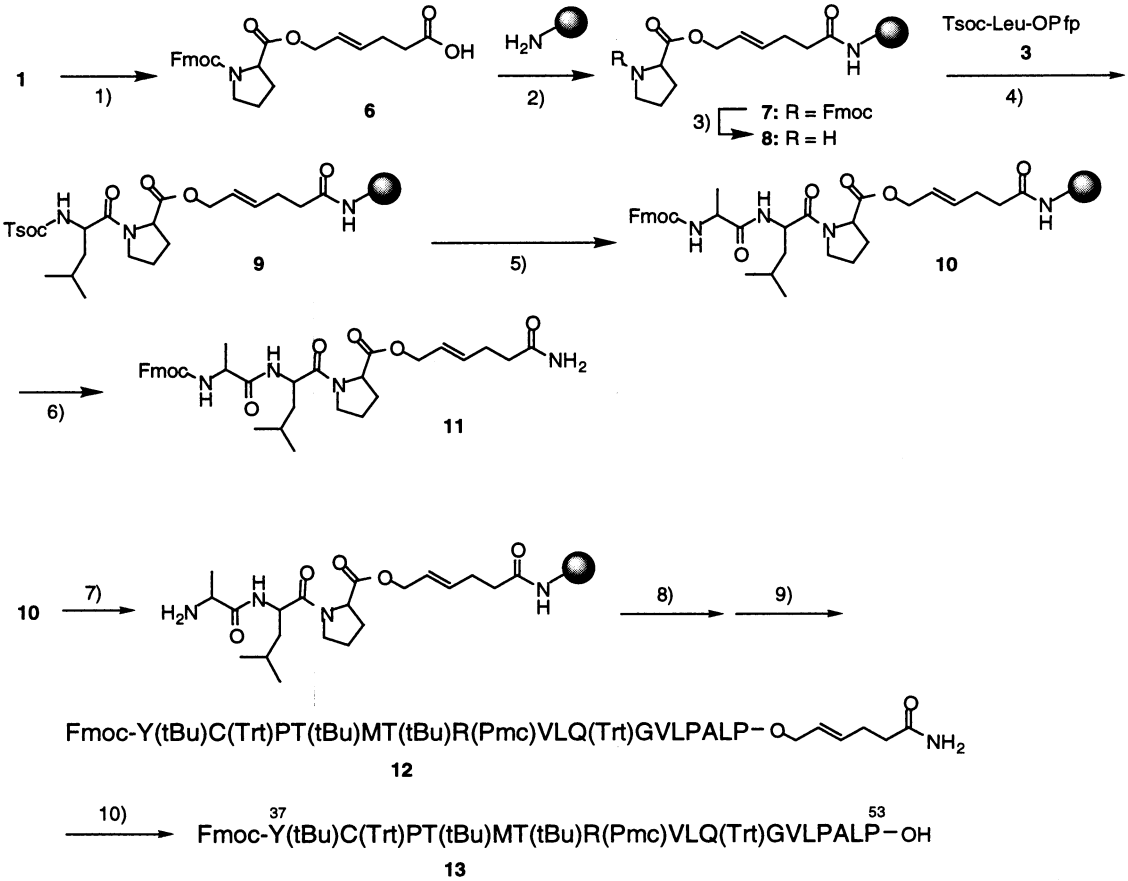
Scheme 3. Preparation of tripeptide in solution phase. (1) DBU (0.1 equiv.), *tert*-dodecanthiol (10 equiv.)/THF rt, 5 h; (2) Pfp-OH, DCC/AcOEt, 0°C, 1 h, then rt, 1.5 h; (3) (*i*-Pr)$_3$SiOTf (1.5 equiv.), 2,6-di-*tert*-butyl-4-methylpyridine (1.7 equiv.), CH_2Cl_2, 0°C, 30 min, then rt, 1 h, 88% over two steps; (4) THF, rt, 30 min, 84%; (5) Fmoc-Ala-F (1.5 equiv.), Bu_4NF (0.1 equiv.)/CH_2Cl_2, rt, 45 min, 84%.

As expected, smooth formation of the desired tripeptide **5** was observed, which was isolated in 84% yield, without any detectable DKP formation. With this success in hand, our attention was subsequently turned to the SPPS of the target sequence **13** (Scheme 4).

