Amino Acids and Peptides. Part 19.[1] Synthesis of β-1- and β-2-Adamantyl Aspartates and their Evaluation for Peptide Synthesis†

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β-1- and β-2-Adamantyl aspartates [H-Asp(O-1-Ada)-OH and H-Asp(O-2-Ada)-OH] have been synthesized and their properties examined. Although the 1-Ada group is labile to TFA, the 2-Ada group is unaffected during TFA treatment, but easily removable by methanesulphonic acid (MSA) at room temperature within 5 min. Both groups are unaffected by treatment with 55% piperidine under conditions which easily cleave the fluoren-9-ylmethoxycarbonyl (Fmoc) group from an α-amino group. Both groups can suppress aspartimide formation as a side reaction under acidic and basic conditions during the synthesis of aspartyl peptides. β-1-or β-2-Adamantyl aspartates may be applicable to solid-phase peptide synthesis in combination with Fmoc or Boc as an N^{α}-protecting group, respectively. Some properties of the aspartimide moiety are described.

Previously, we reported that the reaction between Boc-Asp(OBzl)-ONp and H-Ser-Ser-Thr-Ser-OMe gave Boc-Asp-Ser-Ser-Thr-Ser-OMe (**1**) in crystalline pure form in good yield with a small amount of the desired pentapeptide.[2]‡ This major side reaction during the synthesis of peptides containing aspartyl sequences such as Asp-Gly, Asp-Ser, and Asp-His is well known.[3–6] The aspartimide moiety opens under certain conditions to form mainly β-aspartylpeptide.[5] It is very difficult to remove either the aspartimide derivative or the β-aspartylpeptide from the desired peptide. In order to suppress this side reaction, β-cyclopentyl (Cpe),[7] β-cyclohexyl (Chx),[8] β-cycloheptyl (Chp) and β-cyclo-octyl (Coc),[9] and β-menthyl (Men)[10] esters of aspartic acid were introduced into peptide synthesis, since the steric nature of the β-protecting groups seemed to play an important role in suppressing the side reaction; however these protecting groups did not avoid the side reaction completely. Thus a protecting group for the β-carboxy group of aspartic acid, which could suppress aspartimide formation more strongly during peptide synthesis is required. Such a protecting group should be stable during peptide synthesis and easily removable at the final step without aspartimide formation.

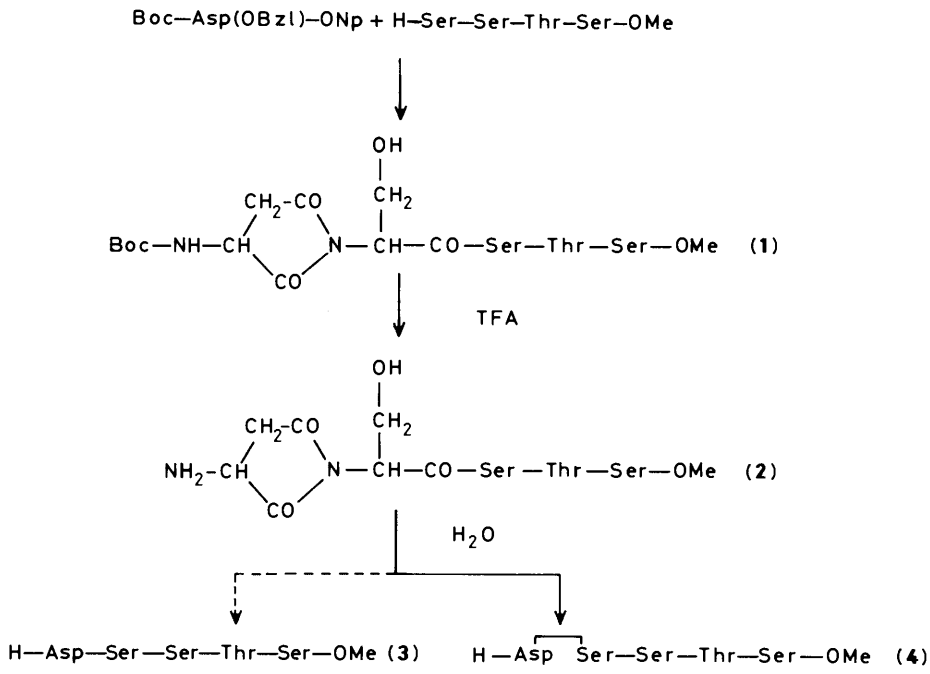
We report here the synthesis of β-1-adamantyl and β-2-adamantyl aspartates [H-Asp(O-1-Ada)-OH and H-Asp(O-2-Ada)-OH] and their evaluation for peptide synthesis, as well as some properties of the aspartimide moiety of Boc-Asp-Ser-Ser-Thr-Ser-OMe (**1**).

Prior to the study of β-1- and β-2-adamantyl aspartates, some properties of the aspartimide moiety of (**1**) were examined. As shown in Scheme 1, treatment with TFA gave H-AspSer-Ser-Thr-Ser-OMe (**2**) in pure form. The product (**2**) was dissolved in M/15-phosphate buffer (pH 7.0) and its rate of conversion into aspartylpeptide was examined by h.p.l.c. As shown in Figure 1, the aspartimide ring opened gradually, to give α- or β-aspartylpeptide completely after 20 h at room temperature. Amino acid analyses of acid and enzymic hydrolysates of H-Asp-Ser-Ser-Thr-Ser-OMe (**3**) and the peptide derived from (**2**) in M/15 phosphate buffer were carried out. In acid hydrolysates of both peptides, the results were in good agreement with the theoretically expected values. In contrast, LAP (Leucine aminopeptidase; EC 3.4.11.1) digests[5,11] gave Asp:Thr:Ser 0:1:2.16 for Asp-Ser-Ser-Thr-Ser-OMe (**3**) with 70.0% average recovery (recovery of Asp was 2.8% when Asp was digested with LAP under the same conditions), demonstrating that all peptide bonds were cleaved by LAP. However, in the aspartylpeptide derived from (**2**) a negligible amount of Ser was recovered, demonstrating that the Asp-Ser bond was not cleaved by LAP and that the Asp-Ser bond formed might be a β-aspartylpeptide bond. The conversion of the aspartimidyl moiety into β-aspartylpeptide was further confirmed by h.p.l.c. For separation of α-aspartyl- and β-aspartylpeptides, compound (**2**) was dissolved in distilled water (pH 6.0) instead of M/15 phosphate buffer (pH 7.0). The aspartimidyl moiety is fairly stable under these conditions even after 6 h at room temperature (Figure 2). The reaction mixture was warmed to 60 °C. As illustrated in Figure 2, after 1 h the aspartimidyl moiety was largely converted into aspartylpeptide, and after 12 h at 60 °C the reaction was almost complete. The product was mainly β-aspartylpeptide, with a small amount of α-isomer. These results are compatible with a previous report.[5]

We chose to synthesize β-1- and β-2-adamantyl aspartates [H-Asp(O-1-Ada)-OH and H-Asp(O-2-Ada)-OH] in the hope that the adamantyl group would be rigid and bulky enough to suppress aspartimide formation. The synthetic scheme is illustrated in Scheme 2. Boc-Asp-OBzl[12,13] and Z-Asp-OBzl[14] were esterified with adamantan-1-ol or adamantan-2-ol according to the procedure of Tam *et al.*[8] with the aid of DCC and DMAP,[15] and/or by the more recent procedure with DCC and *N*-methylimidazole.[16] The DCC–DMAP method gave the corresponding 1- or 2-adamantyl ester in better yield than the DCC–*N*-methylimidazole method. Hydrogenation over Pd afforded Boc-Asp(O-1-Ada)-OH, Boc-Asp(O-2-Ada)-OH, H-Asp(O-1-Ada)-OH, and H-Asp(O-2-Ada)-OH in pure crystalline form, quantitatively. These amino acid derivatives can be easily converted into the corresponding active esters and Z(OMe)-Asp(O-2-Ada)-OH and Fmoc-Asp(O-1-Ada)-OH (Scheme 2).

