A Reductive Acidolysis Final Deprotection Strategy in Solid Phase Peptide Synthesis Based on Safety-Catch Protection[1)]

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A reductive acidolysis final deprotection strategy in solid phase peptide synthesis was developed using a new safety-catch type of semi-permanent protecting groups and new linkers which were derived from 4-methylsulfinylbenzyl protection. This new strategy was based on a two-dimensional protection scheme employing acid-labile temporary and acid-stable but reductive acidolysis-cleavable semi-permanent protecting groups. By using this strategy, we successfully synthesized four model peptides, of which two contained C-terminal amide.

Key words safety-catch protection; reductive acidolysis; solid phase peptide synthesis; silyl chloride

Protection and deprotection steps are important in peptide synthesis. Deprotecting steps can be basically categorized into 1) deprotection of a temporary α-amino group and 2) final deprotection to give the free peptide. The results of peptide syntheses are critically influenced by the combination of these two types of deprotection along with the selection of protecting groups for α-amino and side-chain functional groups. Selectivity is highly important in deprotection of α-amino protecting groups. Undesired cleavage of the side-chain protecting groups is one of the most serious problems during peptide synthesis.[2)]

Two-dimensional protection schemes should satisfy the following requirements: 1) semi-permanent protecting groups must be entirely stable under synthetic conditions, including selective deprotection of temporary protecting groups, 2) all protecting groups must be completely cleavable at the final deprotection stage. Recently, two types of two-dimensional protection schemes have generally been employed in solid phase peptide synthesis (SPPS). One is the combination of weak acid-labile and strong acid-cleavable protections and the other is base-labile temporary and acid-labile semi-permanent protections. However, these schemes do not completely satisfy the requirements. Some of the semi-permanent protecting groups are not entirely stable under repetitive deprotection steps and incomplete final removal of protecting groups is sometimes observed, especially at Arg residues and the C-terminal amide-linkage employed in Fmoc-based SPPS. The 4-methylsulfinylbenzyl (Msob) group was first introduced by Samanen and Brandeis[3)] as a C-terminal carboxyl protecting group for peptides or amino acids. The Msob ester is more stable to acid than the corresponding benzyl ester because of the electron-withdrawing character of the sulfoxide, but is readily removable by reduction of the sulfoxide followed by mild acidolysis (Chart 1). We have already developed a sulfoxide-reducing system using silyl chloride scavengers in trifluoroacetic acid (TFA)[4)] and found that protecting groups derived from Msob could be smoothly reduced to the corresponding sulfide form and then cleaved by mild acidolysis in a one-pot reaction (reductive acidolysis). The Msob-derived groups are more stable than the traditional ones under repetitive N^{α}-selective deblocking conditions, but are easily cleavable under mild conditions at the final deprotection stage in Boc-based SPPS. These features made it possible to employ Msob-derived groups for semi-permanent protection. A strategy employing safety-catch (SC) protection, efficient SPPS[5)] and reductive acidolysis for final deprotection (Fig. 1) is advantageous for minimizing side reactions during elongation of the peptide chain and final deprotection.

In this paper, we describe the development of a series of reductive acidolysis-cleavable safety-catch protecting groups for amine, carboxylic acid, imidazole, hydroxy protection, ester-linkage and amide-linkage to resin, and the demonstration of the usefulness of the new two-dimensional protection scheme by successful synthesis of model peptides.

Results and Discussion

Preparation of Amino Acid Derivatives Bearing Protecting Groups Cleavable by Reductive Acidolysis The

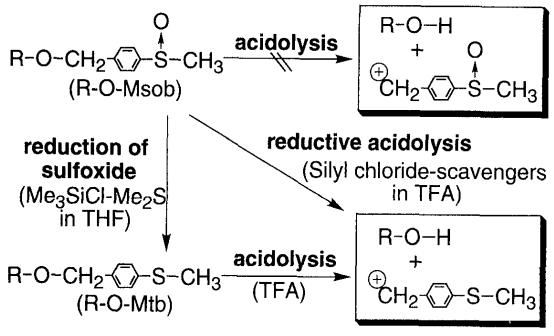

Chart 1. Reductive Acidolysis of Msob Group

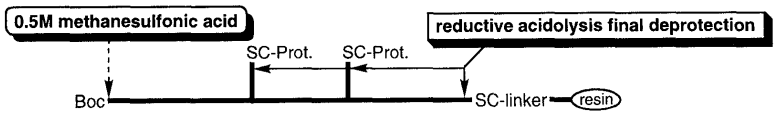

Fig. 1. Reductive Acidolysis Final Deprotection Strategy in Solid Phase Peptide Synthesis Based on Safety-Catch (SC) Protection

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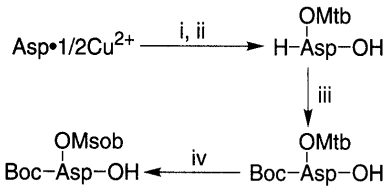

Chart 2. Preparation of Boc–Asp(OMsob)–OH

Reagents: i, Mtb-Br; ii, HCl; iii, Boc_2O; iv, $NaBrO_2$.

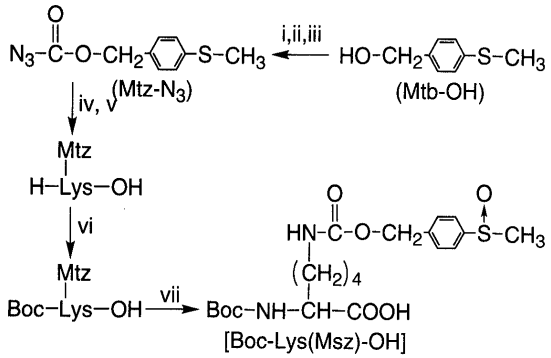

Chart 3. Preparation of Boc–Lys(Msz)–OH

Reagents: i, PhOCOCl; ii, NH_2NH_2; iii, $NaNO_2$; iv, Lys·1/2Cu^{2+}; v, EDTA; vi, Boc_2O; vii, $NaBrO_2$.

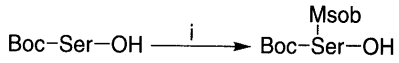

Chart 4. Preparation of Boc–Ser(Msob)–OH

Reagents: i, Msob-Br, NaH.

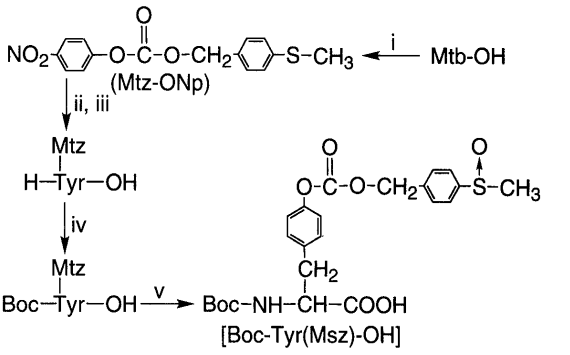

Chart 5. Preparation of Boc–Tyr(Msz)–OH

Reagents: i, ClCOONp; ii, Tyr·1/2Cu^{2+}; iii, EDTA; iv, Boc_2O; v, $NaBrO_2$.

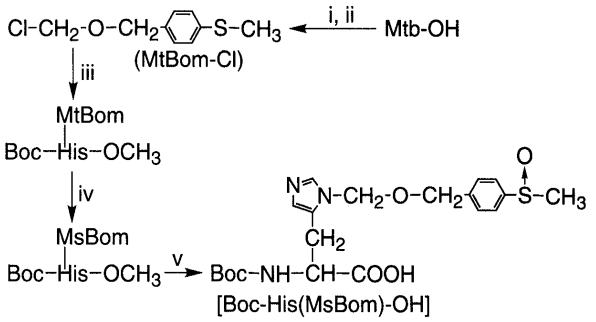

Chart 6. Preparation of Boc–His(MsBom)–OH

Reagents: i, CH_3SCH_2Cl; ii, SO_2Cl_2; iii, Boc–His(Boc)–OCH_3; iv, MCPBA; v, NaOH.

protecting groups were designed by the replacement of the benzyl moiety of known protecting groups with an Msob group.

The ω-carboxyl groups of aspartic acid and glutamic acid were protected as Msob esters. A complex of copper(II) and aspartic acid was allowed to react with 4-methylthiobenzyl bromide (Mtb-Br)[6] in aqueous *N*,*N*-dimethylformamide (DMF) (Chart 2). After removal of copper(II), introduction of the Boc group at the amino group and oxidation of sulfide to sulfoxide using sodium bromite gave the desired Boc–Asp(OMsob)–OH. In the same manner, Boc–Glu(OMsob)–OH was prepared.

The 4-methylsulfinylbenzyloxycarbonyl (Msz) group was employed for the protection of the ε-amino group of lysine, *i.e.*, a urethan type of Msob ester. 4-Methylthiobenzyloxycarbonyl azide (Mtz-N_3), prepared from 4-methylthiobenzyl alcohol in 3 steps, and a complex of copper(II) and lysine were coupled and then treated with EDTA (Chart 3). The resulting Lys(Mtz) was converted to Boc–Lys(Mtz)–OH in a usual manner. Oxidation of this compound gave Boc–Lys(Msz)–OH.

