Amino Acids and Peptides. XXV. Application of Newly Developed β-1- and β-2-Adamantylaspartates to Peptide Synthesis by Solid Phase and Conventional Solution Methods[1,2]

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Newly developed β-1- and β-2-adamantylaspartates [H-Asp(O-1-Ada)-OH and H-Asp(O-2-Ada)-OH] were applied to the synthesis of a C-terminal octapeptide of the β-subunit of human chorionic gonadotropin (hCG) by a conventional solution method and a hexacosapeptide of the α-subunit of insulin receptor (30—55) by a solid-phase method with the objective of suppressing aspartimide formation during the synthesis of aspartylpeptides. The 1-Ada and 2-Ada groups were confirmed to be useful protecting groups for the β-carboxyl function of the Asp residue.

Keywords β-1-adamantylaspartate; β-2-adamantylaspartate; aspartylpeptide; chemical synthesis; aspartimide formation suppression; solid-phase method; conventional solution method

The synthesis and properties of β-1 and β-2-adamantylaspartates [H–Asp(O-1-Ada)–OH (I) and H–Asp(O-2-Ada)–OH (II), shown in Fig. 1] have been reported.[3] The 1-Ada group is susceptible to trifluoroacetic acid (TFA), whereas the 2-Ada group is stable to TFA treatment, but is easily removable by methanesulfonic acid (MSA)[4] or anhydrous HF.[5] Both groups were stable to 50% piperidine in dichloromethane (DCM). Further, both groups can suppress aspartimide formation as a side reaction[6-9] under acidic and basic conditions during the synthesis of aspartylpeptides. Moreover, they can increase the solubility of the peptides in organic solvents.

This report deals with the application of I and II to the synthesis of an octapeptide, H–Ser–Asp–Thr–Pro–Ile–Leu–Pro-Gln–OH,[10] corresponding to the C-terminal sequence 138—145 of the β-subunit of human chorionic gonadotropin (hCG) by the conventional solution method and a hexacosapeptide of the α-subunit of insulin receptor (30—55)[11] by the solid-phase method.

Fig. 1. Structure of β-1- and β-2-Adamantylaspartates
I, β-1-adamantylaspartate; II, β-2-adamantylaspartate.

Previously, H–Ser–Asp–Thr–Pro–Ile–Leu–Pro–Gln–OH was prepared by route A shown in Fig. 2. In this route, in which the Bzl group was employed for protection of the β-carboxyl group of the Asp residue, the hydroxy group of the Thr residue was protected with a *tert*-butyl group in order to suppress aspartimide formation due to steric hindrance.[10] As illustrated in Fig. 2 (routes B and C), Z–Asp(OR)–OH [R: 1-Ada and 2-Ada] and H–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t were coupled by the DCC–HOBt method[12] to afford Z–Asp(OR)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t [R: 1-Ada and 2-Ada]. Although the hydroxy group of the Thr residue was not protected, aspartimide formation was not observed on thin layer chromatography (TLC) during the coupling reaction under basic conditions (triethylamine). After removal of the Z group, the resulting heptapeptide amine was coupled with Z–Ser–N_2H_3 by the azide procedure[13] to give Z–Ser–Asp(OR)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t.

At the final step, two different deprotection methods were employed according to the properties of 1-Ada and 2-

