Synthesis of β-1 and β-2-Adamantylaspartates and their Evaluation for Peptide Synthesis

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β-1 and β-2-Adamantylaspartates, H-Asp(O-1-Ada)-OH and H-Asp(O-2-Ada)-OH respectively, were synthesized and their properties examined, showing their possible application in solid-phase peptide synthesis when used in combination with the fluoren-9-ylmethoxycarbonyl (Fmoc) group, as an N^{α}-protecting group; both these new protecting groups can suppress aspartimide formation as a side reaction under acidic and basic conditions.

Previously, we reported that the reaction between Boc-Asp(OBzl)-ONp and H-Ser-Ser-Thr-Ser-OMe gave Boc-Aspartimidyl-Ser-Ser-Thr-Ser-OMe in crystalline pure form (60% yield) with only a small amount of the desired pentapeptide.[1] This major side reaction during the synthesis of peptides which contain aspartyl sequences such as Asp-Gly, Asp-Ser, and Asp-His is well known.[2—4] In order to suppress this side reaction the β-cyclopentyl (Cpe)[5], β-cyclohexyl (Chx),[6] β-cycloheptyl (Chp), β-cyclo-octyl (Coc),[7] and β-menthyl (Men)[8] esters of aspartic acid were introduced in peptide synthesis, since the steric nature of the β-protecting groups seemed to play an important role in suppressing the side reaction. These protecting groups need to be stable during peptide synthesis and easily removable in the final step to be of any real use.

We report the synthesis of β-1-adamantyl and β-2-adamantyl aspartates, H-Asp(O-1-Ada)-OH and H-Asp(O-2-Ada)-OH respectively, and their evaluation for peptide synthesis. Boc-Asp-OBzl[9] and Z-Asp-OBzl[10] were esterified with adamantan-1-ol and -2-ol respectively, according to the procedure of Tam *et al.*,[6] with the aid of dicyclohexylcarbodiimide (DCC) and 4-*N*-*N*-dimethylaminopyridine (DMAP) or by the more recent procedure using DCC and *N*-methylimidazole.[11] We obtained the corresponding esters in >70% yield. Hydrogenation of the esters gave 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 quantitatively. The stability and susceptibility of the Ada protecting groups to various acids and bases were examined and the results are summarized in Table 1. The 1-Ada group is easily cleaved by CF_3CO_2H (TFA) but is fairly resistant to 7 M HCl/dioxane. The 2-Ada group is stable to the above acids but is cleaved quantitatively by methanesulphonic acid (MSA)[12] within 5 min at room temperature. Both groups are more stable to bases such as 1 M Na_2CO_3 than the benzyl group (5.3% after 5 min, 16.4% after 20 min and 34.4% after 40 min under the same conditions, see Table 1 footnote a), with the 2-Ada group being slightly more sensitive to base than the 1-Ada group, as expected. So the 2-Ada group is unaffected by treatment with TFA under conditions required for N^{α}-deprotection and both groups are unaffected by 55% piperidine treatment under conditions which easily cleave the Fmoc group from the α-amino group.[13] This introduces the posibility of their application in solid-phase peptide synthesis in combination with the Fmoc group as the N^{α}-protecting group.

Next, in order to test the side reaction mentioned above, two model peptides, Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe (R = 1-Ada or 2-Ada) were prepared, as the reaction of

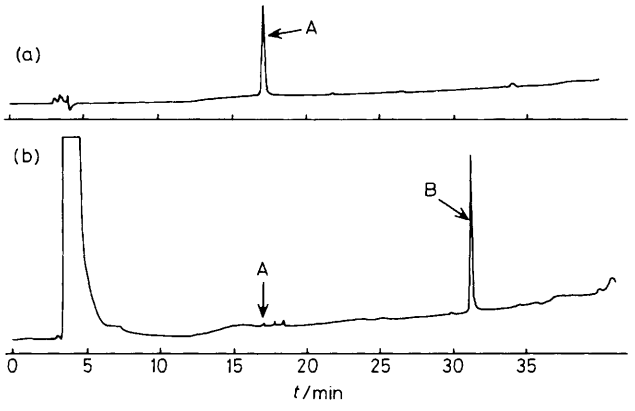
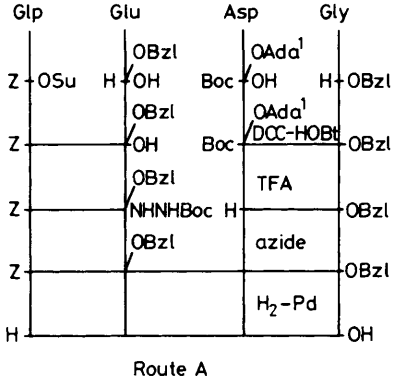


Figure 1. Analytical h.p.l.c. (a) Boc-Aspartimidyl-Ser-Ser-Thr-Ser-OMe (peak A); (b) reaction mixture of Boc-Asp(O-2-Ada)-OSu and H-Ser-Ser-Thr-Ser-OMe in DMF. Peak B is Boc-Asp(O-2-Ada)-Ser-Ser-Thr-Ser-OMe. Column: YMC R-ODS-5 (4.6 × 250 mm); solvent: MeCN (15 → 70%, 30 min, →15%, 10 min)–0.1% TFA; flow rate: 1 ml/min; absorbance: 220 nm.