The protection of the hydroxyl groups of serine and threonine was achieved by employing Msob ether. Boc–Ser(Msob)–OH has already been reported by Futaki *et al.*[7] They employed this protecting group in order to avoid exposure of a sulfated peptide to strong acids. Introduction of Msob was carried out by coupling of Boc–Ser–OH and Msob-Br[6] in the presence of sodium hydride (Chart 4). Boc–Thr(Msob)–OH was prepared in the same manner.

Futaki *et al.*[6] also employed Msob ether as a protection for the hydroxyl group of tyrosine. However, we employed the Msz group for protection of tyrosine from the viewpoint of diminution of side reactions. A carbonate type protection is superior to the corresponding alkyl ether for suppression of alkylation of the phenol ring of tyrosine under deprotection conditions.[8] A complex of tyrosine and copper(II) was coupled with Mtz-ONp (prepared from 4-methylthiobenzyl alcohol), and then the copper(II) was removed by EDTA treatment (Chart 5). After protection of the α-amino group, sodium bromite oxidation gave the desired Boc–Tyr(Msz)–OH.

The imidazole ring of histidine is usually protected by a π-benzyloxymethyl group,[9] which effectively suppresses racemization based on the basicity of the imidazole ring, so we employed the π-4-methylsulfinylbenzyloxymethyl (MsBom) group for protection of the imidazole ring. 4-Methylthiobenzyloxymethyl chloride (MtBom-Cl), prepared from 4-methylthiobenzyl alcohol in 2 steps, was allowed to react with Boc–His(Boc)–OMe[9] and the resulting Boc–His(MtBom)–OMe was oxidized with *m*-chloroperbenzoic acid (MCPBA) (Chart 6). Saponification of this product gave the desired Boc–His(MsBom)–OH.

In the cases of the amino acid derivatives mentioned above, another chiral center was introduced at the sulfur atom of the protecting group. The products were used for synthesis as the diastereomeric mixtures.

Stability of Safety-Catch Protecting Groups The stability of protecting groups based on the Msob group is summarized in Table 1. Seven amino acid derivatives were exposed to acidic or basic conditions and the cleavage of the protecting groups was detected by TLC. The

Table 1. Stability of the Safety-Catch Protecting Groups

Entry	Conditions[a]	Asp(OMsob)	Glu(OMsob)	Lys(Msz)	Ser(Msob)	Thr(Msob)	Tyr(Msz)	His(MsBom)
1	TFA–anisole	–	–	–	–	–	–	–
2	TFA–anisole–EDT	–	–	–	–	–	–	–
3	0.5 M MSA/DOX–DCM (1:9), 2% anisole	–	–	–	–	–	–	–
4	5% Et_3N/DMF	–	–	–	–	–	–	–
5	5% DIEA/DMF	–	–	–	–	–	–	–
6	20% piperidine/DMF	N.D.[c]	N.D.[c]	–	–	–	+	–
7	1 N NaOH/MeOH	N.D.[c]	N.D.[c]	–	–	–	+	–
8	$SiCl_4$–anisole (10 eq each)/TFA[b]	+	+	+	+	+	+	+
9	$SiCl_4$–anisole–thioanisole–EDT (10 eq each)/TFA[b]	+	+	+	+	+	+	+

– Stable, + cleavable as checked by TLC. *a*) 25 °C, 24 h. *b*) 25 °C, 0.5 *h*. *c*) N.D. = not determined.

Table 2. Comparison of the Stability of Protecting Groups to TFA–Anisole at 25 °C for 50 h

	Cleavage ratio (%)[a]		Cleavage ratio (%)[a]		Cleavage ratio (%)[a]
Asp(OMsob)	2	Lys(Msz)	<1	Tyr(Msz)	2
Asp(OcHex)	1	Lys(ClZ)	11	Tyr(Msob)	4
Asp(OBzl)	8	Lys(Z)	83	Tyr(BrZ)	9
Glu(OMsob)	7			Tyr(Cl_2Bzl)	3
Glu(OcHex)	9	Ser(Msob)	3	Tyr(Bzl)	75[b]
Glu(OBzl)	14	Ser(Bzl)	14		
		Thr(Msob)	2	His(MsBom)	2
		Thr(Bzl)	18	His(Bom)	7

a) Recovery of parent amino acid was determined by amino acid analyzer. *b*) By-product was detected on TLC.

safety-catch type protecting groups were stable under the conditions generally used for Boc-based SPPS (entries 1—5), and were smoothly cleaved by reductive acidolysis (entries 8 and 9) using mild acid treatment having sulfoxide-reductive ability. These results show that safety-catch protection based on the Msob group is applicable to Boc-based SPPS.

Semi-permanent protecting groups should be entirely stable under repetitive N^{α}-deprotection conditions, but sometimes slight cleavage of protecting groups raises a problem, especially in Boc-based synthesis. Msob-type protecting groups showed very good stability to acids. The N^{α}-Boc amino acid derivatives listed in Table 2 were treated with TFA–anisole, a selective deprotection reagent for the N^{α}-Boc group, and the amounts of cleavage of side chain-protecting groups were determined by an amino acid analyzer. In most cases, the cleavage ratios of Msob-derived protecting groups were less than those of conventional protections which are widely used for peptide synthesis. The electron-withdrawing character of the sulfoxide of the protecting groups accounted for this high stability, which contributes to the decrease of impurities.

Preparation of Anchoring Linkage for Synthesis of Peptide Containing C-Terminal Free Acid In order to introduce a safety-catch type ester-linkage onto the resin, we designed a new handle reagent, 4-(2,5-dimethyl-4-methylsulfinyl)-4-hydroxybutanoyl-β-alanine (HO–DSB–β-Ala–OH), which has an Msob backbone with three substitutions. The two methyl groups made it easy to release the peptide at the final reductive acidolysis deprotection, and the other substituent was used for anchoring to resin. The handle reagent, HO–DSB–β-Ala–OH, was prepared according to Chart 7. Friedel–Crafts acylation using succinic anhydride and 2,5-dimethylthioanisole prepared from the corresponding thiophenol gave a γ-ketocarboxylic acid. After coupling with H–β-Ala–OBzl and saponification, the ketone moiety was reduced to an alcohol with $NaBH_4$. Oxidation of the sulfide to a sulfoxide using hydrogen peroxide gave a hydroxycarboxylic acid, HO–DSB–β-Ala–OH in 47.5% overall yield. The handle reagent was introduced onto

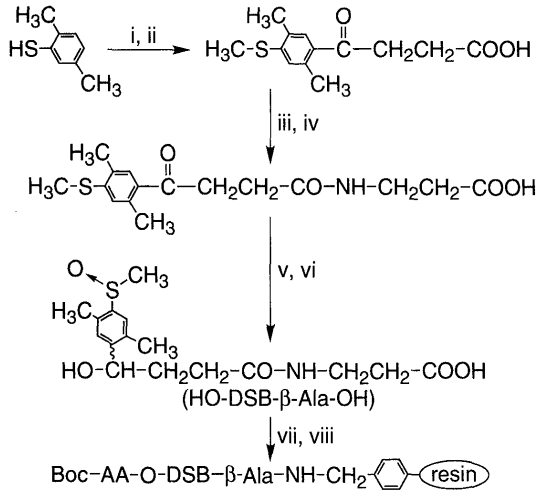

Chart 7. Preparation of Boc–AA–O–DSB–β-Ala–NH–CH_2–C_6H_4-Resin

Reagents: i, CH_3I; ii, succinic anhydride, $AlCl_3$; iii, H–β-Ala–OBzl, DCC; iv, NaOH; v, $NaBH_4$; vi, H_2O_2, AcOH; vii, NH_2–CH_2–C_6H_4-resin, BOP; viii, Boc–AA–OH, DIPCDI, DMAP.

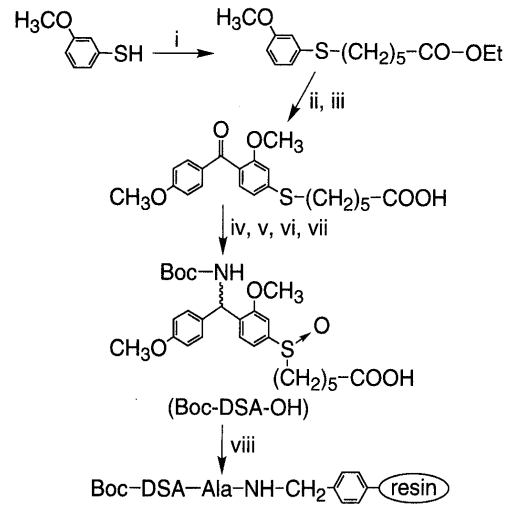

Chart 8. Preparation of Boc–DSA–Ala–NH–CH_2–C_6H_4-Resin

Reagents: i, $Br(CH_2)_5COOEt$; ii, anisoyl chloride, $AlCl_3$; iii, NaOH; iv, NH_2OH; v, Zn, AcOH; vi, Boc_2O; vii, H_2O_2, AcOH; viii, H–Ala–NH–CH_2–C_6H_4-resin, BOP.

aminomethylated polystyrene resin by benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)[10] activation, and the amount of incorporated β-Ala was determined by amino acid analysis of the resulting resin. A C-terminal amino acid was coupled with the HO–DSB–β-Ala–NH–CH_2–C_6H_4-resin by using the diisopropylcarbodiimide (DIPCDI)-4-dimethylaminopyridine (DMAP) method.[11] Since racemization of the loaded amino acid is a known side reaction during this esterification, the amount of the D-isomer of the loaded amino acid was determined by the GITC method.[12] In the case of leucine, 1.7% of D-Leu was incorporated.

