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Studies on Peptides. CXXXII.[1,2] Evaluation of Two β-Carboxyl Protecting Groups of Aspartic Acid, Cycloheptyl and Cyclooctyl, for Peptide Synthesis

Nobutaka Fujii, Motoyoshi Nomizu, Shiroh Futaki, Akira Otaka, Susumu Funakoshi, Kenichi Akaji, Kazuhide Watanabe, and Haruaki Yajima*

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan

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The properties of two aspartic acid β-esters (Asp(OR), R=cycloheptyl (Chp) and cyclooctyl (Coc)) were examined. These two protecting groups were found to be stable to trifluoroacetic acid (0 °C, 2 h), but were cleaved by HF or 1 M trifluoromethanesulfonic acid–thioanisole in trifluoroacetic acid (0 °C, 60 min). Using a model peptide, Z(OMe)–Ala–Asp(OR)–Gly–OBzl, the behavior of the Asp(OR) residue with base or acid was examined. These esters were less susceptible to succinimide formation than the Bzl group in Et_3N treatment. In acid deprotection, succinimide formation from these peptides was less than 7% in both cases.

Keywords——β-cycloheptylaspartate; β-cyclooctylaspartate; acid-catalyzed succinimide formation; base-catalyzed succinimide formation; HF deprotection; trifluoromethanesulfonic acid deprotection

In 1979, β-cyclopentylaspartate (Asp(OCpe)) and β-cyclohexylaspartate (Asp(OChx)) were introduced by Blake[3] and Tam *et al.*,[4] respectively, in order to minimize the major side reaction during the synthesis of peptides containing Asp residues, *i.e.*, base[5] or acid-catalyzed rearrangement[6] to the cyclic imide (succinimide or aspartimide), which is known to be sequence-dependent.[7] These protecting groups also fulfill two other criteria, *i.e.*, they are stable to TFA and cleavable by HF.[8] Thus, their superiority to the Bzl group for solid-phase peptide synthesis was demonstrated by the above authors.[3,4] We have now examined whether more sterically hindered protecting groups than the Cpe or the Chx group can more effectively suppress the side reaction of Asp residues mentioned above.

In the present studies, two Boc–Asp(OR)–OH (R=cycloheptyl (Chp) and cyclooctyl (Coc)) derivatives were prepared according to the procedure described by Tam *et al.*[4] for the preparation of the corresponding Chx derivative. First, the stability of these ester groups to TFA was examined. In an ice-bath, the Boc group was selectively cleaved by TFA within 60 min, but no aspartic acid was detected on thin layer chromatography (TLC), even after treatment for 2 h. However, after 3 h, Asp (12%) was liberated from Asp(OCoc). The result indicated that both groups survive under the usual TFA treatment conditions (60 to 80 min) required for N^{α}-deprotection. However, the Coc group was found to be slightly more acid-sensitive than the Chp group. Next, the susceptibility of these protecting groups to 1 M TFMSA–thioanisole in TFA[9] was examined. Both groups were found to be cleaved quantitatively in an ice-bath within 60 min. We also found that the Cpe and the Chx groups could be cleaved by 1 M TFMSA–thioanisole in TFA under the same conditions. We confirmed that HF is also an effective cleaving reagent for the Chp and the Coc groups. These two protecting groups were cleanly cleaved by HF within 60 min at 0 °C.

Next, in order to test the side reaction mentioned above, two model peptides, Z(OMe)–

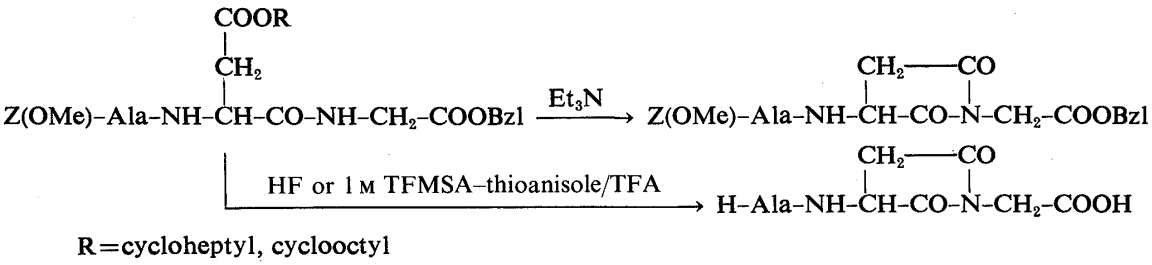

Fig. 1. Succinimide Formation from Z(OMe)–Ala–Asp(OR)–Gly–OBzl

TABLE I. Succinimide Formation (%) from Z(OMe)–Ala–Asp(OR)–Gly–OBzl in Base and Acid Treatments