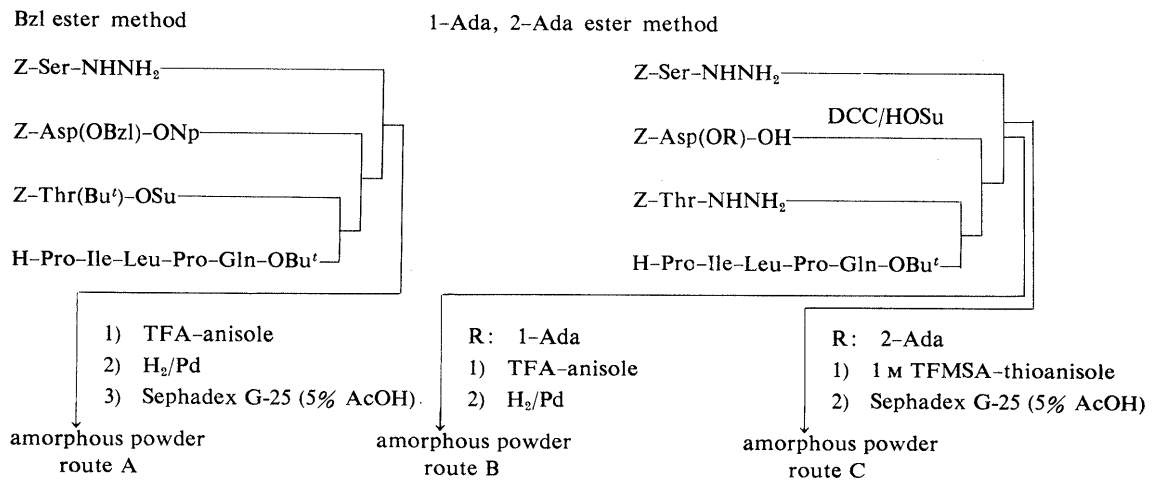

Fig. 2. Synthetic Routes to H–Ser–Asp–Thr–Pro–Ile–Leu–Pro–Gln–OH

This paper is dedicated to Professor Haruaki Yajima on the occasion of his retirement from Kyoto University in March 1989.

Ada. In route B, the 1-Ada and Bu^t ester groups were removed by TFA treatment and then the Z group at the N-terminus was removed by hydrogenation over Pd catalyst to give the desired octapeptide. In route C, all protecting groups were removed by trifluoromethanesulfonic acid (TFMSA)/thioanisole,[14] followed by treatment with slightly alkaline solution (pH 8.0) to reverse the N→O shift to give the desired octapeptide. Octapeptides obtained by routes B and C were compared with the octapeptide obtained by route A[10] on high performance liquid chromatography (HPLC). As shown in Fig. 3, each peptide exhibited a single symmetrical peak at the same retention time. Thus, by using the 1-Ada or 2-Ada group for protection of the β-carboxyl function of the Asp residue, aspartylpeptide could be synthesized without any detectable aspartimide formation.

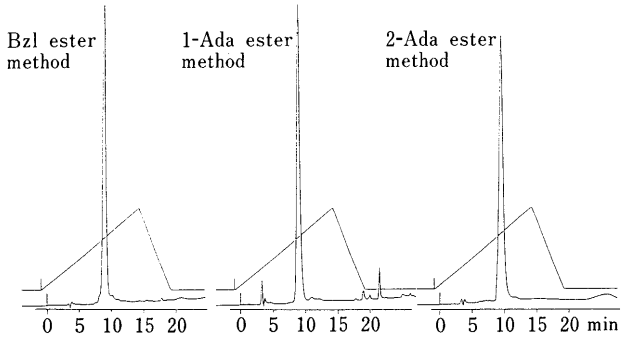

Fig. 3. Comparison of HPLC Profiles of Octapeptides

Column, YMC-PACK A-312 ODS (6×150 mm); solvent, A=H_2O (0.1% TFA) B=CH_3CN (0.1% TFA) gradient (A/B 85/15→15 min→60/40→5 min→85/15); flow rate, 1 ml/min, absorbance measurement, 220 nm.

H-Glu-Gly-His[a]-Leu-Gln[a]-Ile[b]-Leu[b]-Leu-Met-Phe-Lys[b]-Thr-Arg[a]-Pro-Glu-Asp-Phe-Arg[a]-Asp-Leu-Ser-Phe-Pro-Lys-Leu-Ile-OH

Fig. 4. Coupling Procedure for Synthesis of a Hexacosapeptide by the Solid-Phase Method

The amount of Boc-amino acids used for coupling by the symmetrical anhydride method was 4 eq. *a*), DCC-HOBt method (double coupling), *b*), symmetrical anhydride method (double coupling).

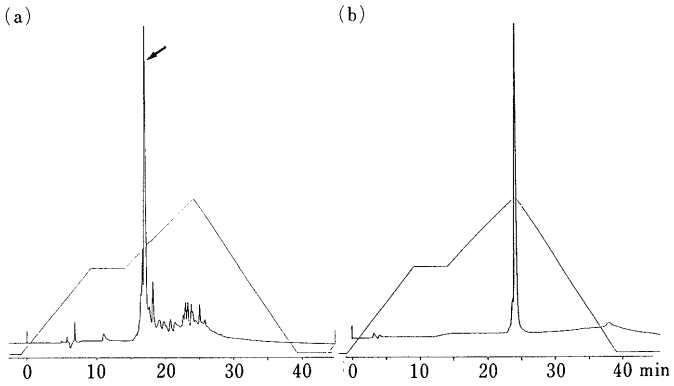

Fig. 5. HPLC of Hexacosapeptide

a) Crude hexacosapeptide. Column, YMC D-ODS-5 (20×250 mm); solvent, A=H_2O (1% TFA) B=CH_3CN (0.1% TFA) gradient (A/B 85/15→10 min→60/40→5 min→60/40→10 min→40/60→15 min→85/15), flow rate, 10 ml/min; absorbance measurement, 220 nm.

