Development of a New Amino-protecting Group, 2-Adamantyloxycarbonyl (2-Adoc), and its Application to the Solid-phase Synthesis of Protected Peptides

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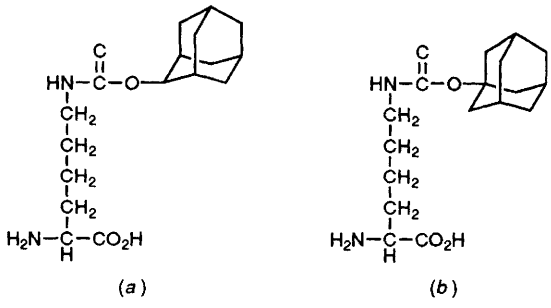
A new ε-amino protecting group, 2-adamantyloxycarbonyl (2-Adoc) has been developed, and its application to the solid phase synthesis of the protected peptide has been demonstrated successfully in combination with N^{α}-fluoren-9-ylmethoxycarbonyl (Fmoc) protection and trifluoroacetic acid (TFA)-cleavable resin support.

Recently, the convergent solid phase strategy, which involves the preparation of partially protected peptide fragments by stepwise solid phase peptide synthesis, followed by purification and their assembly on solid supports, has been employed for the synthesis of large peptides.[1] This strategy is particularly useful for overcoming the difficulties in the synthesis of large peptides, although some problems remain to be solved. In particular, higher solubility of the protected peptide fragment is required, since the fragment must be easily removed by washing with an organic solvent after fragment condensation on the resin. Our studies have therefore been directed to the development of new side-chain protecting groups with the objectives of increasing both the solubility of the peptide fragment in organic solvents and its stability to the conditions during the synthesis of protected peptide fragments for use in convergent solid phase peptide synthesis. This communication deals with the development of the new protecting group, 2-adamantyloxycarbonyl (2-Adoc), which is suitable for ε-amino protection of lysine (Lys) in the convergent strategy.

Previously, we have reported that the 2-adamantyl ester (2-Ada) employed for protection of the β-carboxy function of aspartic acid (Asp) was suitably soluble in organic solvents and stable to acid,[2,3] while the 1-adamantyl ester (1-Ada) was susceptible to acid.[2] We therefore expected that the 2-Adoc group would be stable to trifluoroacetic acid (TFA) and would increase the solubility of protected peptides; 1-adamantyloxycarbonyl (1-Adoc) was not stable to acid.[4]

Firstly, H-Lys(2-Adoc)-OH† (Fig. 1) was prepared from Lys_2–Cu complex and 2-adamantyl chloroformate in the usual manner[5] and its stability and susceptibility to various acids and



(*a*) (*b*)

Fig. 1 Structures of (*a*) Lys(2-Adoc) and (*b*) Lys(1-Adoc)

Fmoc-Cys(Acm)-Lys(2-Adoc)-Cys(Acm)-Thr(Bzl)-Ser(Bzl)-Cys(Acm)-Lys(2-Adoc)-Lys(2-Adoc)-Ser(Bzl)-Cys(Acm)-Cys(Acm)-OH

Fmoc-(GIF 25-35)-OH **1**

Fmoc-Ala-Lys(2-Adoc)-Asp(O-2-Ada)-Cys(Acm)-Val-Cys(Acm)-Lys(2-Adoc)-Gly-OH

Fmoc-(GIF 46-53)-OH **2**

Fig. 2 Amino acid sequences and side-chain protections of the protected peptides Fmoc-(GIF 25–35)-OH (**1**) and Fmoc-(GIF 46–53)-OH (**2**). (Acm = acetamidomethyl, Bzl = benzyl.)

† M.p. 241–245 °C; R_f 0.25 ($CHCl_3$–MeOH–H_2O, 8:3:1, lower phase), $[\alpha]_D$ −10.5° (*c* 1.0, 50% AcOH); satisfactory elemental analyses.