Preparation of Anchoring Linkage for Synthesis of Peptide-Amide We designed a safety-catch linker suitable for peptide-amide synthesis by the incorporation of an alkylsulfinyl group on the benzhydrylamine type linker. We considered that three electron-donating groups are required for the smooth release of peptide-amide by mild acidolysis, since three hydrogens on the benzene rings are substituted with alkyloxy groups in the case of Rink linker[13] or PAL linker[14] which is cleavable by weak acid treatment. A new dialkoxyalkylsulfinylbenzhydrylamine-type handle reagent, 4-(4-methoxyphenyl-Boc-amino)methyl-3-methoxyphenylsulfinyl-6-hexanoic acid (DSA handle reagent) was designed, with two alkyloxy groups and one alkylsulfinyl group on the benzene rings, of which the latter can be converted to an electron-donating alkylthio group. The DSA handle reagent was prepared in seven steps starting from 3-methoxythiophenol (Chart 8). Ethyl 6-(3-methoxyphenyl)thiohexanoate prepared from 3-methoxythiophenol and ethyl 6-bromohexanoate was acylated by Friedel–Crafts reaction with *p*-anisoyl chloride. The saponification of the resulting benzophenone derivative gave 4-(4-methoxybenzoyl)-3-methoxyphenylthio-6-hexanoic acid which was characterized by X-ray crystallography. The product was converted to the oxime, and reduced to a benzhydrylamine. The amine was allowed to react with Boc_2O and then oxidized to sulfoxide to give the DSA handle reagent in 34.1% overall yield. The coupling of the DSA handle with an alanylaminomethylated polystyrene resin gave N–Boc–DSA–Ala–NH–CH_2–C_6H_4-resin. Pátek and Lebl have also reported this type of linker, *i.e.*, a benzhydrylamine derivative containing one alkoxy and two alkylsulfinyl groups.[15]

Table 3. Stability of Anchoring Linkage

Entry	Conditions[b]	Cleavage of Leu (%)[a]	
		Boc–Leu–O–DSB–β-Ala–NH–$CH_2C_6H_4$-resin	Boc–Leu–DSA–Ala–NH–$CH_2C_6H_4$-resin
1	0.5 M MSA/DOX–DCM (1:9), 2% anisole	5	9
2	TFA–anisole	3	9
3	45% TFA/DCM	4	10
4	5% Et_3N/DMF	4	4
5	2% pyridine/DMF	2	8
6	$SiCl_4$–anisole (10 eq each)/TFA	88[c] (91)[d]	69[c] (79)[d]
7	$SiCl_4$–anisole–thioanisole–EDT (10 eq each)/TFA	92[c] (94)[d]	92[c] (95)[d]

a) Determined by amino acid analysis of hydrolyzate of the resulting resin. *b*) 25 °C, 24 h. *c*) 25 °C, 1 h. *d*) 25 °C, 3 h.

Stability of DSB and DSA Linkages The properties of the DSB and DSA linkages were examined using Boc–Leu–O–DSB–β-Ala–NH–CH_2–C_6H_4-resin or Boc–Leu–DSA–Ala–NH–CH_2–C_6H_4-resin. The incorporated β-Ala and Ala were used as internal standards. The stability was determined by amino acid analysis of hydrolyzates of the resin after treatment with various reagents, and the results are shown in Table 3. Both linkages were stable under the acidic conditions used for selective deprotection of the N^{α}-Boc group (entries 1—3), and the basic conditions (entries 4 and 5) used for Boc-based SPPS. The cleavage ratios were 3—10% by treatment with 0.5 M methanesulfonic acid (MSA)/dichloromethane (DCM)–dioxane (DOX) (9:1)–anisole[5,16] or TFA–anisole at 25 °C for 24 h. Under basic conditions, such as 2% pyridine/DMF[5] and 5% triethylamine (TEA)/DMF at 25 °C for 24 h, the recovery of Leu was 92—98%. After treatment with a reductive acidolysis system using tetrachlorosilane–anisole–thioanisole–ethane–1,2-dithiol/TFA–DCM (entry 7), the amount of Leu remaining on the resulting resin was 8% (25 °C, 1 h) or 5—6% (25 °C, 3 h). The two linkages showed almost the same stability, and were suitable for application to Boc-based SPPS.

Synthesis of Model Peptides In order to demonstrate the usefulness of this new strategy based on safety-catch semi-permanent protection and reductive acidolysis final deprotection, four peptides were synthesized as model peptides. γ-Endorphin,[17] a 17-amino acid residue peptide, was synthesized on resin containing the DSB linkage and amino acid derivatives bearing safety-catch protecting groups (Chart 9). Starting from Boc–Leu–O–DSB–β-Ala–NH–CH_2–C_6H_4-resin, a protected γ-endorphin-resin was prepared according to a schedule of efficient SPPS,[5] in which *in situ* neutralization and BOP activation were employed. In the synthesis, the methionine residue was introduced as a sulfoxide form which was convertible to

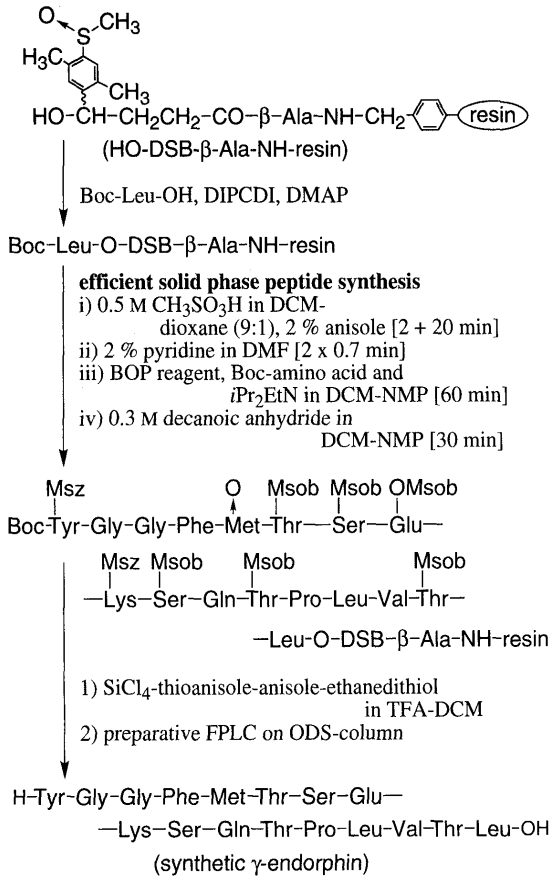

Chart 9. Synthesis of γ-Endorphin

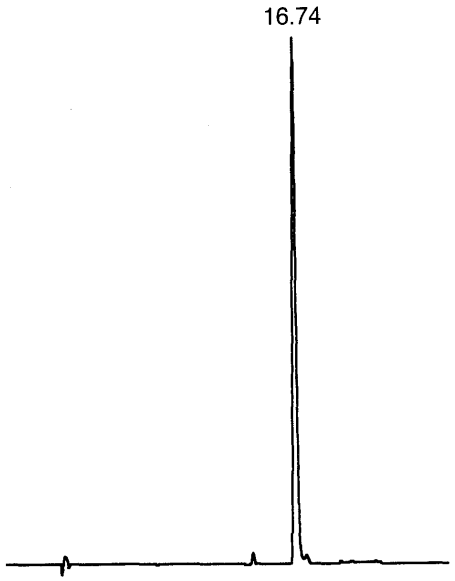

Fig. 2. HPLC Elution Pattern of Crude γ-Endorphin

Conditions: column, YMC AM302 (ODS) (4.6 × 150 mm); eluent, MeCN 10%—60% (30 min) in 0.1% TFA; flow rate, 0.7 ml/min; O.D., 280 nm.

the parent sulfide form by the reductive acidolysis system.[4] After chain elongation, the protected γ-endorphin-resin was treated with tetrachlorosilane–anisole–thioanisole–ethane-1,2-dithiol/TFA–DCM at 25 °C for 3 h, and then the reaction mixture was concentrated *in vacuo* at 25 °C. The crude γ-endorphin was precipitated with ether and extracted with 1 N acetic acid (Fig. 2). The cleavage yield was 82% as determined by amino acid analysis of

