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A New Reductive Acidolysis Final Deprotection Strategy in Solid Phase Peptide Synthesis. Use of a New Safety-Catch Linker[1]

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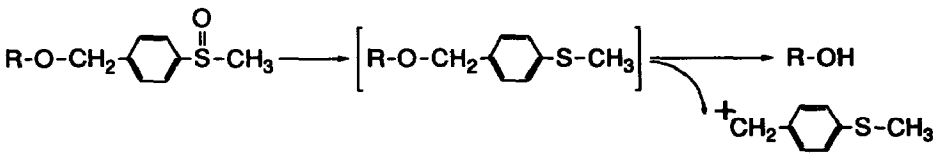
Abstract: A new reductive acidolysis final deprotection strategy in solid phase peptide synthesis was developed using a new safety-catch linker; this new strategy was based on a two-dimensional protection scheme employing acid-labile temporary and acid-stable but reductive acidolysis-cleavable semipermanent protecting groups.

In a two-dimensional protection scheme of peptide synthesis, semi-permanent protecting groups must be entirely stable under synthetic conditions including selective deprotection of temporary protecting groups and completely cleavable at the final deprotection stage. Recently, a series of safety-catch protecting groups have been developed on the basis of 4-methylsulfinylbenzyl (Msob) group.[2-9] These protecting groups are stable to acid because of electron-withdrawing character of the sulfoxide. In a one-pot reaction using tetrachlorosilane-scavengers-trifluoroacetic acid (TFA) system, the Msob-derived protecting groups are smoothly reduced to the corresponding sulfide-form and then cleaved by acidolysis (reductive acidolysis, Scheme 1).[2,5-9] These groups are suitable as semi-permanent protecting groups in solid phase peptide synthesis (SPPS)[10], since the



Scheme 1. Reductive acidolysis using $SiCl_4$-scavengers-TFA

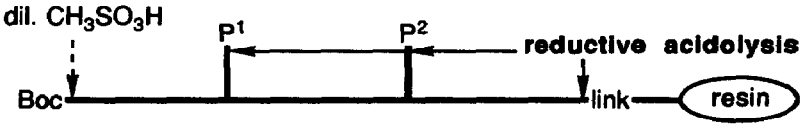
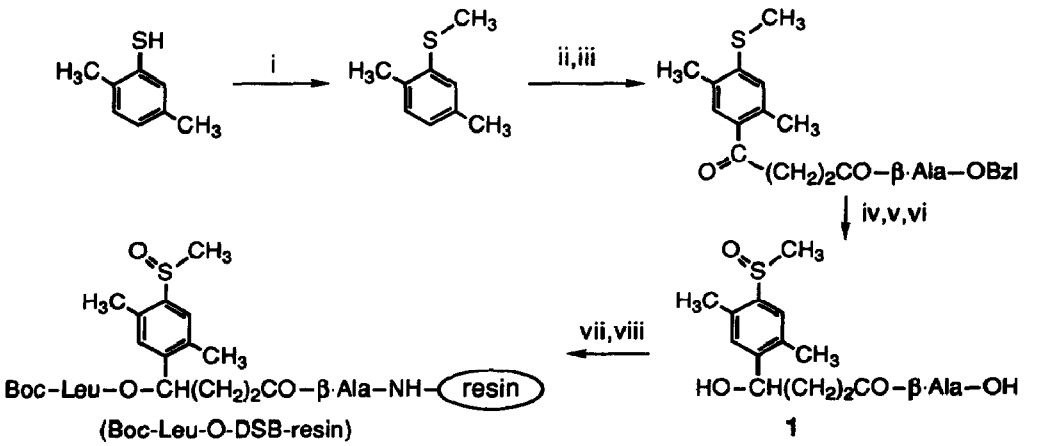


Fig. 1. A two dimensional protection scheme for SPPS by a reductive acidolysis final deprotection strategy

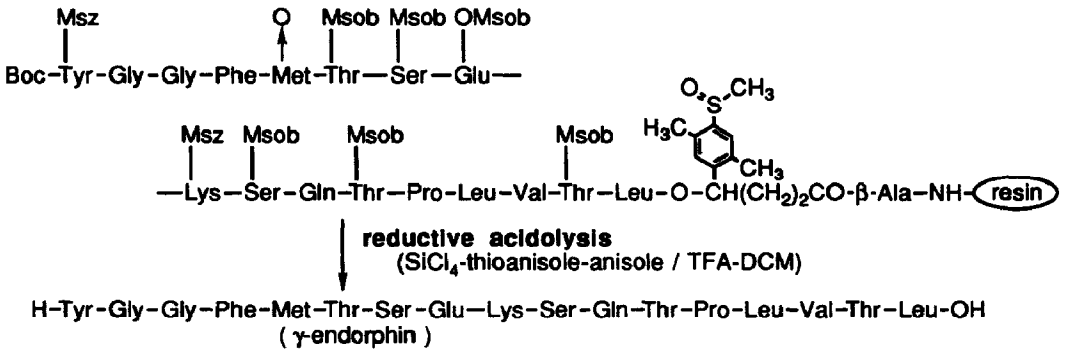
Msob-type groups possess high stability during repetitive acid deblocking cycles, but are cleavable with mild reductive acidolysis.