† Preliminary communication, Y. Okada, S. Iguchi, and K. Kawasaki, *J. Chem. Soc., Chem. Commun.*, 1987, 1532.

‡ All amino acid residues mentioned have the L-configuration. Abbreviations used are those recommended by the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature (*Pure Appl. Chem.*, 1984, **56**, 595): Boc = t-butoxycarbonyl, Z = benzyloxycarbonyl, Fmoc = fluoren-9-ylmethoxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Bzl = benzyl, Su = succinimido, Np = *p*-nitrophenyl, NB = norborn-5-ene-2,3-dicarboximido, DCC = *N,N'*-dicyclohexylcarbodi-imide, HOBt = *N*-hydroxybenzotriazole, DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid, AcOH = acetic acid, BuOH = butan-1-ol, DMF = *N,N*-dimethylformamide, MSA = methanesulphonic acid, TFMSA = trifluoromethanesulphonic acid.



Scheme 1.

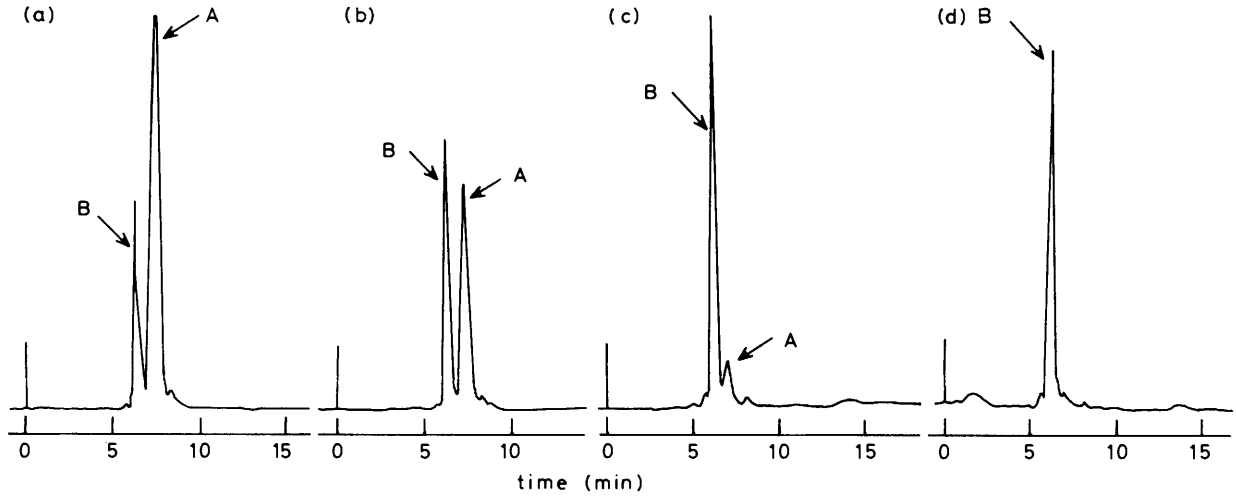


Figure 1. Conversion of the aspartimide derivative (**2**) into the corresponding aspartylpeptide in M/15 phosphate buffer (pH 7.0): (a) after 10 min; (b) after 2 h; (c) after 15 h; (d) after 20 h; peak AH-Asp-Ser-Ser-Thr-Ser-OMe, peak B corresponding aspartylpeptide; column: Asahipak GS-220H (7.6 × 250 mm); solvent M/15 phosphate buffer (pH 7.0)–MeCN (95:5); flow rate 1 ml min^{-1}; absorbance 214 nm

The stability and susceptibility of 1- and 2-adamantyl ester groups to various acids and bases were examined by measuring the amount of regenerated Asp residue with an amino acid analyser; the results are summarized in Table 1, in comparison with those obtained with the cyclohexyl ester group.[8] The 1-Ada group is easily removed by TFA at room temperature, but is fairly resistant to 7M HCl–dioxane. The 2-Ada group is stable to the foregoing acids but is cleaved quantitatively by methanesulphonic acid (MSA)[17] within 5 min at room temperature. Both groups are more stable to bases, such as M Na_2CO_3, than is the benzyl group. The 2-Ada group is slightly more sensitive to bases than the 1-Ada group, as expected. These results show that the 2-Ada group survives under the usual TFA treatment conditions required for N^{α}-deprotection, and that both groups survive treatment with 55% piperidine, under which conditions Fmoc can be easily cleaved from an α-amino group.[18] This indicates the possibility of application of β-1- or β-2-adamantyl aspartate to solid-phase peptide synthesis in combination with Fmoc or Boc as an N^{α}-protecting group, respectively.

In order to study aspartimide formation, the model peptides Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe were prepared from Boc-Asp(OR)-OSu and H-Ser-Ser-Thr-Ser-OMe (R = 1-Ada, 2-Ada, or Chx). The reaction of Boc-Asp(OBzl)-ONp and H-Ser-Ser-Thr-Ser-OMe is known to exhibit a great tendency to form Boc-Asp-Ser-Ser-Thr-Ser-OMe (**1**),[2] and this aspartimidylpeptide appears as a single peak separate from those of

Table 1. Stability of H-Asp(O-1-Ada)-OH, H-Asp(O-2-Ada)-OH, and H-Asp(OChx)-OH in various acids and bases

	% Parent amino acid regenerated															
	H-Asp(O-1-Ada)-OH (5.5 mg, 0.02 mmol) Time (min)						H-Asp(O-2-Ada)-OH (5.5 mg, 0.02 mmol) Time (min)						H-Asp(OChx)-OH (4.3 mg, 0.02 mmol) Time (min)					
	5	20	40	60	120	(24 h)	5	20	40	60	120	(24 h)	5	20	40	60	120	(24 h)
Conditions																		
1.0M HCl (100 equiv.)	0	0	0	0	2	17	0	0	0	0	1							
7.0M HCl–dioxane (200 equiv.)	1	8	15	24	35	82	0	0	0	1	1	20	0	0	0	1	1	
TFA (300 equiv.)	100						0	0	0	0	0							
MSA (400 equiv.)	100						100						74	76	78	85	86	
0.1M NaOH (10 equiv.)	2	4	9	12	24	89	6	24	40	54	81	100	7	33	50	72	75	78
1.0M Na_2CO_3 (100 equiv.)[a]	0	1	1	1	3	15	2	3	7	8	14	87	0	2	3	4	15	
10% Et_3N–H_2O + dioxane (50 equiv.)	0	0	0	0	0	9	1	1	2	2	5	14	0	0	0	0	0	20
10% Et_3N–DMF (70 equiv.)	0	0	0	0	0	0	0	0	0	0	0	0						
10% NMM H_2O (50 equiv.)[b]	0	0	0	0	0	5	0	0	1	1	2	28						
55% piperidine–DMF (500 equiv.)	0	0	0	0	0	0	0	0	0	0	0	0						

[a] Under these conditions, H-Asp(OBzl)-OH was hydrolysed as follows: 5.3% at 5 min; 16.4% 20 min; 34.4% 40 min; 47.7% 60 min; 60.1% 120 min; 100% 24 h. [b] NMM: *N*-methylmorpholine.