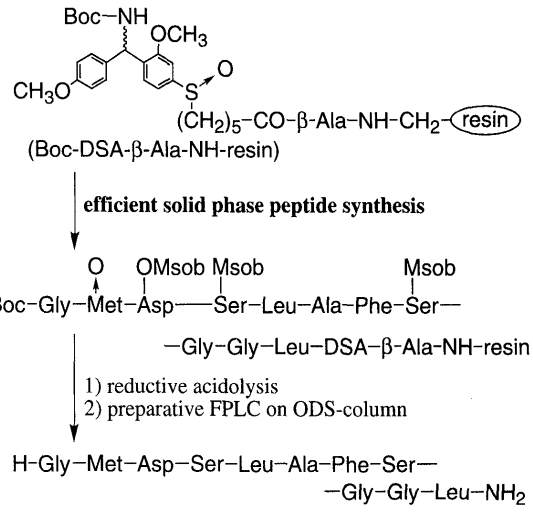

Chart 10. Synthesis of Buccalin

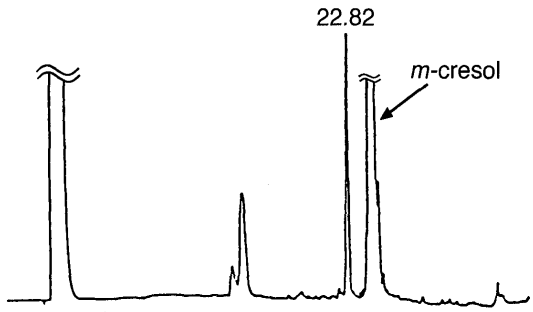

Fig. 3. HPLC Elution Pattern of Crude Buccalin

Conditions: column, YMC AM302 (ODS) (4.6 × 150 mm); eluent, MeCN 10%—60% (30 min) in 0.1% TFA; flow rate, 0.7 ml/min; O.D., 230 nm.

the hydrolyzate of the resulting resin. The crude peptide was purified on a reverse phase-FPLC column to give a homogeneous peptide in 62% overall yield. The synthetic γ-endorphin was identical with a commercially available sample on reverse phase-HPLC.

Another model peptide which has a C-terminal free acid, 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)[18] was synthesized in the same manner using N^{α}-Boc amino acid derivatives *i.e.*, Asp(OMsob), Glu(OMsob), Lys(Msz), Ser(Msob), Thr(Msob), Tyr(Msz), His(MsBom) and Met(O). In this synthesis, the total yield was 25% and the cleavage yield was 70%.

As a model peptide containing a C-terminal amide, buccalin[19] was synthesized; it is an 11-amino-acid peptide-amide (Chart 10). Starting from Boc–Leu–DSA–β-Ala–NH–CH_2–C_6H_4-resin, construction of protected buccalin-resin was achieved by the efficient SPPS. The peptide-resin was treated with tetrachlorosilane–*m*-cresol–thioanisole–ethane-1,2-dithiol/TFA–DCM at 25 °C for 3 h. After purification of the crude sample (Fig. 3), a homogeneous peptide was obtained in 40% overall yield and the cleavage yield was 77%. The formation of succinimide from Asp-containing peptides is a serious side reaction which is dependent on the amino acid sequence. The side reactions at Asp residues bearing various protecting groups were compared by the synthesis of Asp–

Gly-containing peptides.[20] The best results were obtained by the use of Msob or tert-butyl as protecting groups of the Asp residue. In the case of synthesis of buccalin containing the Asp–Ser sequence, which is susceptible to imide formation, no succinimide formation was detected by HPLC analysis.

PHM-27 (H–His–Ala–Asp–Gly–Val–Phe–Thr–Ser–Asp–Phe–Ser–Lys–Leu–Leu–Gly–Gln–Leu–Ser–Ala–Lys–Lys–Tyr–Leu–Glu–Ser–Leu–Met–NH_2)[21] was also synthesized in the same manner using Asp(OMsob), Thr(Msob), Lys(Msz), Tyr(Msz), Glu(OMsob) and His(MsBom). The total yield was 13% and the cleavage yield was 80%.

Conclusion

We have developed a series of safety-catch protecting groups derived from the Msob group, which have good stability under acidic conditions and are easily cleaved by mild acid treatment, such as reductive acidolysis. These groups offer good stability and easy cleavage as semi-permanent protecting groups. In order to apply the concept to solid phase synthesis, two handle reagents were also developed with a safety-catch type of ester or benzhydrylamide linkage to resin. The usefulness of the strategy, consisting of a combination of safety-catch protection and reductive acidolysis final deprotection, was demonstrated by the successful synthesis of four model peptides. This class of protecting groups is expected to be useful for the synthesis of complicated peptides, such as cyclic or branched peptides, in combination with traditional protecting groups.

Experimental

Melting points were determined on a Yanagimoto micro melting apparatus without correction. All compounds except free peptides and resins were routinely checked by TLC with Merck Silica gel $60F_{254}$ precoated plates. Analytical HPLC and amino acid analysis were conducted with a Hitachi L-6200 and a Hitachi L-8500, respectively. FAB-MS was obtained on a JEOL JMS-SX102A spectrometer equipped with the JMA-DA7000 data system. Optical rotation was determined with a HORIBA SEPA-300 polarimeter. Preparative fast protein liquid chromatography (FPLC) and HPLC were conducted with a Pharmacia FPLC system and a Shimadzu LC-4A, respectively. Materials were obtained from commercial suppliers and used without further purification.

Preparation of Amino Acid Derivatives Bearing Safety-Catch Protecting Groups Boc–Asp(OMsob)–OH: $CuCl_2 \cdot 2H_2O$ (0.636 g, 3.77 mmol) in water (3.78 ml) was added to a solution of aspartic acid (0.502 g, 3.77 mmol) in 2 N NaOH (3.78 ml, 7.56 mmol), and the mixture was stirred for 1 h at room temperature. The precipitate was washed with water and dried (0.724 g). Tetramethylguanidine (0.788 ml, 6.28 mmol) was added to a suspension of the above solid and aspartic acid (0.418 g, 3.14 mmol) in DMF–water (2.66 ml–0.32 ml), and the mixture was stirred for 2 h. Then a solution of 4-methylthiobenzyl bromide (1.5 g, 6.91 mmol) in DMF (2.13 ml) was added. The whole was stirred for 14 h, then acetone (25 ml) was added to give a solid. This solid was treated with 1 N HCl in AcOH : dioxane (1 : 3) (16 ml), and the resultant white powder was collected by filtration and washed with acetone (0.744 g). The resulting powder and $NaHCO_3$ (0.491 g, 5.84 mmol) were suspended in water–dioxane (5 ml–5 ml), and di-*tert*-butyl dicarbonate (0.637 g, 2.92 mmol) was added at 4 °C. The mixture was stirred for 14 h, then acidified with citric acid and evaporated. The product was extracted with AcOEt (10 ml) and washed with saturated NaCl. To this solution, water (5 ml), $NaHCO_3$ (0.143 g, 1.7 mmol) and $NaBrO_2 \cdot 3H_2O$ (0.555 g, 2.67 mmol) were added at room temperature. The mixture was stirred for 0.5 h, then $Na_2S_2O_3 \cdot 5H_2O$ (0.302 g, 1.22 mmol) was added and the whole was acidified with 1 N H_2SO_4. The organic layer was washed with 1 N H_2SO_4 and saturated NaCl, dried over Na_2SO_4 and evaporated. Trituration of the residue with hexane gave a solid (0.848 g, 35%), mp 95—96 °C, $[\alpha]_D^{23} = -15.87°$ ($c = 0.38$, DMF). FAB-MS $[M+H]^+ = 386$. *Anal.* Calcd for $C_{17}H_{23}NO_7S$: C, 52.97; H, 6.02; N, 3.63. Found: C, 52.75; H, 6.08; N, 3.53.

Boc–Glu(OMsob)–OH: $CuCl_2 \cdot 2H_2O$ (0.636 g, 3.77 mmol) in water (3.78 ml) was added to a solution of glutamic acid (0.554 g, 3.77 mmol) in 2 N NaOH (3.78 ml, 7.56 mmol), and the mixture was stirred for 1 h at room temperature. The precipitate was washed with water and dried (0.768 g). Tetramethylguanidine (0.788 ml, 6.28 mmol) was added to a suspension of the above solid and glutamic acid (0.462 g, 3.14 mmol) in DMF–water (2.66 ml–0.32 ml), and the mixture was stirred for 2 h. Then, a solution of 4-methylthiobenzyl bromide (1.5 g, 6.91 mmol) in DMF (2.13 ml) was added. The whole was stirred for 14 h, and acetone (25 ml) was added to give a solid. This solid was treated with 1 N HCl in AcOH : dioxane (1 : 3) (15 ml), and the resultant white powder was collected by filtration and washed with acetone (1.047 g). The resulting powder and $NaHCO_3$ (0.687 g, 8.18 mmol) were suspended in water–dioxane (10 ml–10 ml), and di-*tert*-butyl dicarbonate (0.928 g, 4.25 mmol) was added at 4 °C. The mixture was stirred for 14 h, then acidified with citric acid and the solvent was removed. The product was extracted with AcOEt (20 ml) and the organic solution was washed with saturated NaCl. To this solution, water (10 ml), $NaHCO_3$ (0.193 g, 2.29 mmol) and $NaBrO_2 \cdot 3H_2O$ (0.747 g, 3.60 mmol) were added at room temperature. The mixture was stirred for 0.5 h, then $Na_2S_2O_3 \cdot 5H_2O$ (0.406 g, 1.64 mmol) was added and the whole was acidified with 1 N H_2SO_4. The organic layer was washed with 1 N H_2SO_4 and saturated NaCl, dried over Na_2SO_4 and evaporated. Trituration of the residue with hexane gave a solid (0.827 g, 33%), mp 96—102 °C, $[\alpha]_D^{23} = -7.10°$ ($c = 0.49$, DMF). FAB-MS $[M+H]^+ = 400$. *Anal.* Calcd for $C_{18}H_{25}NO_7S \cdot 1/2H_2O$: C, 52.93; H, 6.42; N, 3.43. Found: C, 52.89; H, 6.22; N, 3.33.