b) Purified hexacosapeptide. Column, YMC A-312-ODS (6×150 mm); solvent, A=H_2O (0.1% TFA) B=CH_3CN (0.1% TFA) gradient (A/B 85/15→10 min→60/40→5 min→60/40→10 min→40/60→15 min→85/15); flow rate, 1 ml/min; absorbance measurement, 220 nm.

Table I. Amino Acid Analyses of Purified Hexacosapeptide

Amino acid	Theoretical number	Acid hydrolysate (6 N HCl, 20 h)	Enzymatic hydrolysate (LAP, 24 h)
Asp	2	1.96	1.07
Thr	1	0.89	1.24[a]
Ser	1	0.97	1.42
Gln	1	—	—
Glu	2	3.13	2.04
Pro	2	2.10	1.05
Gly	1	0.91	1.00
Met	1	0.58	0.71
Ile	2	1.62	2.06
Leu	5	4.79	4.77
Phe	3	2.92	2.27
Lys	2	2.05	2.30
NH_3	2	1.17	4.22
His	1	0.94	0.85
Arg	2	1.98	1.62

a) Thr+Gln (overlapping).

Next, Boc–Asp(O-2-Ada)–OH was successfully applied to a solid-phase synthesis of a hexacosapeptide corresponding to amino acid residues 30—55 in the α-subunit of human insulin receptor.[11] The hexacosapeptide was prepared by the solid-phase method in the usual manner using an automatic peptide synthesizer (Applied biosystems 430A peptide synthesizer) according to the coupling procedure shown in Fig. 4. Boc–Asp(O-2-Ada)-OH was introduced onto the peptide resin by the usual symmetrical anhydride method[15] in a good yield (more than 99%). The crude peptide obtained by HF treatment of Boc–Glu(OBzl)–Gly–His(Bom)–Leu–Gln–Ile–Leu–Leu–Met–Phe–Lys(Cl–Z)–Thr(Bzl)–Arg(Tos)–Pro–Glu(OBzl)–Asp(O-2-Ada)–Phe–Arg(Tos)–Asp(O-2-Ada)–Leu–Ser(Bzl)–Phe–Pro–Lys(Cl–Z)–Leu–Ile–PAM-Resin was purified on Sephadex G-25 and then by preparative HPLC (yield 10% from protected peptide resin, Fig. 5a). The analytical HPLC profile of the purified peptide is shown in Fig. 5b. Amino acid ratios in acid and enzymatic (LAP) hydrolysates were in good agreement with the theoretically expected values, as shown in Table I.

Thus, the 1-Ada and 2-Ada groups were confirmed to be useful protecting groups for the β-carboxyl function of the Asp residue in the conventional solution method and solid phase method.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid (110 °C, 18 h, 6 N HCl) and leucine aminopeptidase (LAP, Sigma, Lot No. 33F-0842) hydrolysates were determined with an amino acid analyzer, K-101 AS (Kyowa Seimitsu Co., Ltd.). An Applied Biosystems 430A peptide synthesizer was used for solid-phase peptide synthesis. HPLC was conducted with a Waters M600 instrument [column, YMC-PACK A-312 ODS (6×150 mm) or YMC D-ODS-5 (20×250 mm)]. On TLC (Kieselgel G, Merck), Rf^1, Rf^2, Rf^3 and Rf^4 values refer to the following systems: $CHCl_3$, MeOH and AcOH (90:8:2), $CHCl_3$, MeOH and H_2O, (8:3:1, lower phase), *n*-BuOH, AcOH and H_2O (4:1:5, upper phase) and *n*-BuOH, pyridine, AcOH and H_2O (4:1:1:2), respectively.