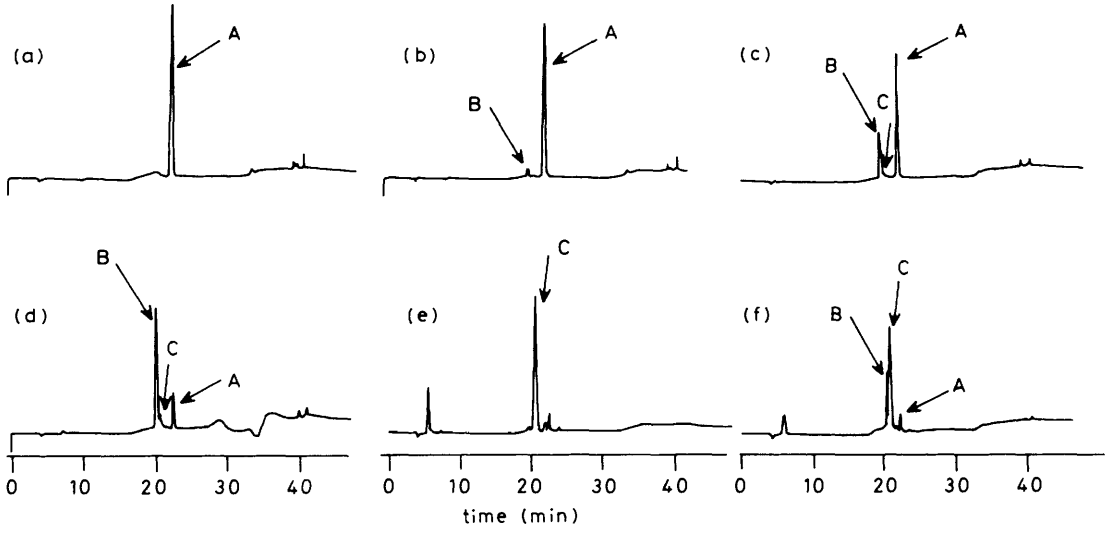
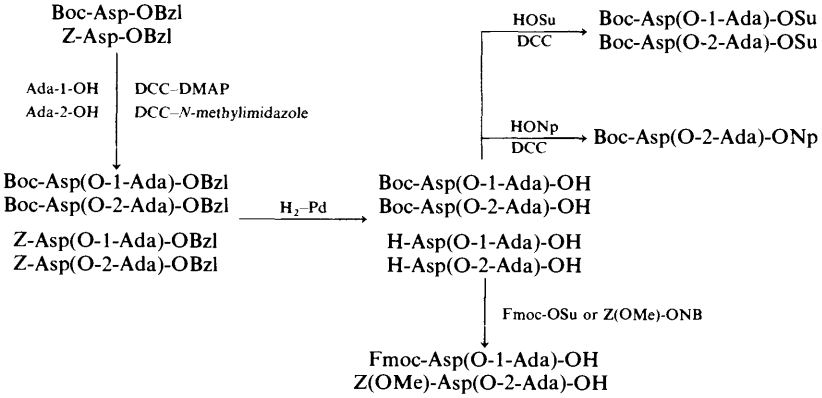


Figure 2. Conversion of the aspartimide derivative (**2**) into the corresponding α- and β-aspartylpeptides in water: (a) after 5 min at room temperature; (b) after 6 h at room temperature; (c) after 1 h at 60 °C; (d) after 12 h at 60 °C; (e) Asp-Ser-Ser-Thr-Ser-OMe (**3**); (f) mixture of (d) and (e); peak A: H-Asp-Ser-Ser-Thr-Ser-OMe (**2**); peak B: H-Asp-Ser-Ser-Thr-Ser-OMe (**4**); peak C: H-Asp-Ser-Ser-Thr-Ser-OMe (**3**); column: YMC PACK A-312 ODS (6.0 × 150 mm); solvent MeCN (0 → 10%, 20 min; → 30%, 10 min) −0.1% TFA; flow rate 1 ml min^{-1}; absorbance 220 nm



Scheme 2.

the desired pentapeptides on h.p.l.c. Boc-Asp(O-1-Ada)-OSu, Boc-Asp(O-2-Ada)-OSu, and Boc-Asp(OChx)-OSu were coupled with H-Ser-Ser-Thr-Ser-OMe in DMF containing Et_3N (1 equiv.). After 12 h at 25 or 30 °C, formation of (**1**) was examined by h.p.l.c.; the results are summarized in Table 2. In the case of the 1-Ada derivative, no aspartimide formation was observed at 25 °C, and only 1.23% was at 30 °C. In the cases of 2-Ada and Chx the extents of aspartimide formation were 3.24 and 3.16% at 25 °C, respectively, and 4.72 and 6.74% at 30 °C, respectively, whereas the figure for the Bzl derivative was 53.4% at 30 °C. The desired peptides were also obtained in good yields, indicating that Boc-Asp(OR)-OH (R = 1-Ada or 2-Ada) can be introduced into a peptide by the OSu active ester method in better yields than in the case of Boc-Asp(OChx)-OSu. From these results, it is clear that our novel protecting groups can strongly suppress aspartimide formation during the synthesis of aspartylpeptides.

Next, in order to test acid-catalysed cyclization,[9,10,19,20] each purified derivative [Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe (R = 1-Ada, 2-Ada, or Chx)] was exposed to HF[21] at 0 °C for 60 min and M TFMSA–thioanisole–TFA[17] at 0 °C for 90 min.

Table 2. Aspartimide formation (%) during peptide synthesis Boc-Asp(OR)-OSu + H-Ser-Ser-Thr-Ser-OMe

	25 °C, 12 h		30 °C, 12 h
R	Aspartimide derivative (%)	Desired peptide (%)	Aspartimide derivative (%)
1-Ada	0	81.9	1.23
2-Ada	3.24	81.0	4.72
Chx	3.16	68.7	6.74
Bzl	*a*		53.4

[a] Not determined.