Mtz–$NHNH_2$: Phenyl chloroformate (19.8 ml, 158 mmol) was added to a solution of 4-methylthiobenzyl alcohol (20 g, 130 mmol) in DCM–pyridine (160 ml–15.6 ml) at −5 °C and the mixture was stirred for 14 h at 4 °C. It was concentrated and the product was extracted with ether. The organic solution was washed with water and concentrated. The residue was taken up in MeOH (200 ml), hydrazine hydrate (15 ml, 264 mmol) was added and the mixture was stirred for 5 h at room temperature and then evaporated. The product was extracted with ether and the organic solution was washed with water, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Addition of hexane gave a solid (27 g, 98%), mp 74—75 °C. EI-MS $[M+H]^+ = 212$. *Anal.* Calcd for $C_9H_{12}N_2O_2S$: C, 50.92; H, 5.70; N, 13.20. Found: C, 51.00; H, 5.62; N, 13.23.

Mtz–N_3: The above product (14 g, 65 mmol) was dissolved in AcOH–water (52 ml–26 ml), sodium nitrite (4.6 g, 66.7 mmol) was added in portions at 4 °C, and then water (26 ml) and ether (150 ml) were added. The organic layer was washed with water, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and concentrated. The product was used immediately for the next reaction.

H–Lys(Mtz)–OH: $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ (24.0 g, 100.4 mmol) was added to a solution of lysine hydrochloride (10.0 g, 54.7 mmol) in water (100 ml), in a boiling water bath. The mixture was stirred for 1 h at the same temperature, and the insoluble material was filtered off. To this solution, $NaHCO_3$ (21.5 g, 256 mmol) and Mtz–N_3 in acetone (200 ml) were added at 4 °C. Stirring was continued for 14 h at room temperature, then removal of the solvent gave a solid. The precipitate was washed with cold water and ethanol and then suspended in a solution of EDTA · 2Na · H_2O (20.4 g, 90 mmol) in water (100 ml). The mixture was stirred for 1 h at 100 °C. Cooling of the mixture gave a white solid (14.6 g, 81%), mp 230—231 °C, $[\alpha]_D^{25} = -10.5°$ ($c = 0.1$, AcOH). FAB-MS $[M+H]^+ = 327$. *Anal.* Calcd for $C_{15}H_{22}N_2O_4S$: C, 55.19; H, 6.79; N, 8.64. Found: C, 55.14; H, 6.78; N, 8.64.

Boc–Lys(Mtz)–OH: Di-*tert*-butyl dicarbonate (6.62 g, 30.3 mmol) was added to a suspension of H–Lys(Msz)–OH (9.0 g, 25.6 mmol) and Et_3N (5.75 ml, 41.4 mmol) in water–THF (50 ml–50 ml) at 4 °C. The mixture was stirred for 14 h at room temperature, then concentrated and acidified with 1 N HCl. The product was extracted with AcOEt. The organic layer was washed with 1 N HCl and saturated NaCl, dried over Na_2SO_4 and concentrated. Trituration of the residue in hexane gave a solid (10.82 g, 92%), mp 85—86 °C, $[\alpha]_D^{24} = -10.44°$ ($c = 0.30$, DMF). FAB-MS $[M+H]^+ = 427$. *Anal.* Calcd for $C_{20}H_{30}N_2O_6S$: C, 56.32; H, 7.09; N, 6.57. Found: C, 56.38; H, 7.21; N, 6.57.

Boc–Lys(Msz)–OH: Water (50 ml), $NaHCO_3$ (1.37 g, 16.4 mmol) and

$NaBrO_2 \cdot 3H_2O$ (5.35 g, 25.7 mmol) were added to a solution of Boc–Lys(Mtz)–OH (10 g, 23.4 mmol) in AcOEt (100 ml) at room temperature. The mixture was stirred for 0.5 h, then $Na_2S_2O_3 \cdot 5H_2O$ (2.9 g, 11.7 mmol) was added and the whole was acidified with 1 N H_2SO_4. The organic layer was washed with 1 N H_2SO_4 and saturated NaCl, and dried over Na_2SO_4. Evaporation of the solvent gave a solid (9.94 g, 96%), mp not clear, $[\alpha]_D^{23} = -8.47°$ ($c = 0.56$, DMF). HRFAB-MS *m/z*: 443.1843 for $[M+H]^+$ (Calcd 443.1852 for $C_{20}H_{30}N_2O_7S$). *Anal.* Calcd for $C_{20}H_{30}N_2O_7S \cdot 1/2H_2O$: C, 53.19; H, 7.06; N, 6.20. Found: C, 53.65; H, 7.17; N, 5.78.

Boc–Ser(Msob)–OH: A solution of Boc–Ser–OH (3 g, 14.6 mmol) in DMF (30 ml) was treated with NaH (60% oil suspension, 1.29 g, 32.2 mmol) and the whole was stirred for 0.5 h at −15 °C, then 4-methylsulfinylbenzyl bromide (4.77 g, 20.5 mmol) was added. Stirring was continued for 3 h at room temperature and the mixture was neutralized with citric acid and concentrated. AcOEt (50 ml) and 5% $NaHCO_3$ (50 ml) were added to the residue and the aqueous layer was collected and acidified with 0.5 N H_2SO_4. The product was extracted with AcOEt and washed with 0.5 N H_2SO_4 and saturated NaCl. The product was dried over Na_2SO_4 and concentrated. The crude product was purified on a silica-gel column with $CHCl_3$–MeOH (10 : 0.5, v/v) and precipitated with ether (3.34 g, 64%), mp 69—70 °C, $[\alpha]_D^{26} = +34.15°$ ($c = 0.41$, MeOH). FAB-MS $[M+H]^+ = 358$. *Anal.* Calcd for $C_{16}H_{23}NO_6S \cdot 1/2H_2O$: C, 52.44; H, 6.33; N, 3.82. Found: C, 52.44; H, 6.50; N, 3.82.

Boc–Thr(Msob)–OH: A solution of Boc–Thr–OH (1.8 g, 8.2 mmol) in DMF (20 ml) was treated with NaH (60% oil suspension, 0.72 g, 18.1 mmol) at −15 °C, the mixture was stirred for 0.5 h at room temperature, and then 4-methylsulfinylbenzyl bromide (2.5 g, 10.7 mmol) was added. The reaction mixture was treated in the same manner as above (1.35 g, 44%), mp 114—116 °C, $[\alpha]_D^{26} = -176.47°$ ($c = 0.6$, MeOH). FAB-MS $[M+H]^+ = 372$. *Anal.* Calcd for $C_{17}H_{25}NO_6S \cdot H_2O$: C, 52.42; H, 6.47; N, 3.60. Found: C, 52.48; H, 6.78; N, 3.50.

Mtz–ONp: 4-Methylthiobenzyl alcohol (1.82 g, 11.81 mmol) was added to a solution of 4-nitrophenyl chloroformate (2.38 g, 11.81 mmol) in pyridine (4.8 ml) at 4 °C. The mixture was stirred for 3 h at room temperature, then poured into water (50 ml). The product was extracted with AcOEt (3 × 10 ml) and the organic solution was washed with 1 N HCl, 5% $NaHCO_3$ and saturated NaCl. The organic layer was dried over Na_2SO_4 and concentrated. The product was solidified by addition of ethanol and reprecipitated from AcOEt–ethanol (2.66 g, 70%), mp 78—79 °C. *Anal.* Calcd for $C_{15}H_{13}NO_5S$: C, 56.42; H, 4.10; N, 4.39. Found: C, 56.38; H, 4.18; N, 4.29.

H–Tyr(Mtz)–OH: A solution of tyrosine (2.78 g, 15.3 mmol) and $CuSO_4 \cdot 5H_2O$ (1.91 g, 7.7 mmol) in 2 N NaOH (15.6 ml, 31.2 mmol) was stirred for 0.5 h at 60 °C under Ar. After cooling of the mixture, AcOH was added to adjust the pH to 5. The precipitate was collected and washed with water and acetone. Mtz–ONp (5.9 g, 18.5 mmol) was added to a solution of the precipitate and $NaHCO_3$. (3.1 g, 36.9 mmol) in DMF–water (7 : 1, 220 ml), and the mixture was stirred for 20 h at room temperature. Concentration of the mixture afforded a solid that was washed with water and acetone, and treated with $EDTA \cdot 2Na \cdot H_2O$ (5.71 g, 15.3 mmol) in water (100 ml) at 100 °C. The mixture was left overnight at 4 °C, then the precipitate was collected and washed with water and acetone (3.76 g, 68%), mp 213—216 °C, $[\alpha]_D^{26} = -16.07°$ ($c = 0.56$, 50% AcOH). FAB-MS $[M+H]^+ = 362$. *Anal.* Calcd for $C_{18}H_{19}NO_5S \cdot 3/4H_2O$: C, 57.66; H, 5.11; N, 3.74. Found: C, 57.54; H, 5.20; N, 3.69.

