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Polypeptide Synthesis Using the *S*-Alkyl Thioester of a Partially Protected Peptide Segment. Synthesis of the DNA-Binding Domain of *c*-Myb Protein (142—193)-NH_2

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A method for polypeptide synthesis using *S*-alkyl thioester of a partially protected peptide segment has been developed. The DNA-binding domain of *c*-Myb protein (142—193) was synthesized by this method to estimate its usefulness. The partially protected peptide segments, Boc-[Lys(Boc)^{143,144,160}]-*c*-Myb protein(142—163)-$SCH_2CH_2CONH_2$ and [Lys(Boc)^{171,182,192}]-*c*-Myb protein(164—193)-NH_2, were highly purified from peptides obtained by a solid-phase method. The thioester in the carboxyl component of the peptide segment was converted to the corresponding *p*-nitrophenyl ester in the presence of silver ions and *p*-nitrophenol and then the two segments were condensed. The coupling reaction proceeded almost completely yielding a highly-purified product after removal of Boc groups.

In our preceding paper,[1] we reported the synthesis of bovine pancreatic trypsin inhibitor (BPTI) consisting of 58 amino acid residues, using partially protected peptide segments prepared from peptides obtained by a solid-phase method. This method was proven to be very effective in reducing the time required for chemical synthesis of polypeptide and to give a highly pure product.

However in this method, it was necessary to protect the side-chain carboxyl groups in peptide segments for selective activation of the terminal carboxyl group. Moreover peptide segments containing side-chain carboxyl groups could be prepared only by the solid-phase method using N^{α}-3-nitro-2-pyridinesulfenyl-amino acids (Npys-amino acids) developed by Matsueda et al.[2]

There arose two problems. The first was that of peptide segment solubility during purification by reversed-phase high-performance liquid chromatography (RPHPLC). Generally, an increase in the number of hydrophobic protecting groups decreases the solubility of a paptide segment in a solvent such as aq acetonitrile. Consequently, the purification of such a peptide by RPHPLC becomes difficult and the recovery yield is usually low. If the number of the protecting groups on a peptide segment can be reduced, the solubility will increase and its purification will be facilitated. The second issue was the complexity of the method for segment preparation. We had to change the procedure for peptide segment preparation depending on the presence or absence of a side-chain carboxyl group. In polypeptide synthesis, it is very important to normalize the procedure for the preparation of a peptide segment.

To overcome these problems, we designed a simpler method based upon our previous protocol and the thiocarboxyl segment strategy, by which Blake et al. achieved perfectly-selective activation of the terminal carboxyl group,[3] and synthesized several proteins.[4,5]

In the new method, the *S*-alkyl thioester of a partially protected peptide segment is used as a building block for polypeptide synthesis. The thioester group should be activated by silver ions. If so, the terminal thioester group will be selectively activated. Furthermore the thioester group does not have such a nucleophilic character as the thiocarboxyl group. Hence, various kinds of protecting groups can be introduced into the side-chain amino groups using a protective reagent such as *N*-(*t*-butoxycarbonyloxy)-succinimide (Boc-ONSu). If an *S*-alkyl thioester of a peptide segment could be synthesized by a solid-phase method with a high yield, a very promising method for polypeptide synthesis would be developed.

To examine the efficiency of this strategy, we attempted to synthesize the DNA-binding domain of *c*-Myb protein consisting of 52 amino acid residues, which is similar in size to BPTI.

In this paper, we describe the preparation of Boc-Gly-SCH_2CH_2CONH-resin, the synthesis of the *S*-alkyl thioester of a partially protected peptide segment, its chemical characteristics and the coupling of the segments and discuss the efficiency of this method.

Results and Discussion

The amino acid sequence of an oncogene product of *c*-Myb protein was deduced from cDNA clones of murine *c-myb* mRNA.[6] This protein binds to DNA at a domain near the N-terminal region.[7] The binding domain was found to have a 3-time tandem repeat of an analogous primary structure.[6] The peptide we synthesized was the third part of the repeat corresponding to the amino acids from 142 to 193. The amino acid sequence is shown in Fig. 1. Two partially protected peptide segments **1** and **2** were prepared and segment coupling of the peptides was carried out at the site indicated by an arrow (Fig. 1) according to the scheme shown in Fig. 2.

Preparation of Boc-Gly-SCH_2CH_2CONH-Resin. Crystalline Boc-Gly-SCH_2CH_2COOH was prepared from Boc-Gly-ONp and $HSCH_2CH_2COOH$. This

142
Val-Lys-Lys-Thr-Ser-Trp-Thr-Glu-Glu-Glu-Asp-Arg-Ile-
↓
Ile-Tyr-Gln-Ala-His-Lys-Arg-Leu-Gly-Asn-Arg-Trp-Ala-
Glu-Ile-Ala-Lys-Leu-Leu-Pro-Gly-Arg-Thr-Asp-Asn-Ala-
193
Ile-Lys-Asn-His-Trp-Asn-Ser-Thr-Met-Arg-Arg-Lys-Val

Fig. 1. Primary sequence of *c*-Myb protein(142—193) deduced from cDNA clone of murine *c-myb* mRNA.[6] Arrow indicates the site of segment coupling.