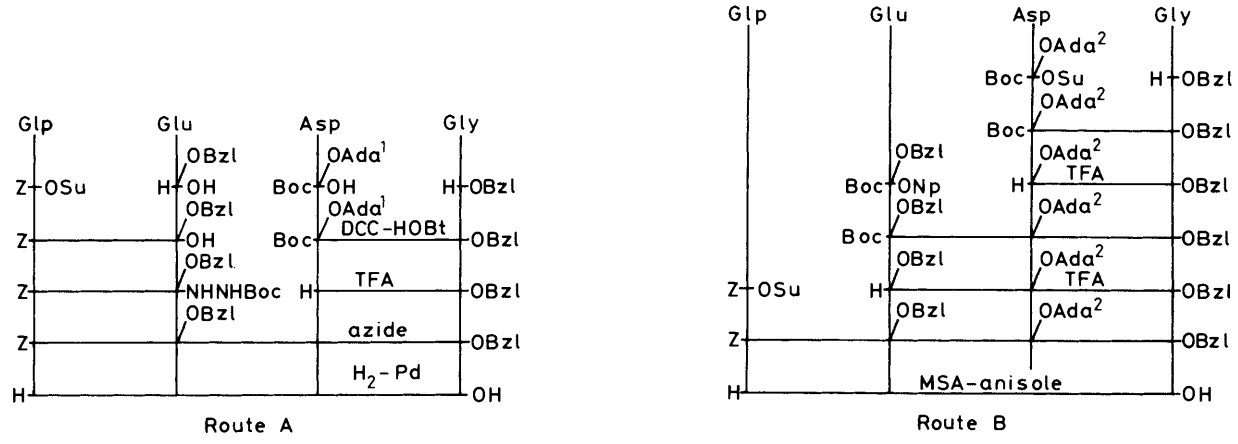
After isolation of the deblocked peptides, the formation of aspartimide was examined by h.p.l.c., with H-Asp-Ser-Ser-Thr-Ser-OMe (**2**) as standard. In the HF and TFMSA–TFA methods, besides the main peak corresponding to the desired deblocked pentapeptide, two minor peaks corresponding to the aspartimide derivative (**2**) and the β-aspartylpeptide, were observed. The β-aspartylpeptide was produced *via* the aspartimide derivative (**2**). The percentages of products are summarized in Table 3. In the case of the 1-Ada derivative, 5.4% side reaction occurred in the HF method and 2.4% in the TFMSA–TFA method. However, TFA treatment alone is enough to obtain the desired product in this case and aspartimide formation will be completely suppressed. In the case of 2-Ada, 2.3% side product was obtained in the HF method and 3.3% in the TFMSA–TFA method. In the case of Chx, 2.5% side reaction occurred in the HF method and 1.9% in the TFMSA–TFA reaction occurred. These results revealed that these protecting groups are able to suppress aspartimide formation during treatment with strong acids even in a sequence which has a great tendency to form aspartimide.

Finally, the usefulness of H-Asp(OR)-OH (R = 1-Ada and 2-Ada) for practical peptide synthesis was examined by using the insulin-releasing tetrapeptide H-Glp-Glu-Asp-Gly-OH[22] as an example, since Asp(OBzl)-Gly was reported to be relatively sensitive to base and to acid.[3–6] Two different routes (A and B in Scheme 3) were used to prepare the tetrapeptide. In route A, Boc-Asp(O-1-Ada)-OH and H-Gly-OBzl were coupled by the DCC–HOBt method[23] to give Boc-Asp(O-1-Ada)-Gly-OBzl in 80% yield. Z-Glp-Glu(OBzl)-OH was coupled with H-Asp-Gly-OBzl by the azide method to give Z-Glp-Glu(OBzl)-Asp-Gly-OBzl. This protected tetrapeptide was hydrogenated over Pd catalyst to give H-Glp-Glu-Asp-Gly-OH quantitatively in pure form. In route B, Boc-Asp(O-2-Ada)-OSu was coupled with H-Gly-OBzl to yield Boc-Asp(O-2-Ada)-Gly-OBzl in 80% yield. Boc-Asp(O-2-Ada)-Gly-OBzl was treated with TFA

Table 3. Products (%)[a] from Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe upon acid treatment

	HF-anisole (0 °C, 60 min)			1M TFMSA–thioanisole–TFA (0 °C, 90 min)		
R	Aspartimide derivative	β-Aspartyl peptide	Desired peptide	Aspartimide derivative	β-Aspartyl peptide	Desired peptide
1-Ada	1.82	3.57	94.6	0.91	1.49	97.6
2-Ada	1.54	0.81	97.7	0.98	2.27	96.8
Chx	1.90	0.65	97.4	0.70	1.21	98.1

[a] Defined as 100 × (product)/(mol aspartimide + mol β-peptide + mol α-peptide).



Scheme 3.

to give H-Asp(O-2-Ada)-Gly-OBzl, which was coupled with Boc-Glu(OBzl)-ONp and Z-Glp-OSu[24] successively to give Z-Glp-Glu(OBzl)-Asp(O-2-Ada)-Gly-OBzl in pure form. All protecting groups were removed by MSA–anisole at room temperature (60 min) to give H-Glp-Glu-Asp-Gly-OH. The final product prepared by either route exhibited a single peak at the same retention time on a $5C_{18}$ h.p.l.c. column. Although the tetrapeptide was obtained in pure form, it did not have any effect on insulin release in rats at the concentration described previously.[22] This is presumably due to the different assay method used (*in vitro* as opposed to *in vivo*[22]).

From these experimental results, we concluded that both Asp(O-1-Ada) and Asp(O-2-Ada) are attractive derivatives for the synthesis of peptides containing aspartyl sequences, such as Asp-Gly, Asp-Ser, *etc.*, sensitive to acid and base. Applications to solid-phase peptide synthesis, especially in combination with Fmoc for α-amino protection seem worthy of examination.

Experimental

M.p.s were determined with a Yanagimoto micro apparatus. Optical rotations were measured with an automatic DIP-360 polarimeter (Japan Spectroscopic Co. Ltd.). Amino acid compositions of acid hydrolysates (6M HCl; 110 °C; 18 h) and LAP digests[11] (Sigma Chemical Co.; from porcine kidney microsome, No. L-0632) were determined with an amino acid analyser (K-101AS, Kyowa Seimitsu). H.p.l.c. was conducted with a Waters M600 instrument [columns YMC-PACK A-312 ODS (6 × 150 mm), YMC-PACK A-302 ODS (4.6 × 150 mm), YMC-PACK R-ODS-5 (4.6 × 250 mm), and YMC-PACK D-ODS-5 (20 × 250 mm)] fitted with a Waters M740 computing integrator to measure peak areas. H.p.l.c. was also conducted with a Waters ALC-GPC-204 system [column Asahipack GS-220H (7.6 × 250 mm)]. On t.l.c. (Kieselgel G, Merck), R_{F1}, R_{F2}, R_{F3}, R_{F4}, R_{F5}, R_{F6}, R_{F7}, and R_{F8}, values refer to (1) $CHCl_3$, (2) $CHCl_3$–Et_2O (4:1), (3) $CHCl_3$–MeOH–AcOH (90:8:2), (4) $CHCl_3$–MeOH–H_2O (8:3:1; lower phase), (5) benzene, (6) BuOH–AcOH–H_2O (4:1:5; upper phase), (7) BuOH–AcOH–pyridine–H_2O (4:1:1:2), and (8) BuOH–AcOH–pyridine–H_2O (1:1:1:1), respectively.