In this communication, we describe a new two-dimensional protection scheme in SPPS employing the safety-catch linkage to the resin and reductive acidolysis final deprotection strategy. This two-dimensional protection scheme is based on a combination of an acid-labile temporary group [Boc (*t*-butoxycarbonyl) in Fig. 1] and acid-stable but reductive acidolysis-labile semipermanent protecting groups (P^1, P^2 and anchoring link in Fig. 1).

In order to introduce a safety-catch type ester linkage to aminomethylated-polystyrene resin, we designed a new handle reagent, 4-(2,5-dimethyl-4-methylsulfinylphenyl)-4-hydroxybutanoic (DSB) acid. Using this reagent, the DSB-resin was prepared according to the scheme shown in Scheme 2. Friedel-Crafts reaction of succinic anhydride and 2,5-dimethylthioanisole prepared from the corresponding thiophenol gave a γ-



Scheme 2. Preparation of DSB-resin. *Reagents;* i, CH_3I; ii, succinic anhydride, $AlCl_3$; iii, H-β-Ala-OBzl, DCC; iv, NaOH; v, $NaBH_4$; vi, H_2O_2; vii, NH_2-resin, diisopropylethylamine, BOP; viii, Boc-Leu, DIPCDI, DMAP.



Scheme 3. Synthesis of γ-endorphin by a reductive acidolysis final deprotection strategy

ketocarboxylic acid. After the coupling with β-Ala-OBzl and saponification, the ketone moiety was reduced to alcohol using $NaBH_4$. The oxidation of the resulting sulfide to sulfoxide using H_2O_2-AcOH gave a hydroxy-carboxylic acid **1** in 47.5 % yield from the corresponding thiophenol. The handle reagent **1** was introduced to an aminomethylated polystyrene-resin by benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluoro-phosphate (BOP)-activation, and the amount of incorporated β-Ala was determined by amino acid analysis of the resulting resin. The C-terminal amino acid was coupled with DSB-resin by diisopropylcarbodiimide (DIPCDI)-*N,N*-dimethylaminopyridine (DMAP) method. Using Boc-Leu-O-DSB-resin thus obtained, the stability of the anchoring linkage was examined. The amount of cleavage by TFA-anisole (25 °C, 24 h) was 3.5 %, whereas 90.5 % of loaded amino acid was cleaved by reductive acidolysis [$SiCl_4$-thioanisole-anisole-TFA (25 °C, 3 h)]. The amount of D-Leu in the liberated amino acid was 1.7 % when determined by GITC method.[11]

In order to demonstrate the usefulness of the new strategy and DSB-resin, we synthesized γ-endorphin (H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH,[12] Scheme 3). Starting from Boc-Leu-O-DSB-resin, the protected peptide-resin was prepared according to the schedule of the efficient SPPS[13] using N^{α}-Boc-amino acids bearing reductive acidolysis-cleavable side chain protecting groups, *i.e.* Tyr(Msz),[6] Met(O), Thr(Msob),[6] Ser(Msob),[4,6] Glu(OMsob)[6] and Lys(Msz)[5] (Msz=4-methylsulfinyl-benzyloxycarbonyl). The protected γ-endorphin-resin [Boc-Tyr(Msz)-Gly-Gly-Phe-Met(O)-Thr(Msob)-Ser(Msob)-Glu(OMsob)-Lys(Msz)-Ser(Msob)-Gln-Thr(Msob)-Pro-Leu-Val-Thr(Msob)-Leu-O-DSB-resin] was deprotected and cleaved from the resin by the treatment with $SiCl_4$-thioanisole-anisole (100 eq. each)/TFA-dichloromethane (DCM) (9:1) at 25 °C for 3 h (the cleavage yield: 82 %). After purification of the crude γ-endorphin using reverse phase FPLC (fast protein liquid chromatography), the total yield based on the starting Boc-Leu-resin was 62 %. The product was identical with the authentic sample purchased from Peptide

Institute Inc. on reverse phase HPLC. Using a new His derivative, His(MsBom) (MsBom=4-methylsulfinylbenzyloxymethyl),[7] we synthesized another model peptide, valosin (H-Val-Gln-Tyr-Pro-Val-Glu-His-Pro-Asp-Lys-Phe-Leu-Lys-Phe-Gly-Met-Thr-Pro-Ser-Lys-Gly-Val-Leu-Phe-Tyr-OH)[14] in the same manner. In this synthesis, the total yield was 26 % and the cleavage yield was 70 %. The synthetic valosin was identical with the authentic sample purchased from Novabiochem on reverse phase HPLC.

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