Z–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t Z–Thr–N_3 (prepared from 215 mg of Z–Thr–N_2H_3, 0.6 ml of 6.4 N HCl/dioxane and 0.07 ml of isopentyl nitrite in the usual manner at −20 °C) in DMF (4 ml) was added to a solution of H–Pro–Ile–Leu–Pro–Gln–OBu^t (prepared from the correspond-

ing protected pentapeptide[10] (725 mg) by catalytic hydrogenation) in DMF (40 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford a solid material, which was collected by filtration, yield 672 mg (81.8%), mp 106 °C with sintering at 94 °C, $[\alpha]_D^{25}$ −110.4° (c=0.7, MeOH), Rf^1, 0.62; Rf^2, 0.97. *Anal.* Calcd for $C_{43}H_{67}N_7O_{11}\cdot 0.5H_2O$: C, 59.4; H, 7.91; N, 11.3. Found: C, 59.2; H, 7.71; N, 11.3.

Z–Asp(O-1-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t Z–Asp(O-1-Ada)–OH, H–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t (prepared from 200 mg of Z–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t by catalytic hydrogenation) and HOSu (32.2 mg) were dissolved in DMF (6 ml) and the solution was cooled with ice-salt, then DCC (57.8 mg) was added. The reaction mixture was stirred at 4 °C for 24 h. After removal of the urea derivative and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford a white precipitate, which was collected by filtration, yield 126 mg (77.0%), mp 116—121 °C. $[\alpha]_D^{25}$ −84.5° (c=0.3, MeOH), Rf^1, 0.54; Rf^3, 0.72. *Anal.* Calcd for $C_{57}H_{86}N_8O_{14}\cdot 0.5H_2O$: C, 61.3; H, 7.86; N, 10.0. Found: C, 61.2; H, 8.06; N, 10.1. Amino acid ratios in an acid hydrolysate: $Asp_{0.96}Thr_{0.83}$–$Glu_{1.00}Pro_{2.06}Ile_{0.74}Leu_{0.83}$ (average recovery 81%).

Z–Asp(O-2-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t The title compound was prepared by the same method as described for the synthesis of Z–Asp(O-1-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t, yield 46.3 mg (28.3%), mp 106—121 °C, $[\alpha]_D^{25}$ −84.5° (c=0.3, MeOH), Rf^1, 0.54; Rf^3, 0.72. *Anal.* Calcd for $C_{57}H_{86}N_8O_{14}\cdot 0.5H_2O$: C, 61.3; H, 7.86; N, 10.0. Found: C, 61.5; H, 7.97; N, 10.5. Amino acid ratios in an acid hydrolysate: $Asp_{0.90}Thr_{1.00}Glu_{1.00}Pro_{2.06}Ile_{0.74}Leu_{0.83}$ (average recovery 81%).

Z–Ser–Asp(O-1-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t Z–Ser–N_3 (prepared from 48 mg of Z–Ser–N_2H_3, 0.06 ml of 6.4 N HCl/dioxane and 0.026 ml of isopentyl nitrite in the usual manner at −20 °C) in DMF (2 ml) was added to a cold solution of H–Asp(O-1-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t (prepared from 70 mg of Z–Asp(O-1-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t by catalytic hydrogenation) in DMF (1 ml). The reaction mixture was stirred at 4 °C for 24 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford a solid mass, yield 45.4 mg (60.3%), mp 115—122 °C, $[\alpha]_D^{25}$ −97.8° (c=0.3, MeOH), Rf^3, 0.66. *Anal.* Calcd for $C_{60}H_{91}N_9O_{16}\cdot 1.5H_2O$: C, 59.0; H, 7.76; N, 10.3. Found: C, 59.0; H, 7.68; N, 10.7. Amino acid ratios in an acid hydrolysate: $Asp_{0.94}Thr_{0.83}$-$Ser_{1.08}Glu_{1.00}Pro_{2.06}Ile_{0.74}Leu_{0.83}$ (average recovery 88.5%).

Z–Ser–Asp(O-2-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t The title compound was prepared in the same manner as described for the synthesis of Z–Ser–Asp(O-1-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t, yield 46.3 mg (61.4%), mp 121—126 °C, $[\alpha]_D^{25}$ −87.8° (c=0.3, MeOH), Rf^3, 0.64. *Anal.* Calcd for $C_{60}H_{91}N_9O_{16}\cdot 1.5H_2O$: C, 59.0; H, 7.76; N, 10.3. Found: C, 59.0; H, 7.68; N, 10.3. Amino acid ratios in an acid hydrolysate: $Asp_{0.90}Thr_{1.00}$-$Ser_{0.90}Glu_{1.00}Pro_{1.85}Ile_{0.79}Leu_{0.90}$ (average recovery 85.5%).