Boc-Asp(O-1-Ada)-OBzl.—Boc-Asp-OBzl (7.05 g, 20 mmol), adamantan-1-ol (3.35 g, 22 mmol) and 4-dimethylaminopyridine (DMAP) (0.24 g, 2.0 mmol) were dissolved in CH_2Cl_2 (200 ml). DCC (4.54 g, 22 mmol) was added to the solution cooled with ice–salt. The mixture was stirred at room temperature overnight. Dicyclohexylurea and solvent were removed, and the residue was dissolved in EtOH (80 ml). *Crystals* which appeared were collected by filtration, yield 6.2 g (68%), m.p. 117—118 °C, $[\alpha]_D^{23}$ +5.2° (*c* 1.0 in $CHCl_3$), R_{F1} 0.47, R_{F2} 0.93 (Found: C, 68.4; H, 7.9; N, 3.2. $C_{26}H_{35}NO_6$ requires C, 68.3; H, 7.7; N, 3.1%).

Boc-Asp(O-1-Ada)-OH.—Boc-Asp(O-1-Ada)-OBzl (2.5 g, 5.46 mmol) was dissolved in MeOH (50 ml) and hydrogenated over Pd. After 4 h, Pd and solvent were removed and light petroleum was added to the residue to afford *crystals* (2.0 g, 100%), m.p. 173—175 °C, $[\alpha]_D^{23}$ +4.6° (*c* 0.5 in MeOH) (Found: C, 61.9; H, 8.2; N, 3.8. $C_{19}H_{29}NO_6$ requires C, 62.1; H, 8.0; N, 3.8%).

Boc-Asp(O-2-Ada)-OBzl.—(1) *DCC-DMAP method.* Boc-Asp-OBzl (5.0 g, 14.5 mmol), adamantan-2-ol (2.34 g, 15.4 mmol) and DMAP (0.171 g, 1.4 mmol) were dissolved in CH_2Cl_2 (50 ml) and cooled with ice–salt. DCC (3.18 g, 15.4 mmol) was added and the mixture was stirred at room temperature overnight. Dicyclohexylurea and solvent were removed, and the residue was dissolved in EtOH (2 ml). *Crystals* which appeared were collected by filtration. From the mother liquor, more product was recovered (total yield 5.0 g, 78%), m.p. 74—75 °C, $[\alpha]_D^{23}$ +8.29° (*c* 1.0 in $CHCl_3$), R_{F2} 0.81, R_{F3} 0.92 (Found: C, 68.2; H, 7.75; N, 3.3. $C_{26}H_{35}NO_6$ requires C, 68.3; H, 7.7; N, 3.1%).

(2) *DCC–N-methylimidazole method.* Boc-Asp-OBzl [from the corresponding DCHA salt (3.08 g, 6.1 mmol) as usual], adamantan-2-ol (0.93 g, 6.1 mmol), and *N*-methylimidazole (0.05 g, 0.61 mmol) were dissolved in CH_2Cl_2 (20 ml) and cooled with ice–salt. DCC (1.26 g, 6.1 mmol) was added and the mixture was stirred at room temperature overnight. After removal of dicyclohexylurea and the solvent, EtOH (10 ml) was added to the residue to afford *crystals* (0.96 g, 34.5%), m.p. 72—74 °C, mixed m.p. 72—74 °C, R_{F2} 0.81, R_{F3} 0.92.

Boc-Asp(O-2-Ada)-OH.—Boc-Asp(O-2-Ada)-OBzl (1.34 g, 2.93 mmol) in MeOH (30 ml) was hydrogenated over Pd for 4 h. After removal of Pd and solvent, EtOH and H_2O were added to the residue to afford *crystals* (0.88 g, 82%), m.p. 111—114 °C, $[\alpha]_D^{29}$ 0° (*c* 1.2 in MeOH) and +21.8° (*c* 1.2 in $CHCl_3$), R_{F3} 0.85, R_{F4} 0.96 (Found: C, 62.0; H, 8.0; N, 3.7. $C_{19}H_{29}NO_6$ requires C, 62.1; H, 8.0; N, 3.8%).

Z-Asp(O-1-Ada)-OBzl.—Z-Asp-OBzl (2.0 g, 5.6 mmol), adamantan-1-ol (0.94 g, 6.2 mmol) and DMAP (0.068 g, 0.56 mmol) were dissolved in CH_2Cl_2 (50 ml) and cooled with ice–salt. DCC (1.27 g, 6.2 mmol) was added and the mixture was stirred at 4 °C overnight. Dicyclohexylurea and solvent were removed, and the residue was extracted with AcOEt. The extract was washed with water, dried (Na_2SO_4), and evaporated. Light petroleum was added to the residue to afford *crystals*, which were collected by filtration and recrystallized from EtOH; yield 2.0 g (73%), m.p. 94—95 °C, $[\alpha]_D^{23}$ +8.56° (*c* 1.0 in $CHCl_3$), R_{F2} 0.92, R_{F5} 0.24 (Found: C, 70.9; H, 6.8; N, 3.1. $C_{29}H_{33}NO_6$ requires C, 70.85; H, 6.8; N, 2.85%).

H-Asp(O-1-Ada)-OH.—Z-Asp(O-1-Ada)-OBzl (1.2 g, 2.44 mmol) in MeOH (40 ml) was hydrogenated over Pd for 4 h. The Pd and solvent were removed, and ether was added to the residue to afford *crystals* (0.60 g, 89%), m.p. 240 °C (decomp.), $[\alpha]_D^{23}$ −15° (*c* 0.6 in MeOH), R_{F6} 0.40, R_{F7} 0.57 (Found: C, 60.4; H, 8.1; N, 5.0. $C_{14}H_{21}NO_4 \cdot 0.5H_2O$ requires C, 60.85; H, 8.0; N, 5.1%).

Z-Asp(O-2-Ada)-OBzl.—Z-Asp-OBzl (2.0 g, 5.6 mmol), adamantan-2-ol (0.85 g, 5.6 mmol), and DMAP (0.073 g, 0.6 mmol) were dissolved in CH_2Cl_2 (50 ml). DCC (1.24 g, 6.0 mmol) was added to the solution cooled with ice–salt. The mixture was stirred at 4 °C overnight. Dicyclohexylurea and solvent were removed, and the residue was extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated. A small amount of MeCN was added to the residue to give crystalline material. After removal of this, the filtrate was evaporated to give oily *material* (2.56 g, 93%), $[\alpha]_D^{25}$ +9.4° (*c* 1.8 in $CHCl_3$), R_{F2} 0.89, R_{F3} 0.94 (Found: C, 70.55; H, 6.8; N, 3.1. $C_{29}H_{33}NO_6$ requires C, 70.85; H, 6.8; N, 2.85%).

H-Asp(O-2-Ada)-OH.—Z-Asp(O-2-Ada)-OBzl (160 mg, 0.325 mmol) in EtOH (10 ml) and H_2O (1 ml) was hydrogenated over Pd for 4 h. After removal of Pd and the solvent, EtOH was added to give *crystals* (37.7 mg, 42%), m.p. 217—221 °C, $[\alpha]_D^{29}$ −11.5° (*c* 0.5 in MeOH), R_{F4} 0.60, R_{F6} 0.47 (Found: C, 60.8; H, 7.9; N, 5.0. $C_{14}H_{21}NO_4 \cdot 0.5H_2O$ requires C, 60.85; H, 8.0; N, 5.1%).