R	Et_3N treatment (25 °C)		Acid treatment (0 °C)	
	20 h	40 h	HF	1 M TFMSA–thioanisole in TFA
Bzl	25.0	36.7	6.8	2.4
Cpe	3.0	6.5	6.0	1.4
Chx	3.2	6.6	4.7	3.0
Chp	3.3	6.3	5.8	3.9
Coc	1.9	5.2	5.4	3.2

Ala–Asp(OR)–Gly–OBzl (R = Chp and Coc) were prepared, since Asp(OBzl)–Gly is known to be most sensitive to base- and acid-catalyzed cyclization.[5-7] In addition, three analogs (R = Bzl, Cpe and Chx) were also prepared for comparison. As regards the latter three protecting groups, succinimide formation from a resin-bound model peptide by base and acid treatment was examined by previous authors,[3,4] but the reaction in solution was not examined. These five protected tripeptide derivatives were synthesized in a stepwise manner without particular difficulty. First, the sensitivity of these derivatives to Et_3N was examined (Fig. 1). At 25 °C, a solution of each protected tripeptide in DMF containing Et_3N (1 eq) was examined by TLC and the corresponding succinimide formed after 20 h and 40 h was measured quantitatively by using a dual-wavelength TLC scanner. As shown in Table I, the Bzl derivative gave the greatest amount of the by-product, as pointed out previously by other authors.[3,4] The other four protecting groups were significantly less susceptible to rearrangement in Et_3N treatment. Within the limits of experimental error, the degrees of rearrangement from the three derivatives, Cpe, Chx and Chp, appear to be roughly equal. A slightly lower susceptibility was noted with the Coc derivative. The results indicated that the two new groups, Chp and Coc, together with Cpe and Chx, tolerate well the basic conditions required for peptide synthesis by the solution method. Z(OMe)–Ala–aspartimidyl-Gly–OBzl, prepared by incubation of Z(OMe)–Ala–Asp(OBzl)–Gly–OBzl with Et_3N at 60 °C for 36 h, was used as a standard in these experiments.

Next, to test acid-catalyzed cyclization (Fig. 1), each derivative was exposed to 1 M TFMSA–thioanisole in TFA or HF in an ice-bath for 120 min, then the succinimide derivative formed was examined quantitatively with a TLC scanner; the results are listed in Table I. H–Ala–aspartimidyl-Gly–OH, derived from the above Et_3N-treated derivative by hydrogenolysis was used as a standard in these experiments. In both acid deprotections, succinimide formation from these peptides was less than 4% in 1 M TFMSA–thioanisole/TFA deprotection and this tendency was slightly less than that of HF deprotection (Table I).

As observed above, the Asp(OR)-peptides protected by secondary alcohols gave less imide than the Asp(OBzl)-peptide, particularly in base treatment as compared to acid

treatment. As described above, base- or acid-catalyzed side reaction of Asp(OR) residues is sequence-dependent.[7] The above experiments were conducted by taking account of the worst conditions likely to be encountered in peptide synthesis. Thus, from the data cited above, the two groups examined here, Chp and Coc, can be judged to be useful in peptide synthesis by the solution method, although these groups were not particularly superior to the Cpe and Chx groups on the basis of this preliminary study.

Experimental

Rf values in TLC performed on silica gel (Kieselgel G, Merck) refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH (10:0.5), Rf_2 $CHCl_3$–MeOH–AcOH (9:1:0.5), Rf_3 $CHCl_3$–MeOH–H_2O (8:3:1), Rf_4 *n*-BuOH–pyridine–AcOH–H_2O (3:1:1:1), Rf_5 *n*-BuOH–AcOH–AcOEt–H_2O (1:1:1:1). After being sprayed with ninhydrin reagent, a plate was heated in an oven (90 °C) for 15 min and the color intensity was measured with a Shimadzu dual-wavelength TLC scanner (model CS-900). Infrared (IR) spectra were measured with a Hitachi 215 grating IR spectrophotometer.

Boc–Asp(OR)–OBzl (R = Chp and Coc)——DCC[10] (1.2 eq) was added to a mixture of Boc–Asp(OH)–OBzl[11] (15.5 mmol each), a corresponding alcohol (1.5 eq each) and 4-dimethylaminopyridine (0.1 eq) in THF (70 ml) and the mixture, after being stirred at 4 °C overnight, was filtered. The solvent was removed by evaporation and the residue was extracted with AcOEt. The organic phase was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. The residue was recrystallized from AcOEt and *n*-hexane. Physical constants and analytical data are listed in Table II.