H–Ser–Asp–Thr–Pro–Ile–Leu–Pro–Gln–OH 1) Z–Ser–Asp(O-1-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t (20 mg) was treated with TFA (0.5 ml) containing anisole (1 drop) in an ice bath for 20 min and then at room temperature for 30 min. After addition of dry ether, the resulting precipitate was collected by centrifugation and dried over KOH pellets *in vacuo* (yield 14 mg). The product in MeOH (8 ml) and H_2O (2 ml) was hydrogenated over Pd catalyst. The deprotected product was lyophilized from a small amount of water, yield 12.4 mg (75.0%), $[\alpha]_D^{25}$ −114.3° (c=0.2, H_2O), Rf^4, 0.11.

2) Z–Ser–Asp(O-2-Ada)–Thr–Pro–Ile–Leu–Pro–Gln–OBu^t (20 mg) was treated with 1 M TFMSA/TFA (0.5 ml) in the presence of thioanisole (61 μl) in an ice bath for 90 min. Ether was added to the solution to afford a precipitate, which was collected by centrifugation and dried over KOH pellets *in vacuo*. This powder in 3% AcOH (1 ml) was applied to a column of Sephadex G-25 (2.2 × 93 cm), which was eluted with the same solvent. Individual fractions (2 g each) were collected. The desired fractions (tube Nos. 223—227) were combined and lyophilized to give a hygroscopic fluffy powder. This powder was dissolved in H_2O. The pH of the solution was adjusted to 8 with 5% NH_4OH. After 30 min at room temperature, the pH of the solution was adjusted to 6 with 5% AcOH and the solvent was removed by lyophilization, yield 6.5 mg (38%), $[\alpha]_D^{25}$ −116.1° (c=0.3, H_2O), Rf^4, 0.11.

Synthesis of a Hexacosapeptide Corresponding to the Amino Acid Residues (30—55) of α-Subunit in Human Insulin Receptor The side-chain protecting groups were Bzl for Ser and Thr, 2-Ada for Asp, Bom for His, 4-Cl–Z for Lys and Tos for Arg. Boc–Ile–PAM–Resin (Lot No. A6 H007, 0.66 g) was placed in an Applied Biosystems 430A peptide synthesizer and treated at 25 °C in the usual manner. The yield of protected peptide polymer was 2.39 g (83.1%). A portion (200 mg) of protected peptide resin was treated with HF (10 ml) at 0 °C for 60 min in the presence of thioanisole (0.6 ml) and *m*-cresol (0.5 ml). After removal of the HF, AcOEt was added to the residue to remove additives. The crude peptide was extracted with 5% AcOH and the solvent was removed by lyophilization. The residue in H_2O (25 ml) was treated with Amberlite IRA-45 (acetate form, approximately 0.5 g) for 30 min. The resin was removed by filtration and washed with a small amount of 3% AcOH. The filtrate and washings were combined and the solvent was removed by lyophilization. The residue in 3% AcOH (2 ml) was applied to a column of Sephadex G-25 (2.1 × 130 cm), equilibrated and eluted with the same solvent. Individual fractions (4 g each) were collected. The desired fractions (tube Nos. 27—39) were combined and lyophilized to afford a white fluffy powder (62 mg). The above purified sample (40 mg) in 0.1% TFA (2.0 ml) was applied to a reversed-phase HPLC column [YMC D-ODS-5 (20 × 250 mm)], which was eluted with CH_3CN containing 0.1% TFA with a gradient of CH_3CN (15% → 10 min → 40% → 5 min → 40% → 10 min → 60% → 15 min → 15%) in 0.1% TFA at a flow rate of 10 ml per min (see Fig. 5a). The eluate corresponding to the main peak (retention time 17 min) was collected and the solvent was removed by lyophilization to give a fluffy powder, yield 11 mg (10% from protected peptide resin), $[\alpha]_D^{25}$ −81.4° (c=0.2, 5% AcOH). The HPLC profile is shown in Fig. 5b. Amino acid ratios in an acid hydrolysate and LAP digest are summarized in Table I.

References and Notes

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