Boc-[Lys(Boc)^{143,144,160}]-c-Myb protein(142-163)-$SCH_2CH_2CONH_2$ (1)

↓ $AgNO_3$ + HONp + 4-methylmorpholine

Boc-[Lys(Boc)^{143,144,160}]-c-Myb protein(142-163)-ONp

↓ [Lys(Boc)^{171,182,192}]-c-Myb protein(164-193)-NH_2 (2)
+ 4-methylmorpholine

Boc-[Lys(Boc)^{143,144,160,171,182,192}]-c-Myb protein(142-193)-NH_2 (3)

↓ TFA cont. $HS(CH_2)_4SH$ (5% v/v)

c-Myb protein(142-193)-NH_2 (4)

Fig. 2. Synthetic route leading to *c*-Myb protein(142—193)-NH_2 (**4**) by segment coupling.

compound was introduced to a *p*-methylbenzhydrylamine resin (MBHA resin) (NH_2-Resin) by a routine procedure similar to the introduction of Boc-amino acids to an MBHA resin. The resulting thioester resin was stable enough to withstand assemblage of the peptide chain corresponding to *c*-Myb protein(142—163).

Preparation of Boc-[Lys(Boc)^{143,144,160}]-*c*-Myb Protein (142—163)-$SCH_2CH_2CONH_2$ (1). Peptide **1** was prepared according to the route shown in Fig. 3. Starting from Boc-Gly-SCH_2CH_2CONH-resin (Gly 0.33 mmol g^{-1}, 1.5 g), a protected *c*-Myb protein (142—163)-SCH_2CH_2CONH-resin was prepared according to the protocol of the system software version 1.40 NMP/HOBt *t*-Boc without any modifications. End capping by acetic anhydride was carried out after each amino-acid introduction reaction. The resin weight increased by 1.4 g. This resin (500 mg from 2.9 g) was treated by anhydrous HF in the presence of *p*-cresol and 1,4-butanedithiol at 0 °C for 90 min. Judging from the elution profile of the crude product by RPHPLC, the thioester was stable under HF-treatment. The crude peptide was purified by RPHPLC to yield 40 mg (14.4 μmol) of highly pure peptide **5**. The yield was 16.7% based on the glycine in the starting resin. The yield of peptide **5** was about one-half that of peptide amide **6** (29.6%) synthesized on the MBHA resin as described later. This could be mainly due to partial aminolysis or hydrolysis of the thioester bond during the chain elongation reaction. The yield of peptide **5** was acceptable at the present stage, but further investigation of the chemical design as well as the preparation method of the thioester anchoring group is still required to increase the peptide thioester yield. But the thioester in peptide **5** was quite stable during purification on RPHPLC or during prolonged storage at 4 °C. The introduction reaction of Boc groups to peptide **5** proceeded almost quantitatively and did not accompany any side-reaction when