Boc-Asp(O-1-Ada)-OSu.—Boc-Asp(O-1-Ada)-OH (0.97 g, 2.64 mmol) and HOSu (0.365 g, 3.17 mmol) were dissolved in

CH_2Cl_2 (15 ml) and DMF (6 ml). DCC (0.654 g, 3.17 mmol) was added with cooling (ice–salt). The mixture was stirred at 4 °C overnight. Dicyclohexylurea and solvent were removed, and the residue was extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated. Addition of a small amount of EtOH to the residue gave *crystals* (0.423 g, 34.5%), m.p. 122—125 °C, $[\alpha]_D^{29}$ −4.95° (*c* 0.7 in $CHCl_3$), R_{F2} 0.41, R_{F4} 0.71 (Found: C, 59.8; H, 7.0; N, 6.1. $C_{23}H_{32}N_2O_8$ requires C, 59.5; H, 6.9; N, 6.0%).

Boc-Asp(O-2-Ada)-OSu.—Boc-Asp(O-2-Ada)-OH (5.0 g, 13.6 mmol) and HOSu (1.72 g, 15 mmol) were dissolved in AcOEt (60 ml) and DMF (4 ml). DCC (3.09 g, 15 mmol) was added with cooling (ice–salt). The mixture was stirred overnight. Dicyclohexylurea and solvent were removed, and the residue was extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated. Light petroleum was added to the residue to afford a solid mass (4.9 g, 77.5 %), m.p. 109—114 °C. For analysis, this material (1 g) was recrystallized from EtOH (3 ml) to give *crystals* (0.50 g), m.p. 130—132 °C, $[\alpha]_D^{29}$ −7.75° (*c* 0.5 in $CHCl_3$), R_{F3} 0.85 (Found: C, 59.3; H, 6.9; N, 6.0. $C_{23}H_{32}N_2O_8$, requires C, 59.5; H, 6.9; N, 6.0%).

Boc-Asp(O-2-Ada)-ONp.—Boc-Asp(O-2-Ada)-OH (400 mg, 1.09 mmol) and *p*-nitrophenol (176 mg, 1.27 mmol) were dissolved in CH_2Cl_2 (15 ml). DCC (269 mg, 1.27 mmol) was added with cooling (ice–salt). The mixture was stirred at room temperature overnight. Dicyclohexylurea and solvent were removed, and the residue was extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated to give oily material. This product in $CHCl_3$ (3 ml) was applied to a silica gel column (1.2 × 33 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (40 ml each) were collected and the eluate (tubes 4–5) was evaporated to leave an oily *residue* (346 mg, 65%), $[\alpha]_D^{27}$ +18.4° (*c* 0.7, in $CHCl_3$), R_{F2} 0.72, R_{F3} 0.94 (Found: 61.9; H, 6.7, N, 5.7. $C_{25}H_{32}N_2O_8$ requires C, 61.5; H, 6.6; N, 5.7%).

Fmoc-Asp(O-1-Ada)-OH.—H-Asp(O-1-Ada)-OH (100 mg, 0.36 mmol) was suspended in water (1 ml) containing Et_3N (0.05 ml, 0.36 mmol). Fmoc-OSu[25] (121 mg, 0.36 mmol) in MeCN (1 ml) was added and the mixture was stirred at room temperature for 1 h. After removal of the solvents, 1M HCl (30 ml) was added and the oily material was extracted with ether. The extract was washed with water, dried ($MgSO_4$), and evaporated to leave oily material. This was applied to a silica gel column (1.3 × 17 cm), equilibrated and eluted with $CHCl_3$. The eluate (200—300 ml) was evaporated to give an amorphous *powder* (144.5 mg, 82%), $[\alpha]_D^{30}$ +0.2° (*c* 1.0 in MeOH) and +36.6° (*c* 1.0 in $CHCl_3$), R_{F3} 0.50 (Found: C, 70.5; H, 6.5; N, 2.9. $C_{29}H_{31}O_6N{\cdot}0.25\ H_2O$ requires C, 70.5; H, 6.4; N, 2.8%).

Z(OMe)-Asp(O-2-Ada)-OH.—H-Asp(O-2-Ada)-OH (100 mg, 0.36 mmol) and Z(OMe)-ONB (139 mg, 0.41 mmol) were dissolved in MeCN (3.0 ml) and DMF (3.0 ml) containing Et_3N (0.10 ml, 0.71 mmol). The mixture was stirred at room temperature overnight. The solvent was removed and the residue in $CHCl_3$ (1.0 ml) was applied to a silica gel column (1.2 × 40 cm), equilibrated and eluted with $CHCl_3$. The eluate (500—700 ml) was evaporated to leave an oily *material* (138 mg, 89%), $[\alpha]_D^{26}$ −5.5° (*c* 0.56 in MeOH), R_{F3} 0.72, R_{F4} 0.92 (Found: C, 62.7; H, 7.0; N, 2.9. $C_{23}H_{29}NO_7{\cdot}0.5H_2O$ requires C, 62.7; H, 6.9; N, 3.2%).

Examination of Stability and Sensitivity of H-Asp(OR)-OH (R = 1-Ada, 2-Ada, or Chx) *to Base and Acid.*—H-Asp(OR)-OH (0.02 mmol) was dissolved in acid or base (Table 2) at room temperature. Samples for amino acid analysis were prepared as follows. (1) In the case of basic solution; 10 μl of each solution was diluted with 0.1—1M HCl (90 μl) to adjust the pH to about 2. This solution (10—20 μl) was injected into the amino acid analyzer and the amount of regenerated Asp residue was measured as a function of the time. (2) In the case of acidic solution: 10 μl of each solution was diluted with water or 0.025—0.5M Na_2CO_3 to adjust the pH to about 2. This solution (10—20μl) was used for amino acid analysis.

H-Asp-Ser-Ser-Thr-Ser-OMe (**2**).—Compound (**1**) (70 mg, 0.12 mmol) was dissolved in TFA (1.0 ml) and stored at 0 °C for 30 min and at room temperature for 30 min. Ether was added to yield a precipitate, which was collected by filtration and washed with ether, giving an amorphous *powder* (70 mg, 98%), $[\alpha]_D^{25}$ −8.7° (*c* 0.6 in DMF) (Found: C, 38.1; H, 5.0; N, 10.8. $C_{18}H_{29}N_5O_{11}{\cdot}CF_3CO_2H{\cdot}1.5H_2O$ requires C, 38.0; H, 5.3; N, 11.1%).

H-Ser-Ser-Thr-Ser-OMe.—Z-Ser-Ser-Thr-Ser-OMe[2] (402 mg, 0.94 mmol) in DMF (10 ml) was hydrogenated over Pd for 8 h. After removal of Pd and the solvent, ether and EtOH (4:1) were added to afford *crystals* (299 mg, 95%), m.p. 181—184 °C (decomp.) (from EtOH), $[\alpha]_D^{28}$ −24.0° (*c* 0.5 in MeOH), R_{F4} 0.2, R_{F7} 0.08 (Found: C, 40.0; H, 6.85; N, 13.4. $C_{14}H_{26}N_4O_9{\cdot}1.5H_2O$ requires C, 39.9; H, 6.95; N, 13.3%).