Thus, aforementioned **1** was converted to free acid **6** (2.5 equiv. Et_3SiH, 50% TFA/CH_2Cl_2,[10] 96%) that was attached to Sieber amide resin[11] (Novabiochem 0.52 mmol/g) through activation by HBTU-HOBt-DIEA in *N*-methylpyrrolidone (NMP). Resin bound proline **7** was *N*-deprotected with 20% piperidine in NMP. Resultant **8** was reacted with Pfp ester **3** (2 equiv.) in NMP (rt, 30 min). Subsequent coupling with Fmoc-Ala-F (1.5 equiv.) was successfully carried out in CH_2Cl_2 in the presence of Bu_4NF (0.1 equiv.) to give **10**, again with no appreciable DKP formation. Cleavage from the resin by 2% TFA in CH_2Cl_2 afforded, after chromatographic purification, tripeptide **11** in 87% yield.[12]

To synthesize the complete 37–53 fragment of hCG β-subunit, resin-bound tripeptide **10** was subjected to automated SPPS, carried out by an ABI 431A Peptide Synthesizer with Fmoc-protocol (System software Ver. 2.0, coupling by HBTU/HOBt, end capping by Ac_2O). After acidic cleavage, the target sequence was isolated as C-terminal ester **12** in 30% overall yield after HPLC purification (Inertsil prep-sil, GL Science Inc.).[13] Dramatic improvement is obvious from Fig. 1; HPLC profiles revealed the clean formation of **12** (R_t 25.71 min, m/z 3133 as M+Na^+), in stark contrast with the result obtained by conventional protocol, which solely relies upon the Fmoc chemistry. In the latter case, only a trace amount, if any, of the desired product was formed. The major product (R_t 13.10 min) proved to have m/z 2923 (M+Na^+), which corresponds to 37–51 segment and apparently derives from DKP formation. Removal of the linker was accomplished by treatment with $Pd(Ph_3P)_4$ (0.2 equiv.) and $PhNHCH_3$ (10 equiv.) in DMSO (rt, 4 h) to afford the C-terminal free acid **13** in 70% yield.

In conclusion, we have developed a novel strategy in SPPS having C-terminal proline, by which DKP formation can be completely suppressed.



Scheme 4. Solid-phase synthesis of hCG fragment. (1) 50% TFA, Et_3SiH (2.5 equiv.)/CH_2Cl_2, rt, 1.5 h, 95%; (2) HBTU, HOBt, DIEA/NMP, rt, 1 h; (3) 20% piperidine/NMP; (4) NMP, rt, 30 min; (5) Fmoc-Ala-F (2.0 equiv.), Bu_4NF (0.1 equiv.)/CH_2Cl_2, rt, 2 h; (6) 2% TFA/CH_2Cl_2; (7) 20% piperidine/NMP; (8) Fmoc-AA, HBTU, HOBt, DIEA/NMP; (9) 20% piperidine/NMP; (10) MeNHPh (10 equiv.), $Pd(PPh_3)_4$ (0.2 equiv.)/DMSO, rt, 4 h.

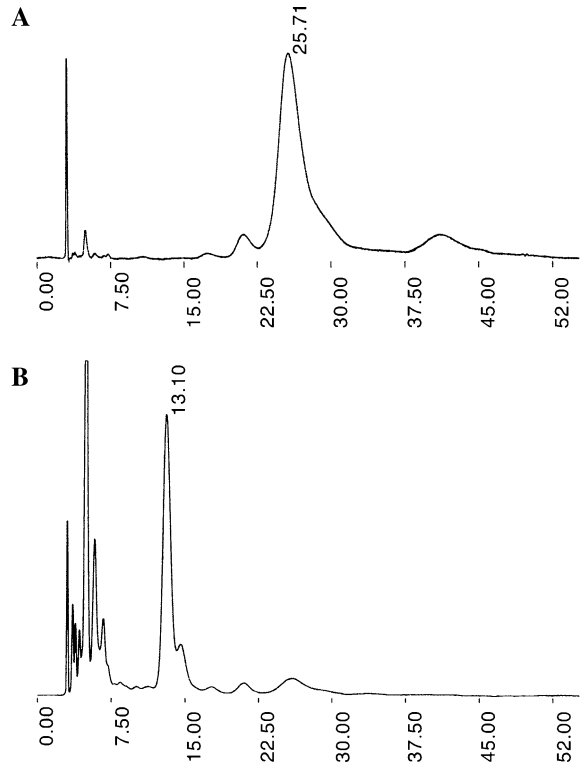


Figure 1. HPLC profiles of peptides prepared as shown in Scheme 4 (A) and conventional method (B).

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12. Yields were calculated based upon initial loading of the resin.
13. Inertsil prep-sil (GL Science Inc.), ϕ 10×250 mm, $CHCl_3$/MeOH 96/4, 2 ml/min, peaks detected at 254 nm.