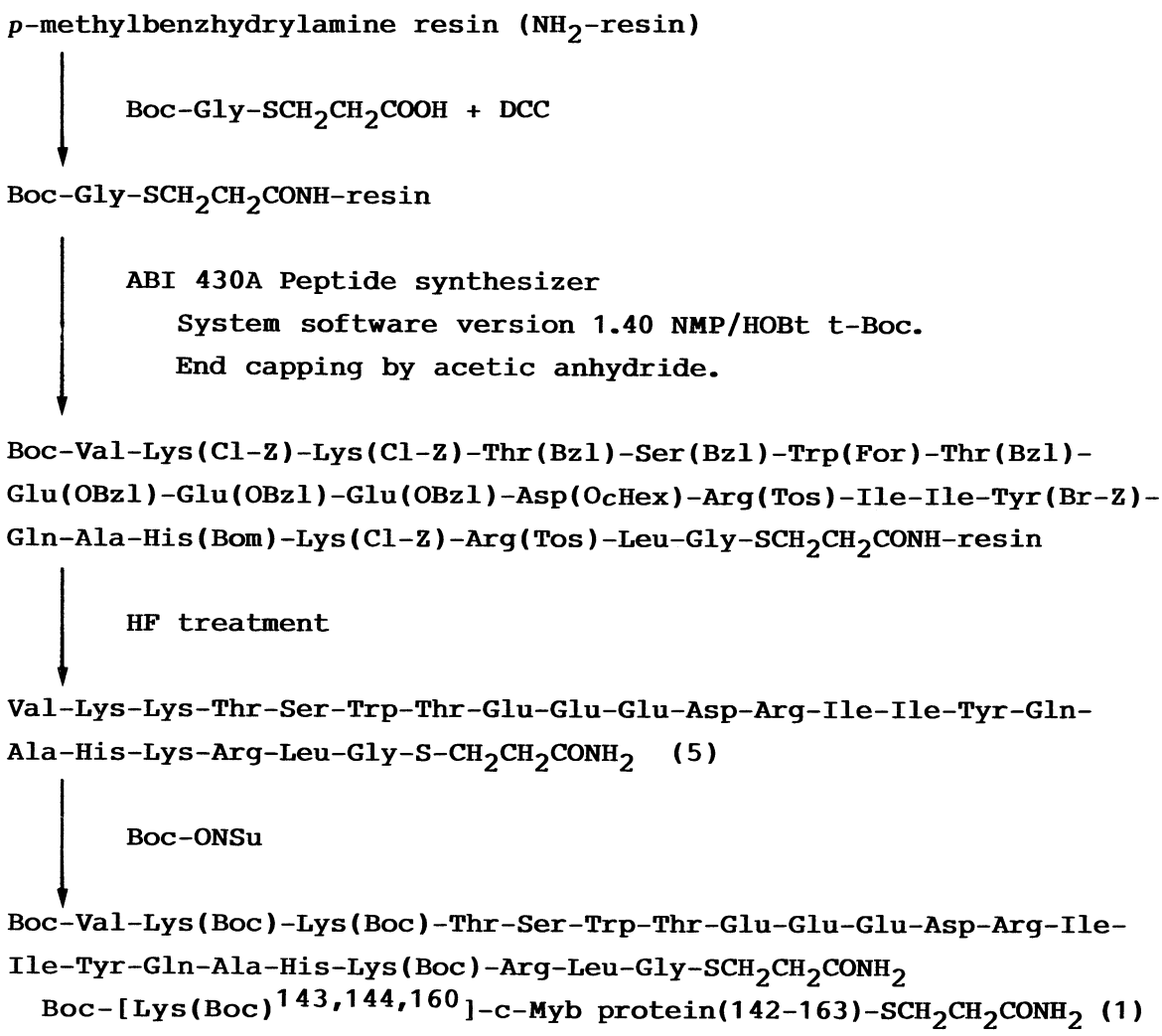

Fig. 3. Synthetic scheme of Boc-[Lys(Boc)^{143,144,160}]-*c*-Myb protein(142—163)-$SCH_2CH_2CONH_2$.

Boc-ONSu was used as the protective reagent. The yield of peptide **1** was practically quantitative.

Preparation of [Lys(Boc)^{171,182,192}]-*c*-Myb Protein(164—193)-NH_2 (2). The preparation of peptide amide **2** was carried out according to the scheme shown in Fig. 4. This procedure functions well in the synthesis of BPTI.[1] A peptide chain of *c*-Myb protein (164—193) was assembled on 0.78 g of MBHA resin (NH_2 0.64 mmol g^{-1}) according to the same protocol described for the synthesis of peptide **1**. After the completion of chain assembly, a 2,2,2-trichloroethoxycarbonyl (Troc) group was introduced to protect the terminal amino group using *N*-(2,2,2-trichloroethoxycarbonyloxy)-succinimide (Troc-ONSu). The final weight of the protected peptide resin was 3.2 g. An aliquot of the resin (0.8 g) was treated with HF in the presence of *p*-cresol and 1,4-butanedithiol at 0 °C for 90 min to yield a crude product (580 mg), which was purified on RPHPLC to give 140 mg of highly purified peptide amide **6**. The yield was 29.6% based on the amino group in the starting MBHA resin. Boc groups were introduced to peptide amide **6** according to the conditions described for the preparation of peptide **1**. The reaction proceeded completely to result in peptide amide **7** at a yield of 82%. The Troc group of peptide amide **7** was removed by zinc dust treatment in 50% aq acetic acid under nitrogen with mild sonication at room temperature for 1 h. Peptide amide **2** was obtained at a yield of 65% without any serious side reactions. But it must be noted that the almost total decomposition of peptide amide **7** was occurred when the peptide solution was sonicated for 4 h under insufficient replacement by nitrogen gas.

Preparation of *c*-Myb Protein(142—193)-NH_2 (4). As shown in Fig. 2 the thioester group in peptide **1** was converted to the corresponding active ester in the presence of *p*-nitrophenol (HONp) and $AgNO_3$ in dimethyl sulfoxide (DMSO). To the DMSO solution, peptide amide **2** and 4-methylmorpholine (NMM) were added and the solution was stirred. The