Boc–Asp(OR)–OH (R = Chp and Coc)——The above Bzl ester (4.3 mmol each) dissolved in MeOH (10 ml) containing a few drops of AcOH was hydrogenated over a Pd catalyst at room temperature for 3 h, until the Bzl ester was completely cleaved (monitored by TLC). The catalyst was removed by filtration, the filtrate was concentrated and the residue was recrystallized from *n*-hexane. Physical constants and analytical data are listed in Table II.

Treatment of Boc–Asp(OR)–OH (R = Chp and Coc) with TFA——The above Asp derivative (2.5 mg each) was exposed to TFA–anisole (4:1, 12.5 μl) for 120 min. The Boc group was cleaved from each derivative within 60 min. Rf_5 0.75 (H–Asp(OChp)–OH), 0.78 (H–Asp(OCoc)–OH). Within 120 min, no spot corresponding to Asp was observed on TLC. After 180 min, Asp (12%) derived from the Coc derivative was detected with the TLC scanner.

Treatment of Boc–Asp(OR)–OH (R = Chp and Coc) with HF——Boc–Asp(OR)–OH (5 mg each) was treated with HF (approximately 1 ml) in the presence of thioanisole (10 eq) in an ice-bath for 60 min. After evaporation of the HF, the residue was washed with ether and applied to an amino acid analyzer. Recoveries of Asp were 91% and 92% respectively. Besides Asp, no other peak was observed.

Treatment of Boc–Asp(OR)–OH (R = Cpe, Chx, Chp and Coc) with 1 M TFMSA–Thioanisole/TFA——Boc–Asp(OR)–OH (5 mg each) was treated with 1 M TFMSA–thioanisole/TFA (0.3 ml) in the presence of *m*-cresol (10 eq) in an ice-bath for 60 min, then dry ether was added. The residue was examined in an amino acid analyzer. Recoveries of Asp were 94%, 95%, 95% and 100%, respectively. Besides Asp, no other peak was detected.

Boc–Asp(OR)–Gly–OBzl (R = Bzl, Cpe, Chx, Chp and Coc)——These dipeptide esters were prepared by the DCC procedure.[10] Except for Boc–Asp(OBzl)–Gly–OBzl (yield 82%, Rf_1 0.57, mp 95—97 °C, $[\alpha]_D^{20}$ −5.4° in MeOH. *Anal.* Calcd for $C_{29}H_{36}N_2O_8$: C, 65.14; H, 5.66; N, 5.24. Found: C, 65.32; H, 5.74; N, 5.17.), they were obtained as

TABLE II. Characterization of Boc–Asp(OR)–OH and Its Bzl Ester

	Yield (%)	mp (°C)	$[\alpha]_D^{20}$ (MeOH)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
Boc–Asp(OChp)–OBzl	91	52—54	−3.2	$C_{23}H_{33}NO_6$	65.84 (65.86	7.93 8.00	3.34 3.36)
Boc–Asp(OChp)–OH	82	96—98	−3.4	$C_{16}H_{27}NO_6$	58.30 (58.61	8.20 8.45	4.25 4.42)
Boc–Asp(OCoc)–OBzl	60	40—41	−17.5	$C_{24}H_{35}NO_6$	66.49 (66.58	8.14 8.41	3.23 3.62)
Boc–Asp(OCoc)–OH	67	84—86	−2.0	$C_{17}H_{29}NO_6$	59.45 (59.55	8.51 8.68	4.08 4.37)

Table III. Characterization of Z(OMe)–Ala–Asp(OR)–Gly–OBzl

R	Yield (%)	mp (°C)	$[\alpha]_D$		Formula	Analysis (%) Calcd (Found)		
						C	H	N
Bzl	52	132—135	−16.6	DMF	$C_{32}H_{35}N_3O_9$	63.46 (63.31	5.83 5.76	6.94 7.02)
Cpe	42	120—122	−19.8	MeOH	$C_{30}H_{37}N_3O_9$	61.74 (61.88	6.39 6.37	7.20 7.29)
Chx	59	130—132	−10.2	MeOH	$C_{31}H_{39}N_3O_9 \cdot 1/2\,H_2O$	61.37 (61.47	6.64 6.49	6.92 7.35)
Chp	51	115—117	−23.3	MeOH	$C_{32}H_{41}N_3O_9$	62.83 (62.89	6.76 6.80	6.87 7.11)
Coc	45	103—105	−22.8	MeOH	$C_{33}H_{43}N_3O_9 \cdot H_2O$	62.44 (62.41	7.15 6.98	6.62 6.92)

oily products. R=Cpe: yield 60%, Rf_2 0.85; R=Chx: yield 84%, Rf_2 0.89; R=Chp: yield 55%, Rf_2 0.96; R=Coc: yield 58%, Rf_2 0.95.