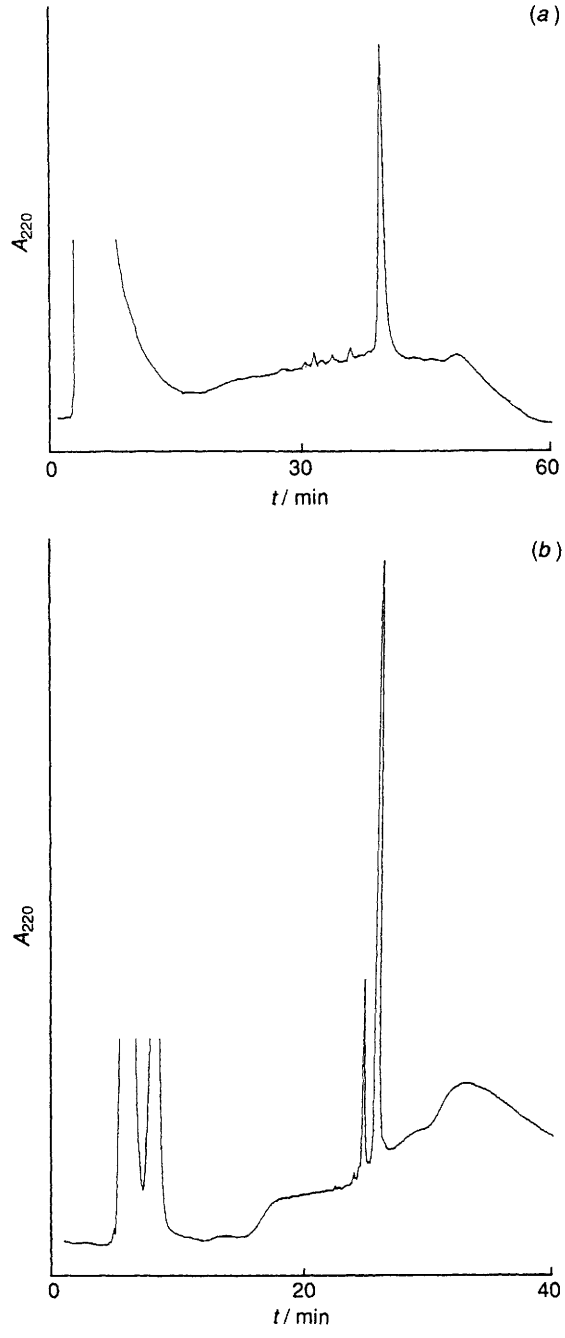


Fig. 3 Analytical HPLC of the crude products: (*a*) Fmoc-(GIF 25–35)-OH: column, YMC-Pack A-302 ODS (4.6 mm × 150 mm); solvent, a = H_2O (0.05% TFA), b = MeCN (0.05% TFA); gradient (a : b) 80 : 20 for 5 min, 80 : 20 to 20 : 80 in 25 min, 20 : 80 for 10 min, and then return to 80 : 20 in 10 min. (*b*) Fmoc-(GIF 46–53)-OH: column, YMC-Pack PROTEIN RP (4.6 mm × 250 mm); solvent, a = H_2O (0.05% TFA), b = MeCN (0.05% TFA); gradient (a : b) 80 : 20 for 5 min, 80 : 20 to 10 : 90 in 15 min, 10 : 90 for 10 min, and then return to 80 : 20 in 10 min.

bases as examined by measurements of regenerated Lys concentration with an amino acid analyser after each treatment. The 2-Adoc group was stable to 7.6 mol dm^{-3} HCl in dioxane, TFA, 25% HBr in acetic acid and 1 mol dm^{-3} trimethylsilyl bromide–thioanisole–TFA for up to 24 h, as expected. The 2-Adoc group could be removed by trifluoromethanesulfonic acid or anhydrous HF in a few minutes at 0 °C, but it was cleaved very slowly by methanesulfonic acid (MSA), and so MSA treatment was not practical for the deprotection of 2-Adoc. It was stable to 20% piperidine–dimethylformamide (DMF), fluoren-9-ylmethoxycarbonyl (Fmoc)-deprotecting reagent, and 10% triethylamine–DMF, 10% $NaHCO_3$ and 2 mol dm^{-3} NaOH, for up to 24 h. We therefore conclude that the 2-Adoc group is suitable for ε-amino protection of Lys in the convergent strategy in combination with N^{α}-Fmoc protection and TFA-cleavable resin support.

In order to evaluate the 2-Adoc group for solid phase peptide synthesis, we synthesized two protected peptides corresponding to the sequences 25–35 (**1**) and 46–53 (**2**) of metallothionein-like growth inhibitory factor (GIF)[6] by the stepwise solid phase method in combination with N^{α}-Fmoc protection,[7] TFA-cleavable resin and TFA-stable side-chain protecting groups including 2-Adoc (Fig. 2). Fmoc-Lys(2-Adoc)-OH‡ was prepared from H-Lys(2-Adoc)-OH and *N*-(fluoren-9-ylmethoxycarbonyl) succinimide (Fmoc-OSu) as usual.[8] The desired sequences were constructed on Wang resin[9] by Bop reagent[10]-mediated coupling, and then cleaved from the support by TFA–phenol (95 : 5) to retain N^{α}-Fmoc and the side-chain protecting groups. The protected peptide fragments obtained were highly homogeneous on amino acid analyses§ and reversed-phase HPLC without any purification as shown in Fig. 3. These results demonstrated that no side reaction occurred during 20% piperidine deprotection and TFA cleavage. These fragments were easily soluble in DMF, and might be employed to construct the whole GIF molecule by the convergent solid phase strategy.

The results obtained here show that the newly developed 2-Adoc group is suitable for ε-amino protection in convergent solid phase peptide synthesis in combination with Fmoc chemistry in terms of good chemoselectivity and high solubility. The 2-Adoc group is also useful for peptide synthesis by the orthogonal protection strategy both in solution and solid phase method.

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‡ Amorphous powder; R_f 0.70 ($CHCl_3$–MeOH–H_2O 8 : 3 : 1 lower phase), $[\alpha]_D$ −14.2 (*c* 1.0, DMF); satisfactory elemental analyses.

§ Fmoc-(GIF 25–35)-OH; Thr 0.96 (1), Ser 1.70 (2), Lys 3.00 (3), Cys was not determined.

Fmoc-(GIF 46–53)-OH; Asp 1.17 (1), Gly 1.00 (1), Ala 0.98 (1), Val 1.08 (1), Lys 2.21 (2), Cys was not determined.