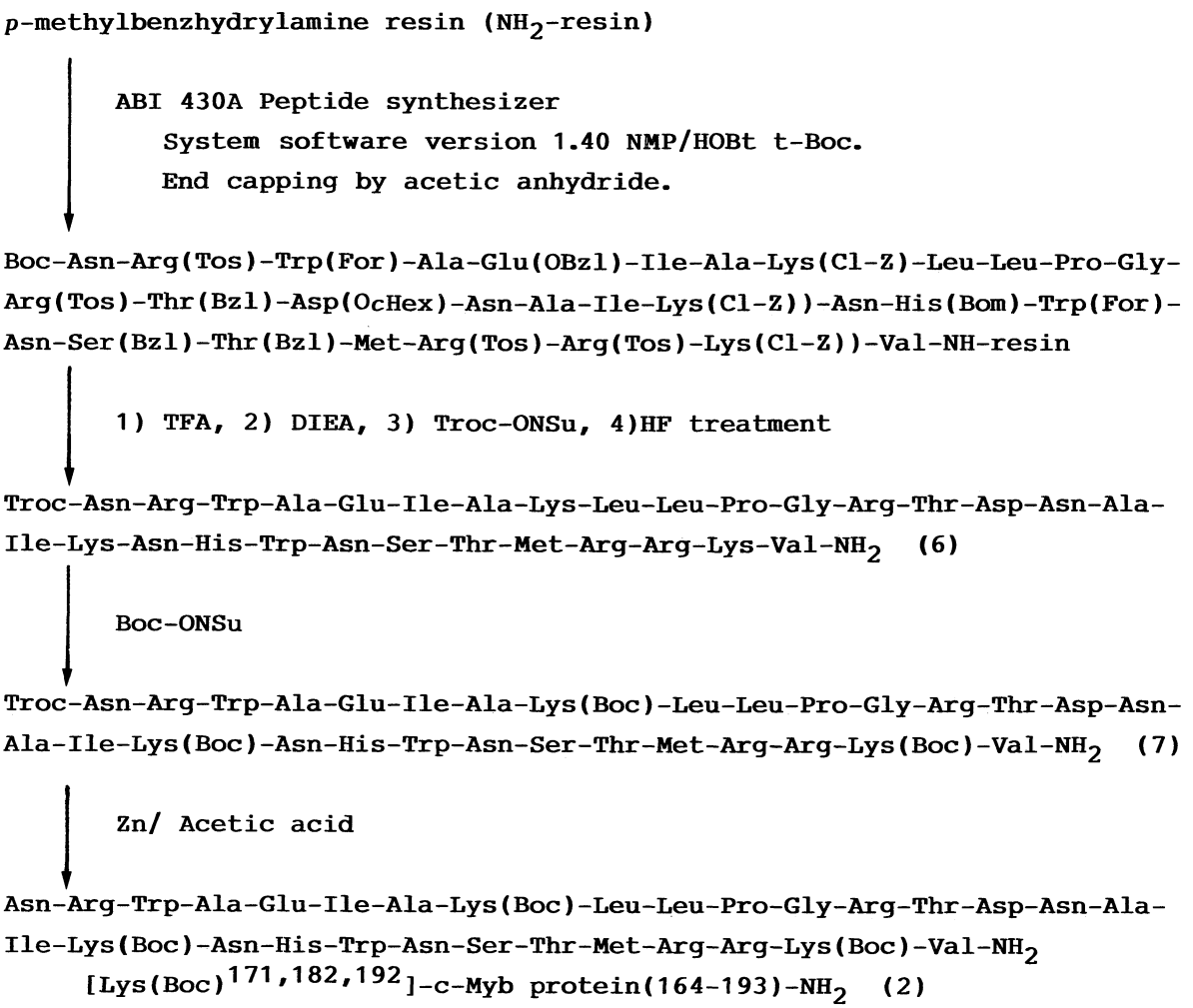

Fig. 4. Synthetic scheme of [Lys(Boc)^{171,182,192}]-*c*-Myb protein(164—193)-NH_2.

concentrations of peptides **1** and **2** were 16 mM# and 10 mM, respectively. The coupling reaction was monitored by RPHPLC. After 3 d, condensation reaction was almost complete (Fig. 5). Silver ions were removed as AgCl. The product was precipitated by adding ethyl acetate, collected by centrifugation, suspended in dioxane and freeze-dried. The powder was treated with TFA containing 1,4-butanedithiol at room temperature for 10 min. After removal of TFA, a product was isolated by RPHPLC (Fig. 6). The yield was 1.1 μmol, 50%. This yield is quite high, if the nonspecific adsorption of peptide amide **4** during the isolation process is taken into account. *N*-Hydroxysuccinimide ester corresponding to peptide **1** gave results as good as those of *p*-nitrophenyl ester with regard to the segment condensation reaction between peptides **1** and **2**. However in this experiment, we employed *p*-nitrophenyl ester, which was mild in reactivity but considerably more stable in the presence of moisture. Methionine was not damaged by silver ions. No serious side reaction was observed during segment coupling under this minimum protection strategy.