General Procedure for Synthesis of Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe (R = 1-Ada, 2-Ada, or Chx) *and Examination of Aspartimide Formation.*—Boc-Asp(OR)-OSu (R = 1-Ada, 2-Ada, or Chx) (0.193 mmol) and H-Ser-Ser-Thr-Ser-OMe (60 mg, 0.152 mmol) were dissolved in DMF (2 ml) containing Et_3N (0.02 ml, 0.143 mmol). The mixture was stirred at 25 or 30 °C for 12 h. This mixture (20 μl) was diluted with MeCN (200 μl), and a portion (10 μl) was subjected to h.p.l.c. [YMC-PACK R-ODS-5 (4.6 × 250 mm); MeCN (15 → 70%, 30 min; →15%, 10 min)—0.1% TFA; flow rate 1 ml min^{-1}; absorbance 220 nm]. The retention times were: Boc-Asp-Ser-Ser-Thr-Ser-OMe (**1**), 15.24 min; Boc-Asp-(OR)-Ser-Ser-Thr-Ser-OMe (R = 1-Ada) 32.01 min; (R = 2-Ada) 32.37 min; (R = Chx) 27.96 min. The amounts of the aspartimide derivative (%) and the desired peptides (%) are summarised in Table 2. In order to isolate the desired pentapeptide, after removal of the solvent, AcOEt (8 ml) was added to give a precipitate, which was collected, washed with water and ether, and dried *in vacuo*. This powder contained the impure aspartimide derivative (**1**). The powder in DMF (2 ml) was applied to a Sephadex LH-20 column (1.7 × 142 cm) equilibrated and eluted with DMF. Individual fractions (5 g each) were collected. The desired peptide was contained in tubes 15—17. However, tube 15, besides the desired peptide, contained the aspartimide derivative (**1**). The pure pentapeptide was therefore isolated by preparative reversed-phase h.p.l.c. on a YMC-PACK D-ODS-5 column (20 × 250 mm) (flow rate 13 ml/min^{-1}; retention times Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe (R = 1-Ada) 25.37 min; (R = 2-Ada) 25.645 min; (R = Chx) 20.85 min] in the same solvent system as just described; yields, m.p.s, $[\alpha]_D$ value, elemental analyses, and R_F values are summarized in Table 4.

General Procedure for Deprotection of the Pentapeptide and Examination of Aspartimide Formation.—(1) HF *method.* Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe (R = 1-Ada, 2-Ada, or Chx) (10 mg) in HF (1 ml) containing anisole (0.1 ml) was stirred at 0 °C for 1 h. After removal of HF, dry ether was added to afford a precipitate, which was collected by centrifugation and washed with ether. This compound was dissolved in water and subjected to h.p.l.c. [YMC-PACK A-302 ODS (4.6 × 150 mm); MeCN (0%, 5 min; →10%, 20 min; →0%, 1 min; 0% 14 min) −0.1%

Table 4. Yields, m.p.s, $[\alpha]_D$ values, elemental analyses, and R_F values of Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe purified by h.p.l.c.

R	Yield (%)	M.p. (°C)	$[\alpha]_D$ (DMF)	Formula	Analyses Calc. (Found) C	H	N	T.l.c.[a] R_F
1-Ada	57.5	193—197	+2.0 (*c* 0.2)	$C_{33}H_{53}N_5O_{14}\cdot 1.5H_2O$	51.4 (51.3	7.3 7.0	9.1 9.1)	0.83
2-Ada	56.6	191—196	−2.6 (*c* 0.2)	$C_{33}H_{53}N_5O_{14}$	53.3 (53.0	7.2 7.2	9.4 9.1)	0.82
Chx	49.5	209—211	−3.8 (*c* 0.2)	$C_{29}H_{49}N_5O_{14}\cdot 1.5H_2O$	48.5 (48.2	7.3 6.95	9.7 9.8)	0.82

[a] Solvent BuOH–AcOH–H_2O (4:1:5; upper phase).

TFA; flow rate 1 ml min^{-1}; absorbance 220 nm; retention times Asp-Ser-Ser-Thr-Ser-OMe (**4**), 11.14 min; H-Asp-Ser-Ser-Thr-Ser-OMe (**3**), 13.18 min; H-Asp-Ser-Ser-Thr-Ser-OMe (**2**), 17.78 min]. The results are summarized in Table 3.

(2) *TFMSA–TFA method.* Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe (R = 1-Ada, 2-Ada, or Chx) (5 mg) in M TFMSA–TFA (0.2 ml) containing thioanisole (25 μl) was stirred at 0 °C for 90 min. Ether and light petroleum (1:1) were added, to afford a precipitate, which was collected by centrifugation and washed with ether. This compound was dissolved in water and subjected to h.p.l.c. as in (1). The results are summarized in Table 3.

Boc-Asp(O-1-Ada)-Gly-OBzl.—Boc-Asp(O-1-Ada)-OH (2.0 g, 5.44 mmol), H-Gly-OBzl [from H-Gly-OBzl·Tos-OH (2.02 g) and 5% Na_2CO_3 (50 ml)] and HOBt (0.74 g, 5.44 mmol) were dissolved in DMF (70 ml). DCC (1.35 g, 6.53 mmol) was added with cooling (ice–salt). The mixture was stirred at room temperature for 1 day. Dicyclohexylurea and solvent were removed, and the residue was extracted with AcOEt. The extract was washed with 5% AcOH, 5% Na_2CO_3, and water, dried (Na_2SO_4), and evaporated. The residue in $CHCl_3$ (5 ml) was applied to a silica gel column (2.3 × 40 cm), equilibrated and eluted with $CHCl_3$. The eluate (900—1 500 ml) was evaporated to leave an *oil* (2.6 g, 85%), $[\alpha]_D^{23}$ −5.6° (*c* 0.6 in MeOH), R_{F3} 0.85 (Found: C, 65.4; H, 7.6; N, 5.5. $C_{28}H_{38}N_2O_7$ requires C, 65.4; H, 7.4; N, 5.4%).

Z-Glp-Glu(OBzl)-OH.—Z-Glp-OSu (4.46 g, 12 mmol) and H-Glu(OBzl)-OH (3.42 g, 14 mmol) were dissolved in DMF (60 ml) containing Et_3N (3 ml, 21.6 mmol). The mixture was stirred at room temperature for 2 days. The solvent was removed and the residue was dissolved in 5% $NaHCO_3$ and washed with AcOEt. The water layer was acidified with conc. HCl. The oily material was extracted with AcOEt. The extract was washed with water, dried (Na_2SO_4), and evaporated. Light petroleum was added to the residue to give a *solid*, which was recrystallized from ether; yield 4.60 g (78%), m.p. 115—119 °C, $[\alpha]_D^{23}$ −21.8° (*c* 0.5 in MeOH), R_{F3} 0.33, R_{F4} 0.67 (Found: C, 61.3; H, 5.4; N, 6.0. $C_{25}H_{26}N_2O_8\cdot 0.5H_2O$ requires C, 61.1; H, 5.5; N, 5.7%).

Z-Glp-Glu(OBzl)-NHNHBoc.—Z-Glp-Glu(OBzl)-OH (4.6 g, 9.53 mmol), NH_2NHBoc (1.32 g, 10 mmol), and HOBt (1.35 g, 10 mmol) were dissolved in DMF (50 ml). DCC (2.36 g, 11 mmol) was added with cooling (ice–salt). The mixture was stirred at room temperature overnight. Dicyclohexylurea and solvent were removed, and the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried (Na_2SO_4), and evaporated. Ether was added to the residue to give *crystals* (5.3 g, 93%), m.p. 135 °C with sintering at 115 °C, $[\alpha]_D^{23}$ −52.6° (*c* 1.0 in MeOH), R_{F3} 0.42, R_{F4} 0.81 (Found: C, 60.4; H, 6.3; N, 9.5. $C_{30}H_{36}N_4O_9$ requires C, 60.4; H, 6.1; N, 9.4%).