Boc–Tyr(Mtz)–OH: A solution of di-*tert*-butyl dicarbonate (1.06 g, 4.9 mmol) in THF (10 ml) was added to a suspension of H–Tyr(Mtz)–OH (1.47 g, 4.1 mmol) and Et_3N (0.85 ml, 6.1 mmol) in water (10 ml) at 4 °C. The mixture was stirred for 14 h at room temperature, then concentrated and acidified with 0.5 N H_2SO_4. The product was extracted with AcOEt (50 ml) and the organic solution was washed with 0.5 N H_2SO_4 and saturated NaCl, dried over Na_2SO_4, concentrated and precipitated from hexane. The product was reprecipitated from AcOEt–hexane (1.5 g, 80%), mp 82—83 °C, $[\alpha]_D^{26} = +6.74°$ ($c = 0.45$, MeOH). FAB-MS $[M+H]^+ = 462$. *Anal.* Calcd for $C_{23}H_{27}NO_7S$: C, 59.27; H, 5.84; N, 3.01. Found: C, 59.07; H, 5.93; N, 3.05.

Boc–Tyr(Msz)–OH: $NaHCO_3$ (78 mg, 0.9 mmol) and a solution of $NaBrO_2 \cdot 3H_2O$ (0.766 g, 3.7 mmol) in water (10 ml) were added to a solution of Boc–Tyr(Mtz)–OH (1.42 g, 3.1 mmol) in AcOEt (10 ml), and the reaction mixture was stirred vigorously at room temperature for 0.5 h. $Na_2S_2O_3 \cdot 5H_2O$ (1.53 g, 6.2 mmol) was added and the whole was acidified with 0.5 N H_2SO_4. The organic layer was washed with 0.5 N H_2SO_4 and saturated NaCl, and dried over Na_2SO_4. After concentration, the product was precipitated from hexane (1.18 g, 80%), mp 61—64 °C, $[\alpha]_D^{26} = -167.8°$ ($c = 0.44$, MeOH). FAB-MS $[M+H]^+ = 478$. *Anal.* Calcd for $C_{23}H_{27}NO_8S \cdot 3/4H_2O$: C, 56.26; H, 5.54; N, 2.85. Found: C, 56.45; H, 5.79; N, 2.88.

MtBom–SMe: NaH (60% oil suspension, 1.87 g, 38.9 mmol), sodium iodide (2.9 g, 19.5 mmol) and chloromethyl methyl sulfide (1.63 ml, 19.5 mmol) were added to a solution of 4-methylthiobenzyl alcohol (3 g, 19.5 mmol) in 1,2-dimethoxyethane (30 ml) at 4 °C. The reaction was continued for 1 h at 4 °C and 3 h at room temperature. The mixture was poured into ice-water (120 ml) and then extracted with ether (50 ml). The organic layer was washed with saturated NaCl, dried over Na_2SO_4 and concentrated to give an oily product (4.17 g, 93%). *Anal.* Calcd for $C_{10}H_{14}OS_2$: C, 56.03; H, 6.58. Found: C, 55.58; H, 6.61.

Boc–His(MtBom)–OMe: A solution of SO_2Cl_2 (2.42 ml, 30.1 mmol) in DCM (30 ml) was added dropwise to a solution of MtBom–SMe (6.45 g, 30.1 mmol) in DCM (30 ml) at −78 °C. The mixture was stirred for 0.5 h at the same temperature, then Boc–His(Boc)–OMe · HCl (7.63 g, 18.8 mmol) was added at 4 °C and the whole was stirred for 14 h at room temperature, concentrated and extracted with AcOEt. The organic solution was washed with 5% $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4, and evaporated. The residue was subjected to silica gel column chromatography with $CHCl_3$–MeOH (20 : 0.5). The fraction containing the desired product was collected and evaporated. Trituration of the residue with hexane gave a solid (6.59 g, 81%), mp 108—109 °C, $[\alpha]_D^{19} = -14.06°$ ($c = 0.64$, MeOH). FAB-MS $[M+H]^+ = 436$. *Anal.* Calcd for $C_{21}H_{29}N_3O_5S \cdot 1/4H_2O$: C, 57.32; H, 6.83; N, 9.55. Found: C, 57.09; H, 6.94; N, 9.26.

Boc–His(MsBom)–OMe: *m*-Chloroperoxybenzoic acid (0.82 g, 3.79 mmol) was added to a solution of Boc–His(MtBom)–OMe (1.65 g, 3.79 mmol) in $CHCl_3$ (16 ml) at −78 °C. The mixture was stirred for 0.5 h at the same temperature and then Me_2S (0.179 ml, 3.79 mmol) was added. After removal of the solvent, the residue was extracted with AcOEt. The organic solution was washed with 5% $NaHCO_3$ and saturated NaCl and dried over Na_2SO_4. After removal of the solvent, trituration of the residue with hexane gave a solid (1.51 g, 88%), mp 68—69 °C, $[\alpha]_D^{21} = -15.84°$ ($c = 0.51$, DMF). FAB-MS $[M+H]^+ = 453$. *Anal.* Calcd for $C_{21}H_{29}N_3O_6S \cdot 1/2H_2O$: C, 54.76; H, 6.57; N, 9.12. Found: C, 54.80; H, 6.67; N, 8.83.

Boc–His(MsBom)–OH: A solution of Boc–His(MsBom)–OMe (0.5 g, 1.11 mmol) in MeOH (5 ml) was treated with 1 N NaOH (1.33 ml, 1.33 mmol) at 4 °C. The mixture was stirred for 1 h at 4 °C, then acidified with 1N HCl and the product was absorbed on Diaion HP-20. The resin was rinsed with water and eluted with MeOH (4 × 20 ml). The eluate was evaporated to afford a residue, which was dissolved in $CHCl_3$. This solution was dried over Na_2SO_4 and concentrated. Trituration of the residue with ether gave a solid (0.42 g, 87%), mp 95—100 °C $[\alpha]_D^{19} = -17.3°$ ($c = 0.52$, DMF). FAB-MS $[M+H]^+ = 438$. *Anal.* Calcd for $C_{20}H_{27}N_3O_6S \cdot H_2O$: C, 52.73; H, 6.42; N, 9.23. Found: C, 52.80; H, 6.49; N, 9.26.

Stability of Protecting Groups The N^{α}-Boc–amino acid derivatives (1 mg) bearing a safety-catch protecting group were exposed to the reagents (100 μl) listed in Table 1. Cleavage of the protecting group was detected by TLC analysis. The results are summarized in Table 1.

Comparison of the Stability of Protecting Groups to TFA–Anisole: The N^{α}-Boc–amino acid derivatives (1 mg) listed in Table 2 were treated with TFA–anisole (100 μl–2 μl) at 25 °C for 50 h. The mixtures were diluted with water and the amounts of parent amino acid were determined with the amino acid analyzer. The results are shown in Table 2.

Preparation of DSB Handle Reagent 4-(2,5-Dimethyl-4-methylthiophenyl)-4-ketobutanoic Acid: A solution of 2,5-dimethylthiophenol (1 ml, 7.38 mmol) and methyl iodide (1.01 ml, 14.8 mmol) in DMSO (5 ml) was treated with NaH (60% oil suspension, 0.65 g, 16.2 mmol). The mixture was stirred for 14 h, then poured into ice-water (50 ml), and the product was extracted with ether. The organic solution was washed with saturated NaCl, dried over Na_2SO_4 and concentrated. This product was dissolved in DCM (25 ml), then succinic anhydride (0.74 g, 7.38 mmol) and anhydrous aluminum chloride (2.26 g, 16.2 mmol) were added at 4 °C. The mixture was refluxed with stirring for 1 h, then evaporated. Ice-water (50 ml) and concentrated HCl (20 ml) were added to the residue. The product was extracted with AcOEt, and the organic solution was washed with 1 N HCl, 5% $NaHCO_3$ and saturated NaCl, then concentrated. Addition of hexane gave a solid (1.44 g, 77%), mp

115—116 °C. FAB-MS $[M+H]^+=253$. *Anal.* Calcd for $C_{13}H_{16}O_3S$: C, 61.88; H, 6.39. Found: C, 61.73; H, 6.46.