Confirmation of Synthetic *c*-Myb Protein(142—193)-NH_2 (4). The amino acid composition of the purified peptide amide **4** obtained from RPHPLC is shown in Table 1. Peptide amide **4** was eluted as a symmetrical peak by ion-exchange chromatography (Fig. 7). These data suggest that highly pure peptide amide **4** was obtained from this synthesis. Two-dimensional NMR spectroscopy of peptide amide **4** also confirmed the purity of this peptide.[8]

Evaluation of the Newly-Developed Method. Partially protected peptide segments **1** and **2** were successfully prepared and all the peptide segments were well characterized by amino acid analysis and fast atom bombardment mass spectrometry. The thioester group of peptide **1** was activated by silver ions and converted to the corresponding active ester. The terminal carbonyl group was selectively activated by a thioester strategy exactly the same as the thiocarboxyl

1 M=1 mol dm^{-3}

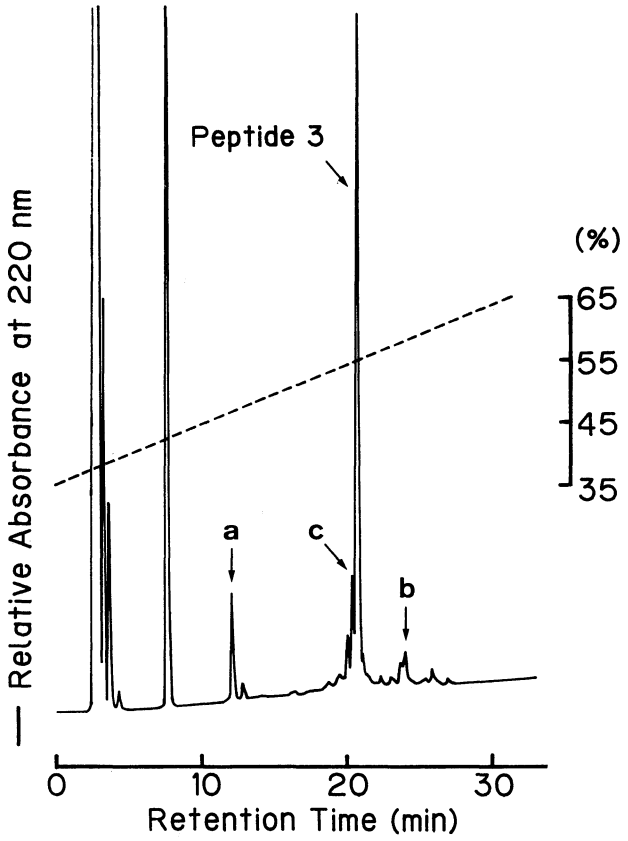

Fig. 5. HPLC profile of the reaction mixture of peptide amide **3** after 3-day reaction. Chromatography was carried out on Cosmosil 5C18 at a flow rate of 1 ml min^{-1} at 40°C. Broken line indicates the acetonitrile concentration in 0.1% aq TFA solution. Arrow **a** indicates the elution position of peptide amide **2**. Arrow **b** indicates the elution position of the active ester of peptide **1**. Arrow **c** indicates the elution position of the hydrolyzed product of peptide **1**.

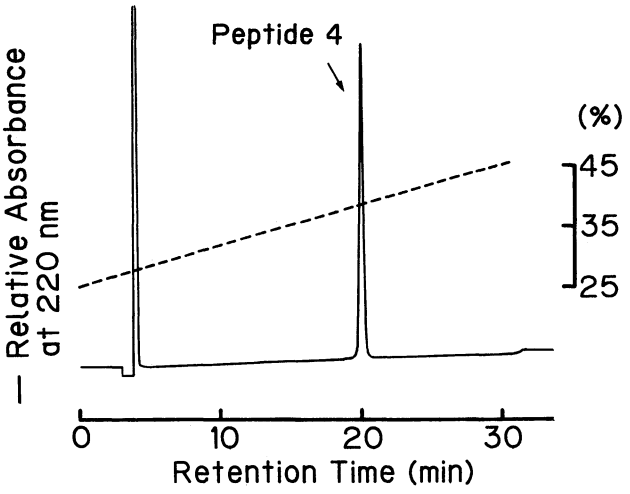

Fig. 6. HPLC profile of synthetic *c*-Myb protein-(142—193)-NH_2 (**4**) after preparative RPHPLC purification. Chromatography was carried out on Cosmosil 5C18 at a flow rate of 1 ml min^{-1} at 40°C. Broken line indicates the acetonitrile concentration in 0.1% aq TFA solution.

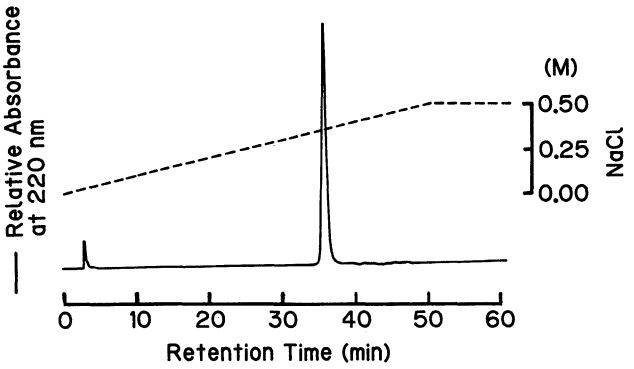

Fig. 7. Ion-exchange chromatogram of synthetic *c*-Myb protein(142—193)-NH_2 on TSK gel CM-5PW. Broken line indicates the concentration of NaCl in buffer.