Z-Glp-Glu(OBzl)-Asp-Gly-OBzl.—6M HCl–dioxane (1.6 ml, 9 mmol) was added to Z-Glp-Glu(OBzl)-NHNHBoc (1.79 g, 3 mmol) with cooling (ice). After 10 min, this solution was diluted with DMF (1.6 ml) and cooled to −20 °C. Isopentyl nitrite (0.420 ml, 3.0 mmol) was added to give the corresponding azide in the usual manner. This azide solution was combined with H-Asp-Gly-OBzl·TFA [from Boc-Asp(O-1-Ada)-Gly-OBzl (1.54 g, 3.0 mmol) and TFA (3.5 ml, 30 mmol) containing anisole] in DMF (10 ml) containing Et_3N (1.26 ml, 9.0 mmol). The mixture was stirred at 4 °C overnight. The solvent was removed and the residue was extracted with AcOEt. The extract was washed with M HCl and water, dried (Na_2SO_4), and evaporated. Ether was added to the residue to give gelatinous *material* (0.82 g, 37%), m.p. 154—161 °C, $[\alpha]_D^{23}$ −49.8° (*c* 0.5 in MeOH), R_{F3} 0.16, R_{F4} 0.88 (Found: C, 61.0; H, 5.45; N, 7.7. $C_{38}H_{40}N_4O_{12}$ requires C, 61.3; H, 5.4; N, 7.5%).

H-Glp-Glu-Asp-Gly-OH.—Z-Glp-Glu(OBzl)-Asp-Gly-OBzl (200 mg, 0.27 mmol) in MeOH (10 ml) and DMF (6 ml) was hydrogenated over Pd. After 9 h, Pd and solvent were removed. Ether was added to the residue to give solid *material* (92 mg, 80%), amorphous, $[\alpha]_D^{23}$ −42.8° (*c* 0.5 in H_2O), R_{F7} 0.21, R_{F8} 0.50 (Found: C, 44.1; H, 5.5; N, 13.1. $C_{16}H_{22}N_4O_{10}\cdot 0.25H_2O$ requires C, 44.2; H, 5.2; N, 12.9%). Amino acid ratios in an acid hydrolysate were Asp:Glu:Gly 1.00:2.04:0.98 (average recovery 93.6%).

Boc-Asp(O-2-Ada)-Gly-OBzl.—H-Gly-OBzl [from H-Gly-OBzl·Tos (1.35 g, 4.0 mmol) and Na_2CO_3 (0.21 g, 2.0 mmol)] and Boc-Asp(O-2-Ada)-OSu (1.68 g, 3.6 mmol) were dissolved in AcOEt (20 ml) and DMF (3 ml) containing Et_3N (0.21 ml, 1.5 mmol) and the mixture was stirred at room temperature overnight. The solvent was removed and the residue in $CHCl_3$ (3 ml) was applied to a silica gel column (2.1 × 33 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (100 ml each) were collected. The eluate (tubes 3—4) was evaporated to an oily *material* (1.53 g, 83%), $[\alpha]_D^{26}$ −11.8° (*c* 1.0 in MeOH), R_{F1} 0.17, R_{F2} 0.72 (Found: C, 65.6; H, 7.7; N, 5.3. $C_{28}H_{38}N_2O_7$ requires C, 65.4; H, 7.4; N, 5.4%).

Boc-Glu(OBzl)-Asp(O-2-Ada)-Gly-OBzl.—A solution of Boc-Asp(O-2-Ada)-Gly-OBzl (422 mg, 0.82 mmol) in TFA (0.93 ml, 8.2 mmol) containing anisole (0.18 ml, 1.64 mmol) was stored at 0 °C for 30 min and at room temperature for 30 min. TFA was removed by evaporation and the residue was dried (KOH) *in vacuo*. The resultant H-Asp(O-2-Ada)-Gly-OBzl·TFA and Boc-Glu(OBzl)-ONp (376 mg, 0.82 mmol) were dissolved in AcOEt (10 ml) containing Et_3N (0.23 ml, 1.64 mmol). The mixture was stirred at room temperature for 1 day. The solution was washed with 5% Na_2CO_3, 10% citric acid, and water, dried (Na_2SO_4), and evaporated. The oily residue in $CHCl_3$ (3 ml) was applied to a silica gel column (2.5 × 30 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (100 ml each) were collected. The eluate (tubes 6—9) was evaporated to leave an oily

residue (450 mg, 75%), $[\alpha]_D^{26}$ −19.0° (*c* 0.5 in MeOH), R_{F2} 0.38, R_{F3} 0.88 (Found: C, 65.3; H, 6.9; N, 5.55. $C_{40}H_{51}N_3O_{10}$ requires C, 65.5; H, 7.0; N, 5.7%).

Z-Glp-Glu(OBzl)-Asp(O-2-Ada)-Gly-OBzl.—A solution of Boc-Glu(OBzl)-Asp(O-2-Ada)-Gly-OBzl (380 mg, 0.52 mmol) in TFA (0.6 ml, 5.26 mmol) containing anisole (0.2 ml, 1.85 mmol) was kept at 0 °C for 30 min and at room temperature for 1 h. Light petroleum was added and the solution was cooled with ice to give a solid mass, which was isolated by decantation and dried (KOH) *in vacuo*. The resultant powder and Z-Glp-OSu (190 mg, 0.53 mmol) were dissolved in AcOEt (10 ml) containing Et_3N (0.22 ml, 1.6 mmol). The mixture was stirred at room temperature overnight. After concentration to a small volume, ether was added to give *crystals*, which were collected by filtration and washed with EtOH; yield 251 mg (55%), m.p. 148—150 °C, $[\alpha]_D^{29}$ −39.2° (*c* 0.5 in MeOH), R_{F3} 0.63, R_{F4} 0.45 (Found: C, 65.5; H, 6.2; N, 6.4. $C_{48}H_{54}N_4O_{12}$ requires C, 65.6; H, 6.2; N, 6.4%).

H-Glp-Glu-Asp-Gly-OH.—A solution of Z-Glp-Glu(OBzl)-Asp(O-2-Ada)-Gly-OBzl (90 mg, 0.1 mmol) in MSA (1 ml) containing anisole (0.15 ml) was kept at 0 °C for 30 min and at room temperature for 60 min. Addition of ether gave solid material, which was dissolved in water and washed with ether. The water layer was lyophilized to give an MSA salt as an amorphous powder (53.0 mg, 100%), R_{F7} 0.27, R_{F8} 0.50. Amino acid ratios in an acid hydrolysate: Asp:Glu:Gly 1.01:2.01:1.00 (average recovery 99%). This tetrapeptide exhibited a single peak at the same retention time as the tetrapeptide prepared by route A [h.p.l.c. on YMC-PACK A-312 ODS (6.0 × 150 mm); solvent MeCN (0 → 30%, 20 min; →0%, 10 min)–0.1% TFA; flow rate 1 ml min^{-1}; absorbance 220 nm; retention time 18.59 min).

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