4-(2,5-Dimethyl-4-methylthiophenyl)-4-ketobutanoyl-β-alanine Benzyl Ester: DCC (1.3 g, 6.28 mmol) was added to a solution of above product (1.44 g, 5.71 mmol), β-alanine benzyl ester, *p*-toluenesulfonate (2 g, 5.71 mmol) and Et_3N (0.79 ml, 5.71 mmol) in DMF (10 ml) at 4 °C. The mixture was stirred for 14 h at room temperature, then an insoluble by-product was filtered off and the solution was concentrated. The product was extracted with AcOEt. The organic layer was washed with 1 N HCl, 5% $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4 and concentrated. Addition of hexane gave a solid (2.28 g, 966%), mp 94—95 °C. FAB-MS $[M+H]^+=414$. *Anal.* Calcd for $C_{23}H_{27}NO_4S$: C, 66.80; H, 6.58; N, 3.39. Found: C, 66.88; H, 6.68; N, 3.33.

4-(2,5-Dimethyl-4-methylthiophenyl)-4-ketobutanoyl-β-alanine: The above product (2.17 g, 5.24 mmol) was dissolved in MeOH–dioxane (10 ml–10 ml), 1 N NaOH (6.28 ml, 6.29 mmol) was added, and the mixture was stirred for 1 h at 4 °C. After removal of the solvent, the product was solidified by addition of 1 N HCl and hexane. The crude product was reprecipitated from MeOH–hexane (1.29 g, 76%), mp 134—135 °C. FAB-MS $[M+H]^+=324$. *Anal.* Calcd for $C_{16}H_{21}NO_4S\cdot 1/5H_2O$: C, 58.76; H, 6.60; N, 4.28. Found: C, 58.97; H, 6.55; N, 4.21.

4-(2,5-Dimethyl-4-methylthiophenyl)-4-hydroxybutanoyl-β-alanine: A solution of above product (1.19 g, 3.66 mmol) in MeOH (10 ml) was treated with $NaBH_4$ (0.55 ml, 14.6 mmol) at 4 °C. The mixture was refluxed for 1 h and then evaporated. To the residue, 1 N H_2SO_4 and AcOEt were added. The organic layer was washed with 1 N H_2SO_4 and saturated NaCl, dried over Na_2SO_4 and concentrated. Addition of ether–hexane (1 : 1) gave a solid (0.98 g, 82%), mp 96—97 °C. FAB-MS $[M]^+=325$. *Anal.* Calcd for $C_{16}H_{23}NO_4S\cdot 1/4H_2O$: C, 58.24; H, 7.18; N, 4.25. Found: C, 58.25; H, 7.21; N, 4.10.

4-(2,5-Dimethyl-4-methylsulfinylphenyl)-4-hydroxybutanoyl-β-alanine (HO–DSB–β-Ala–OH): A solution of the above product (0.3 g, 0.92 mmol) in AcOH (6 ml) was treated with 30% H_2O_2 (102 ml, 1.02 mmol) at room temperature. The mixture was stirred for 1 h, then Me_2S (15 μl, 204 μmol) was added and the whole was evaporated to dryness. The product was used in the next reaction without purification, HRFAB-MS *m/z*: 342.1398 for $[M+H]^+$ (Calcd 342.1375 for $C_{16}H_{24}NO_5S$).

HO–DSB–β-Ala–NH–CH_2–C_6H_4-Polystyrene Resin: An aminomethylated polystyrene resin (hydrochloride form) (1 g, 0.77 meq/g) was rinsed with 5% diisopropylethylamine–DMF (×2) and DMF (×5). A solution of HO–DSB–β-Ala–OH (prepared above) in DMF–DCM (5 ml–5 ml), HOBt·H_2O (141 mg, 0.92 mmol), BOP (0.408 g, 0.92 mmol) and diisopropylethylamine (0.161 ml, 0.92 mmol) were added to the resin. The mixture was vortexed for 2 h at 25 °C and then the resin was rinsed with DMF (×5) and DCM (×5) to afford 1.19 g of the desired product (0.556 meq/g).

Boc–Amino Acid–O–DSB–β-Ala–NH–CH_2–C_6H_4-Polystyrene Resin: DMF–DCM (1 : 1), N^{α}-Boc protected amino acid (5 eq), 4-dimethylaminopyridine (0.1 eq) and diisopropylcarbodiimide (5 eq) were added to HO–DSB–β-Ala–NH–CH_2–C_6H_4-polystyrene resin (0.3—0.6 meq/g, 1 eq). The mixture was vortexed for 1 h at 25 °C and then the resin was rinsed with DMF and DCM to afford the desired product (loading of amino acid, 0.2—0.5 meq/g).

Preparation of DSA Handle Reagent Ethyl 3-Methoxyphenylthio-6-hexanoate: Ethyl 6-bromohexanoate (1.58 ml) was added to a mixture of 3-methoxythiophenol (1 ml, 8.06 mmol) and potassium *tert*-butoxide (0.995 g) in DMF (10 ml) at 0 °C. The mixture was stirred for 1 h at room temperature, then filtered and the filtrate was concentrated. The product was extracted with AcOEt and the organic solution was washed with 5% $NaHCO_3$, 1 N HCl and saturated NaCl, dried over Na_2SO_4 and concentrated to give an oily product (2.15 g, 95%): EI-MS $[M]^+=282$. *Anal.* Calcd for $C_{15}H_{22}O_3S\cdot 1/4H_2O$: C, 62.79; H, 7.73. Found: C, 62.79; H, 7.79.

Ethyl 4-(4-Methoxybenzoyl)-3-methoxyphenylthio-6-hexanoate: The above product (2.15 g 9.61 mmol) was dissolved in DCM (20 ml) at 0 °C, and anisoyl chloride (1.69 ml) and aluminum chloride (2.33 g) were added to the solution. The mixture was stirred for 1.5 h at room temperature, then evaporated and poured into ice water. The oily product was obtained in the same manner as described above (2.54 g, 80%). EI-MS $[M]^+=416$.

4-(4-Methoxybenzoyl)-3-methoxyphenylthio-6-hexanoic Acid: A mixture of the above product (2.02 g 4.85 mmol) and 4 N NaOH (12.12 ml) in MeOH (20 ml) was stirred at 40 °C for 1 h, and then concentrated. The residue was acidified with 6 N HCl, then the product was extracted with AcOEt. The organic solution was washed with 1 N HCl and saturated NaCl, dried over Na_2SO_4 and evaporated to afford a solid (1.60 g, 85%), mp 119—122 °C. EI-MS $[M]^+=388$. *Anal.* Calcd for $C_{21}H_{24}O_5S\cdot 1/5H_2O$: C, 64.33; H, 6.17. Found: C, 64.51; H, 6.00.

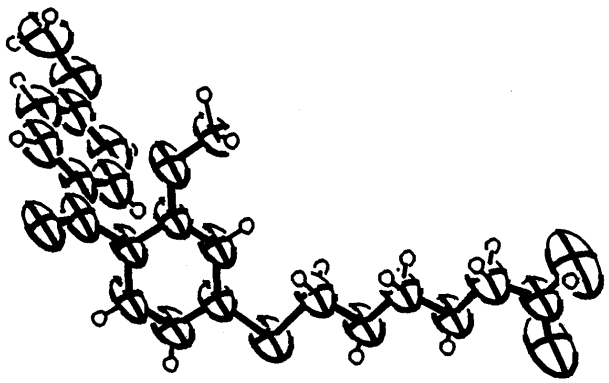

Fig. 4. X-Ray Crystal Structure of 4-(4-Methoxybenzoyl)-3-methoxyphenylthio-6-hexanoic Acid

A crystal of this compound was analyzed by the X-ray diffraction method and the configuration of the anisoyl moiety was confirmed (Fig. 4).

4-(4-Methoxyphenyl-Boc-aminomethyl)-3-methoxyphenylthio-6-hexanoic Acid: A solution of the above carboxylic acid (1.00 g 2.57 mmol) and *N*-hydroxylamine hydrochloride (5.40 g) in ethanol–pyridine (16 ml–4 ml) was stirred at 85 °C for 3 h and then evaporated. The product was extracted with AcOEt and the organic solution was washed with 5% $NaHCO_3$, 1 N HCl and saturated NaCl, dried over Na_2SO_4 and concentrated to give an oily product (0.865 g). Zinc powder (2.00 g) was added to a solution of the above product in AcOH (10 ml) and the reaction was continued for 1 h at 50 °C. The mixture was filtered, the filtrate was concentrated, and the residue was dissolved in DMF (10 ml). To this solution, TEA (0.484 ml) and di-*tert*-butyl dicarbonate (0.456 g) were added at 0 °C. The whole was stirred for 1 h at room temperature and then concentrated. The oily product was obtained in the same manner as above (0.745 g, 59%). EI-MS $[M]^+=489$.

4-(4-Methoxyphenyl-Boc-aminomethyl)-3-methoxyphenylsulfinyl-6-hexanoic Acid (Boc–DSA–OH): The above product (0.745 g 1.52 mmol) was dissolved in AcOH (10 ml) and then 30% H_2O_2 (0.259 ml) was added at room temperature. The mixture was stirred for 1 h at the same temperature, then Me_2S (0.078 ml) was added and the whole was concentrated. The residue was extracted with AcOEt. The organic solution was washed with 1 N HCl and saturated NaCl, and dried over Na_2SO_4. The crude product was purified on a silica gel column (2.5 × 20 cm) with $CHCl_3$–MeOH (20 : 0.5, v/v). Removal of the solvent gave a solid (0.629 g, 90%), mp 66—69 °C. FAB-MS $[M+Na]^+=528$. *Anal.* Calcd for $C_{26}H_{35}NO_7S\cdot 1/2H_2O$: C, 60.68; H, 6.86; N, 2.72. Found: C, 60.65; H, 6.98; N, 2.74.