Table 1. Amino Acid Composition of c-Myb Protein(142—193)-NH_2

	Peptide **4**	Expected
Asp	6.08	6
Thr	3.93	4
Ser	1.78	2
Glu	5.13	5
Pro	0.93	1
Gly	2.15	2
Ala	4	4
Val	2.05	2
Met	1.09	1
Ile	3.19	4
Leu	3.03	3
Tyr	1.01	1
His	2.16	2
Lys	6.11	6
Trp	2.34	3
Arg	6.07	6

strategy. As a result, protection of the side-chain carboxyl groups was unnecessary. Only the functional groups that required protection were amino groups in this synthesis. The thioester group did not react with Boc-ONSu though the thiol of the thiocarboxyl group was modified. If a peptide thioester is employed, various kinds of protecting groups may be introduced to the side chain, as well as α-amino groups after purification of a peptide segment on RPHPLC. Protecting groups such as the Boc group enhance the solubility of a partially protected peptide segment in DMSO or *N,N*-dimethylformamide (DMF) more than the citraconoyl group. In the thiocarboxyl segment strategy, side-chain amino groups were protected by citraconoyl groups.[4,5]

Only one protecting group existed on peptide **5** or **6** when purified on RPHPLC. Consequently, purification was much simpler compared with BPTI synthesis.[1] Therefore long peptide segments were easily prepared. The yield of segment coupling was also satisfactory.

In conclusion, the partially protected peptide

thioester is a promising building block for polypeptide synthesis.

Materials and Methods

MBHA resin hydrochloride and Boc-amino acid derivatives were purchased from the Peptide Institute Inc. (Minoh, Osaka). Solvents and reagents used for solid-phase peptide synthesis were purchased from Applied Biosystems Japan (Tokyo). Dimethyl sulfoxide used for segment coupling was silylation grade (Pierce, Rockford, IL). Analytical RPHPLC was performed on Cosmosil 5C18 (4.6×250 mm) (Nacalai Tesque, Kyoto) and preparative RPHPLC was on YMC-Gel ODS S-5 AM-type (10×250 mm or 20×250 mm) (YMC, Kyoto). Amino acids were analyzed on an L-8500 amino acid analyzer (Hitachi Ltd., Tokyo) after hydrolysis by 4 M methanesulfonic acid at 110 °C for 24 h in an evacuated sealed tube. Peptide mass number was determined by fast atom bombardment mass spectrometry using a JMS-HX100 (Jeol Ltd., Tokyo) equipped with a JMA-3100 mass data system. Peptide weight was calculated based upon the amino acid analysis data. Ultrasonication was carried out using a Branson Model B-220.

Synthesis

Peptide Chain Elongation on a Solid-Support. Solid-phase synthesis of a peptide segment was carried out on a peptide synthesizer 430A (Applied Biosystems Inc., Foster City, CA.) employing the 0.5 mmol scale standard protocol of the benzotriazole active ester method of the system software version 1.40 NMP/HOBt *t*-Boc. End capping by acetic anhydride was performed after each amino-acid introduction reaction. The side-chain protecting groups of Boc-amino acids used were *o*-chlorobenzyloxycarbonyl (Cl-Z) for the N^{ε} of Lys, *o*-bromobenzyloxycarbonyl (Br-Z) for the phenolic OH of Tyr, benzyl (Bzl) for the alcoholic OH of Thr and Ser, cyclohexyl ester (O*c*Hex) for the β-carboxyl group of Asp, benzyl ester (OBzl) for the γ-carboxyl group of Glu, benzyloxymethyl (Bom) for the N^{π} of imidazole of His, tosyl (Tos) for the N^{g} of Arg and formyl (For) for the N^{i} of Trp.

Preparation of Boc-Gly-SCH_2CH_2COOH. To a solution of Boc-Gly-ONp (1.5 g, 5 mmol) dissolved in DMF (50 ml), 3-mercaptopropionic acid (0.5 g, 5 mmol) and *N,N*-diisopropylethylamine (DIEA) (1.0 g, 7.5 mmol) were added with stirring at room temperature for 15 h. After evaporation of the solvent under reduced pressure, the product was dissolved in ethyl acetate. The ethyl acetate layer was washed with 0.1 M HCl (2×) and water saturated with NaCl (5×) and dried over sodium sulfate. The ethyl acetate solution was concentrated. An oil obtained was dissolved in ether. To the ethereal solution cyclohexylamine (CHA) (450 mg, 4.5 mmol) was added to give crystals, which were recrystallized from hot ethyl acetate: Boc-Gly-$SCH_2CH_2COOH \cdot$CHA; 1.70 g, 94.0%, mp 112.5—123.2 °C. This CHA salt (1.66 g) was suspended in ethyl acetate and CHA was extracted by dilute aq citric acid. The organic layer was washed with water saturated with NaCl (3×) and dried over sodium sulfate. After concentration of the solution, hexane was added to give Boc-Gly-SCH_2CH_2COOH (1.11 g, 91.6%), mp 104—106 °C. Found: H, 6.60; C, 45.49; N, 5.40%. Calcd for $C_{10}H_{17}O_5NS$: H, 6.51; C, 45.61; N, 5.32%.