Z(OMe)–Ala–Asp(OR)–Gly–OBzl (R=Bzl, Cpe, Chx, Chp and Coc)——These tripeptide esters were prepared by the Su condensation[12] of Z(OMe)–Ala–OH (1.1 eq) with TFA-treated samples of Boc–Asp(OR)–Gly–OBzl derivatives (1.0 eq) as usual and the products were purified by recrystallization from AcOEt and ether. Yield, physical constants and analytical data are listed in Table III.

Treatment of Z(OMe)–Ala–Asp(OR)–Gly–OBzl (R=Bzl, Cpe, Chx, Chp and Coc) with Et_3N——In the presence of Et_3N (1 eq), a solution of Z(OMe)–Ala–Asp(OR)–Gly–OBzl (8.3 mmol each) in DMF (50 μl) was kept standing at 25 °C. After 20 and 40 h, an aliquot was examined by TLC in the solvent system of $CHCl_3$–MeOH (10:0.5). Each spot was measured with a dual-wavelength TLC scanner and the results (average of two experiments) are shown in Table I.

Z(OMe)–Ala–Asp–Gly–OBzl——In order to obtain a standard sample for use in the above experiment, a solution of Z(OMe)–Ala–Asp(OBzl)–Gly–OBzl (2.0 g) in DMF (20 ml) containing Et_3N (1.5 eq) was incubated at 60 °C for 36 h and loss of the starting material was followed by TLC. The solvent was removed by evaporation and the residue was recrystallized from AcOEt and ether; yield 1.23 g (75%), mp 108—110 °C, $[\alpha]_D^{20}$ −18.0° (c=0.6, MeOH), Rf_1 0.31. *Anal.* Calcd for $C_{25}H_{27}N_3O_8$: C, 60.35; H, 5.47; N, 8.45. Found: C, 60.18; H, 5.48; N, 8.46.

H–Ala–Asp–Gly–OH——Z(OMe)–Ala–Asp(OBzl)–Gly–OBzl (100 mg) was dissolved in AcOH (10 ml) and hydrogenated over a Pd catalyst for 5 h. The solution was filtered, the filtrate was concentrated, and the residue was precipitated from H_2O with EtOH; yield 30 mg (68%), $[\alpha]_D^{25}$ −23.9° (c=0.2, H_2O), Rf_4 0.25. *Anal.* Calcd for $C_9H_{15}N_3O_6$: C, 41.38; H, 5.78; N, 16.09. Found: C, 41.00; H, 5.60; N, 16.09.

H–Ala–Asp–Gly–OH——Z(OMe)–Ala–Asp–Gly–OBzl (100 mg) was hydrogenated over a Pd catalyst as described above. The product was precipitated from H_2O with EtOH; yield 31 mg (64%), $[\alpha]_D^{20}$ +16.0° (c=0.2, H_2O), Rf_4 0.38. IR (Nujol): 1776, 1784 (–CO–) cm^{-1}. *Anal.* Calcd for $C_9H_{13}N_3O_5 \cdot 2H_2O$: C, 38.71; H, 5.41; N, 15.50. Found: C, 38.62; H, 5.51; N, 14.91.

Treatment of Z(OMe)–Ala–Asp(OR)–Gly–OBzl with 1 M TFMSA–Thioanisole/TFA——Each tripeptide derivative (5 mg) was treated with 1 M TFMSA–thioanisole/TFA (250 μl) in the presence of *m*-cresol (30 eq) in an ice-bath for 120 min. The product was precipitated with ether and examined by TLC using the above two samples as references. The results are shown in Table I.

Treatment of Z(OMe)–Ala–Asp(OR)–Gly–OBzl with HF——Each tripeptide derivative (5 mg) was treated with HF (*ca.* 1 ml) in the presence of thioanisole (30 eq) in an ice-bath for 120 min. HF was removed by evaporation and the residue was examined by TLC as described above. The results are shown in Table I.

References and Notes

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2) Ala, Asp and their derivatives used in this investigation are of the L-configuration. The following abbreviations are used: Boc=*tert*-butoxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, DCC=dicyclohexylcarbodiimide, TFA=trifluoroacetic acid, TFMSA=trifluoromethanesulfonic acid, THF=

tetrahydrofuran, DMF = dimethylformamide, Su = *N*-hydroxysuccinimidyl.

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