Boc–Leu–DSA–Ala–NH–CH_2–C_6H_4-polystyrene Resin: Starting from an aminomethylated polystyrene resin hydrochloride (100 mg 0.83 meq/g), Boc–Ala–OH, Boc–DSA handle reagent and Boc–Leu–OH were coupled by the efficient SPPS method to afford 141 mg of resin (0.406 meq/g).

Examination of the Stability of the DSB and DSA Linkages Boc–Leu–O–DSB–β-Ala–NH–CH_2–C_6H_4-polystyrene resin (5 mg, 0.23 meq/g) or Boc–Leu–DSA–Ala–NH–CH_2–C_6H_4-polystyrene resin (5 mg, 0.41 meq/g) was exposed to a reagent (500 μl) listed in Table 3. After the treatment, the resin was washed with DCM, DMF, water, DMF, DCM and MeOH, and then dried. An aliquot of the resin was hydrolyzed using 12 N HCl–propionic acid (1 : 1, v/v) at 110 °C for 24 h. Each hydrolyzate was diluted with water and the ratio of leucine to internal standard amino acids was measured with an amino acid analyzer. The results are summarized in Table 3.

In the case of entry 6 (3 h) for Boc–Leu–O–DSB–β-Ala–NH–CH_2–C_6H_4-polystyrene resin, the filtrate was concentrated and the ratio of D-Leu in the residue was determined by the GITC method; 1.7% of D-Leu was detected, and had presumably been formed during esterification of Boc–Leu–OH by the carbodiimide–DMAP method.

SPPS of Protected Peptide Resins Using Boc-amino acids bearing reductive acidolysis-cleavable side chain-protecting groups, protected

peptide-resins were prepared by the efficient SPPS, that is 1) selective deprotection of the N^{α}-Boc group using a mild deprotection system, 0.5 M MSA/DCM–DOX (9 : 1)–2% anisole (1 and 20 min), 2) weak base-wash with 2% pyridine/DMF (2 × 0.7 min), 3) coupling using Boc–amino acid (2 eq), BOP (2 eq) and diisopropylethylamine (4 eq) in DCM–*N*-methylpyrrolidone (NMP) (1 : 2) (60 min), and 4) capping with 0.3 M decanoic anhydride in DCM–NMP (1 : 1) (30 min).

Protected γ-endorphin-resin (227 mg) was obtained starting from Boc–Leu–O–DSB–β-Ala–NH–CH_2–C_6H_4-polystyrene resin (150 mg 0.23 meq/g) and had the following amino acid composition: Thr, 2.69; Ser, 1.60; Glu, 1.78; Gly, 1.80; Val, 0.99; Met, 0.77; Leu, 2.00; Tyr, 0.83; Phe, 0.90; Lys, 0.96; Pro, 0.96 (cont. of Leu 0.131 meq/g).

Protected valosin-resin (332 mg) was obtained starting from Boc–Tyr(Msz)–O–DSB–β-Ala–NH–CH_2–C_6H_4-polystyrene resin (100 mg 0.55 meq/g) as described above, and had the following amino acid composition: Asp, 0.96; Thr, 0.83; Ser, 0.99; Glu, 1.88; Gly, 2.10; Val, 2.61; Met, 0.92; Leu, 2.00; Tyr, 1.88; Phe, 2.69; Lys, 2.70; His, 0.80; Pro, 2.75 (cont. of Leu 0.14 meq/g).

Protected buccalin-resin (414 mg) was obtained starting from Boc–Leu–DSA–β-Ala–NH–CH_2–C_6H_4-polystyrene resin (285 mg 0.48 meq/g), and had the following amino acid composition: Asp, 1.10; Ser, 1.15; Gly, 3.10; Ala, 1.00; Met, 0.62; Leu, 2.14; Phe, 1.00 (cont. of Leu 0.26 meq/g).

Protected PHM-27-resin (335 mg) was prepared starting from Boc–Met(O)–DSA–Ala–NH–CH_2–C_6H_4-polystyrene resin (147 mg, 0.45 meq/g), and had the following amino acid composition: Asp, 2.34; Thr, 0.83; Ser, 3.49; Glu, 2.04; Gly, 2.30; Ala, 5.11; Val, 1.00; Met, 0.81; Leu, 5.45; Tyr, 1.19; Phe, 2.08; Lys, 3.34; His, 1.00 (cont. of His 0.15 meq/g).

Deprotection and Purification of Synthetic Peptides Synthesis of γ-Endorphin: The protected γ-endorphin-resin (50 mg, 6.5 μmol) was suspended in TFA–DCM (1.5–0.15 ml) in the presence of anisole (34 μl), thioanisole (77 μl) and ethane-1,2-dithiol (55 μl). Tetrachlorosilane (153 μl) was added and the mixture was stirred for 3 h at 25 °C, then concentrated at room temperature. The product was precipitated with dry ether and extracted with 1 N AcOH. An aliquot of the resulting resin was used for analysis of the cleavage ratio in the same manner as used to examine the stability of the linkages. The cleavage yield was 82%. Purification of the crude sample on a YMCgel ODS-AQ120A S-50 column (1.6 × 50 cm) with acetonitrile (0—60%) in 0.1% TFA gave a homogeneous product (7.1 mg, 62% based on the starting Boc–Leu–O–DSB–β-Ala–NH–CH_2–C_6H_4-polystyrene resin), which was identical with an authentic sample on HPLC and included all of the amino acids in the expected ratios: Thr, 3.03; Ser, 1.94; Glu, 2.21; Gly, 2.00; Val, 1.07; Met, 0.91; Leu, 2.25; Tyr, 0.93; Phe, 0.98; Lys, 1.03; Pro, 1.10 (recovery of Leu 89%). HPLC was run on a YMC AM302 column (4.6 × 150 mm) [t_R: 16.69 min, on gradient elution with CH_3CN (10—60%, 30 min) in 0.1% TFA, 0.7 ml/min, O.D. 280nm].

Synthesis of Valosin: The protected valosin-resin (100 mg, 14 μmol) was treated essentially as above. Purification afforded a homogeneous product (10.4 mg, 25% based on the starting Boc–Tyr(Msz)–O–DSB–β-Ala–NH–CH_2–C_6H_4-polystyrene resin), which was identical with an authentic sample on HPLC and included all of the amino acids in the expected ratios: Asp, 1.02; Thr, 0.88; Ser, 0.94; Glu, 1.95; Gly, 2.06; Val, 2.87; Met, 0.95; Leu, 2.00; Tyr, 1.96; Phe, 2.87; Lys, 2.84; His, 0.99; Pro, 3.10 (recovery of Leu 79%). HPLC was run on a YMC AM302 column (4.6 × 150 mm) [t_R: 18.80 min, on gradient elution with CH_3CN (20—70%, 30 min) in 0.1% TFA, 0.7 ml/min, O.D. 230 nm].

Synthesis of Buccalin: The protected buccalin-resin (50 mg, 13 μmol) was treated with *m*-cresol (35 μl), thioanisole (77 μl), ethane-1,2-dithiol (55 ml) and tetrachlorosilane (153 ml) in TFA–DCM (1.5–0.15 ml) for 3 h at 25 °C. The reaction mixture was worked up as described above. Purification of the crude product gave a homogeneous product (7.0 mg, 40% based on the starting Boc–Leu–DSA–β-Ala–resin), which was identical with an authentic sample on HPLC and included all of the amino acids in the expected ratios: Asp, 0.97; Ser, 1.80; Gly, 2.94; Ala, 1.00; Met, 0.93; Leu, 2.01; Phe, 1.00 (recovery of Ala 87%). HPLC was run on a YMC AM302 column (4.6 × 150 mm) [t_R: 22.80 min, on gradient elution with CH_3CN (10—60%, 30 min) in 0.1% TFA, 0.7 ml/min, O.D. 230 nm].

Synthesis of PHM-27 was achieved using the protected PHM-27-resin (100 mg 15 μmol) in the same manner as described for buccalin. The synthetic PHM-27 was obtained (7.4 mg, 13% yield calculated from Boc–Met(O)–DSA–Ala–resin), identical with an authentic sample. The amino acid composition was: Asp, 2.07; Thr, 0.95; Ser, 4.07; Glu, 1.96; Gly, 2.23; Ala, 2.00; Val, 0.95; Met, 1.05; Leu, 4.97; Tyr, 1.02; Phe, 1.82; Lys, 3.00; His 1.00 (recovery of His 72 %). HPLC was run on a YMC AM302 column (4.6 × 150 mm) [t_R: 21.78 min, on gradient elution with CH_3CN (20—70%, 30 min) in 0.1% TFA, 0.7 ml/min, O.D. 230 nm].

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