Preparation of Boc-Gly-SCH_2CH_2CONH-Resin. MBHA resin hydrochloride (NH_2 0.47 mequiv g^{-1}, 3.4 g) was washed with 5% DIEA/dichloromethane (DCM) (2×5 min) and DCM (3×1 min), successively. Boc-Gly-SCH_2CH_2COOH (718 mg, 2.74 mmol) dissolved in 20 ml of DCM and a 0.5 M solution of dicyclohexylcarbodiimide (DCC) in DCM (4 ml) were successively added to the reaction vessel, which was shaken overnight. The resin obtained was treated with 5% acetic anhydride in DCM (2×5 min) and dried after washing the resin with 2-propanol (3×2 min) and DCM (3×1 min) to give 3.77 g of a resin (Gly: 0.33 mmol g^{-1}).

***c*-Myb Protein (142—163)-$SCH_2CH_2CONH_2$ (5).** Starting from Boc-Gly-SCH_2CH_2CONH-resin (1.5 g), peptide chain elongation was carried out by a 430A peptide synthesizer to give 2.9 g of a protected peptide resin. An aliquot of the resin (500 mg) was treated with anhydrous HF (11 ml) containing *p*-cresol (0.5 ml) and 1,4-butanedithiol (1.7 ml) at 0 °C for 90 min. After evaporation of HF, the residual solid was washed with ether (3×). Crude peptide was extracted with 10% aq acetic acid and freeze-dried to give a powder (192 mg). This crude product was purified on RPHPLC to give peptide **5** (40 mg, 14.4 μmol, 16.7%). Found *m/z* 2774.9 $(M+H)^+$. Calcd *m/z* 2774.6 $(M+H)^+$. Amino acid analysis of peptide **5**: $Asp_{1.04}Thr_{1.94}Ser_{0.92}Glu_{4.27}Gly_{1.04}Ala_1Val_{1.02}Ile_{1.23}Leu_{0.97}Tyr_{0.92}His_{1.08}Lys_{2.85}Trp_{0.65}Arg_{1.89}$.

Boc-[Lys(Boc)^{143,144,160}]-*c*-Myb Protein(142—163)-$SCH_2CH_2CONH_2$ (1). Peptide **5** (25 mg, 9.0 μmol) was dissolved in DMSO (0.4 ml) containing triethylamine (TEA) (9.6 μl). Boc-ONSu (15 mg, 72 μ mol) was added to the solution and stirred at room temperature for 2.5 h, after which ethyl acetate and ether were added. The precipitate was washed with ethyl acetate and lyophilized from a dioxane suspension to give peptide **1** (25.8 mg, 90%). Found *m/z* 3174.8 $(M+H)^+$. Calcd *m/z* 3174.8 $(M+H)^+$. Amino acid analysis of peptide **1**: $Asp_{1.00}Thr_{1.98}Ser_{0.90}Glu_{4.39}Gly_{1.07}Ala_1Val_{0.97}Ile_{1.25}Leu_{0.97}Tyr_{0.93}His_{1.08}Lys_{2.82}Trp_{0.63}Arg_{1.84}$.

Troc-*c*-Myb Protein(164—193)-NH_2 (6). Starting from MBHA resin hydrochloride (NH_2 0.64 mequiv g^{-1}, 0.78 g), chain elongation reaction was carried out. After completion of a peptide chain elongation cycle, the peptide resin was treated with 55% trifluoroacetic acid (TFA) in DCM for 5 and then 15 min, followed by neutralization with 5% DIEA in DCM for 5 min twice. Troc-ONSu (440 mg, 1.5 mmol) was allowed to react with the terminal amino group at room temperature for 15 h. The peptide resin thus obtained was 3.2 g. An aliquot of the resin (0.8 g) was treated with anhydrous HF (17.2 ml) containing *p*-cresol (0.8 ml) and 1,4-butanedithiol (2.7 ml) at 0 °C for 90 min. After evaporation of HF, the residual solid was washed with ethyl acetate (3×) and with ether (3×). The peptide amide was extracted with 5% aq acetic acid and lyophilized to give a crude product (580 mg). This peptide was purified on RPHPLC to obtain peptide amide **6** (140 mg, 37 μmol, yield 29.6%). Found *m/z* 3749.3 $(M+H)^+$. Calcd *m/z* 3749.1 $(M+H)^+$. Amino acid analysis of peptide amide **6**: $Asp_{5.10}Thr_{1.93}Ser_{0.79}Glu_{1.29}Pro_{0.75}Gly_{1.12}Ala_3Val_{1.10}Met_{1.01}Ile_{1.94}Leu_{1.94}His_{1.07}Lys_{3.06}Trp_{1.75}Arg_{3.78}$.

Troc-[Lys(Boc)^{171,182,192}]-*c*-Myb Protein(164—193)-NH_2 (7). Peptide amide **6** (24 mg, 6.5 μmol) was dissolved in DMSO (200 μl) containing TEA (7.8 μl) and Boc-ONSu (13 mg, 60 μmol) and stirred for 2.5 h. Peptide amide **7** was obtained according to the procedure of the preparation of peptide **1**: Yield 21 mg, 5.2 μmol, 80%. Found *m/z* 4050.0

$(M+H)^+$. Calcd m/z 4049.3 $(M+H)^+$. Amino acid analysis of peptide amide **7**: $Asp_{5.16}Thr_{1.93}Ser_{0.87}Glu_{1.15}Pro_{0.91}Gly_{1.09}Ala_3Val_{1.03}Met_{1.12}Ile_{2.01}Leu_{1.97}His_{1.07}Lys_{2.85}Trp_{1.30}Arg_{3.81}$.

[Lys(Boc)^{171,182,192}]-*c*-Myb Protein(164—193)-NH_2 (2). Peptide amide **7** (40 mg) was dissolved in a mixture of acetic acid (1 ml) and water (1 ml). Zinc (100 mg) was added under a nitrogen atmosphere and the solution was sonicated for 1 h at room temperature. After centrifugation of the reaction mixture, the supernatant was applied to an ODS column. The isolated product was freeze-dried to give peptide amide **2**: Yield 25 mg, 6.4 μmol, 65%. Found m/z 3875.5 $(M+H)^+$. Calcd m/z 3875.3 $(M+H)^+$. Amino acid analysis of peptide amide **2**: $Asp_{5.23}Thr_{1.95}Ser_{0.90}Glu_{1.37}Pro_{0.78}Gly_{1.25}Ala_3Val_{1.06}Met_{1.15}Ile_{2.05}Leu_{1.99}His_{1.12}Lys_{3.00}Trp_{1.39}Arg_{3.82}$.

***c*-Myb Protein(142—193)-NH_2 (4).** Peptide **1** (11.5 mg, 3.6 μmol) was dissolved in DMSO (220 μl) containing HONp (6.5 mg, 47 μmol) and NMM (0.3 μl, 2.7 μmol). $AgNO_3$ (2.4 mg, 14 μmol) was added and the solution was stirred at room temperature in the dark. After 2 h, peptide amide **2** (8.6 mg, 2.2 μmol) and NMM (0.6 μl, 5.5 μmol) were added. After stirring overnight, NMM (0.3 μl, 2.7 μmol) was added and the solution was stirred for 3 d. To the solution, solid NaCl (1.2 mg) was added. The precipitate formed was removed by centrifugation. Ethyl acetate was added to the supernatant. The precipitate formed was collected by centrifugation and washed with ethyl acetate. The peptide obtained was suspended in dioxane and freeze-dried to give a crude product of peptide amide **3**, which was treated with TFA (200 μl) containing 1,4-butanedithiol (10 μl) at room temperature for 10 min. TFA was removed by N_2 air flush. The residual solid was washed with dry ether (2×) and isolated by RPHPLC to give peptide amide **4** (6.9 mg, 1.1 μmol); yield: 50% based on peptide amide **2** used for coupling reaction. Amino acid analysis of peptide amide **4**: $Asp_{6.08}Thr_{3.93}Ser_{1.78}Glu_{5.13}Pro_{0.93}Gly_{2.15}Ala_4Val_{2.05}Met_{1.09}Ile_{3.19}Leu_{3.03}Tyr_{1.01}His_{2.16}Lys_{6.11}Trp_{2.34}Arg_{6.07}$.

Purity Check by Ion-Exchange Chromatography. Peptide amide **4** purified on RPHPLC was subjected to a TSK gel CM-5PW column (TOSO, Tokyo) (7.5 mm×75 mm) equilibriated with 0.05 M sodium phosphate buffer (pH 8.0) and chromatographed by a linear 0—0.5 M NaCl gradient in buffer over 50 min at a flow rate of 1 ml min^{-1}.

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References

1) S. Aimoto, N. Mizoguchi, H. Hojo, and S. Yoshimura, *Bull. Chem. Soc. Jpn.*, **62**, 524 (1989).
2) R. Matsueda and R. Walter, *Int. J. Pept. Protein Res.*, **16**, 392 (1980).
3) J. Blake, *Int. J. Pept. Protein Res.*, **17**, 273 (1981).
4) J. Blake, *Int. J. Pept. Protein Res.*, **27**, 191 (1986).
5) J. Blake, D. Yamashiro, K. Ramasharma, and C. H. Li, *Int. J. Pept. Protein Res.*, **28**, 468 (1986).
6) T. J. Gonda, N. M. Gough, A. R. Dunn, and J. de Blaquiere, *EMBO J.*, **4**, 2003 (1985).
7) K-H. Klempnauer and A. E. Sippel, *EMBO J.*, **6**, 2719 (1987).
8) The NMR study on the solution structure of synthetic *c*-Myb protein(142—193) will be reported elsewhere by Y. Nishimura et al.