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

	% Parent amino acid regenerated											
	H-Asp(O-1-Ada)-OH (5.5 mg, 0.02 mmol) *t*/min						H-Asp(O-2-Ada)-OH (5.5 mg, 0.02 mmol) *t*/min					
Conditions	5	20	40	60	120	(24 h)	5	20	40	60	120	(24 h)
1.0 M HCl (2 ml, 100 equiv.)	0	0	0	0	2	17	0	0	0	0	1	
7.0 M HCl/dioxane (0.5 ml, 200 equiv.)	1	8	15	24	35	82	0	0	0	1	1	20
TFA (0.5 ml, 200 equiv.)	100						0	0	0	0	0	
MSA (0.5 ml, 400 equiv.)	100						100					
0.1 M NaOH (2 ml, 10 equiv.)	2	4	9	12	24	89	6	24	40	54	81	100
1.0 M Na_2CO_3 (2 ml, 100 equiv.)[a]	0	1	1	1	3	15	2	3	7	8	14	87
10% $NH_2NH_2 \cdot H_2O$ (2 ml, 200 equiv.)[b]	0	0	0	0	0	9	1	1	2	2	5	14
10% Et_3N/H_2O + dioxane (1.5 ml, 50 equiv.)	0	0	0	0	0	2	0	0	0	1	2	20
10% Et_3N/dioxane (2 ml, 70 equiv.)	0	0	0	0	0	0	0	0	0	0	0	0
10% NMM/H_2O (1 ml, 50 equiv.)[c]	0	0	0	0	0	5	0	0	1	1	2	28
55% piperidine/DMF (2 ml, 70 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] Hydrazide derivative was not observed on t.l.c. [c] NMM: *N*-methylmorpholine.



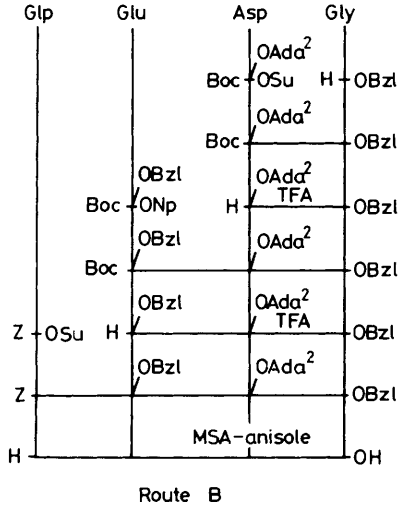


Figure 2. Synthetic routes to tetrapeptide. OAda[1]: β-1-Adamantyl ester; OAda[2]: β-2-Adamantyl ester.

Boc-Asp(OBzl)-ONp and H-Ser-Ser-Thr-Ser-OMe gave Boc-Aspartimidyl-Ser-Ser-Thr-Ser-OMe[1] in 60% yield. This peptide appeared as a single peak separate from those of the desired pentapeptides on h.p.l.c. [$5C_{18}$ column, solvent: MeCN and water containing 0.1% TFA. The % of MeCN was increased from 15% to 70% for 30 min and then decreased to 15% for 10 min]. Boc-Asp(O-1-Ada)-OH and Boc-Asp(O-2-Ada)-OH were coupled with H-Ser-Ser-Thr-Ser-OMe in DMF containing Et_3N (1 equiv.), by DCC-HOBt (HOBt = hydroxybenzotriazole) and OSu active ester methods respectively. After 12 h at room temperature, aspartimide formation in each reaction mixture was examined by t.l.c. and h.p.l.c. In both the above cases, aspartimide formation was negligible in contrast to more than 60% formation in the case of the OBzl protecting group[1] (Figure 1).

Next, to test acid-catalysed cyclization, each derivative [Boc-Asp(OR)-Ser-Ser-Thr-Ser-OMe (R = 1-Ada or 2-Ada)] was exposed to HF at 0 °C, or MSA at room temperature, for 60 min (the latter conditions were previously reported to have a tendency to facilitate the formation of imide derivatives from Asp-containing peptides[14]). After isolation of the deblocked peptides by addition of ether to the reaction mixture, the formation of aspartimide was examined by h.p.l.c. [solvent: MeCN (0 → 10%, 20 min, →30%, 10 min)–0.1% TFA] using H-Aspartimidyl-Ser-Ser-Thr-Ser-OMe prepared from Boc-Aspartimidyl-Ser-Ser-Thr-Ser-OMe by TFA treatment, as a standard sample. Only a negligible amount of aspartimide derivative was formed in each case.

Finally, insulin-releasing tetrapeptide, H-Glp-Glu-Asp-Gly-OH,[15] was prepared using β-1-adamantyl or β-2-adamantylaspartate protection by two different routes (A and B) as shown in Figure 2. There was a 70% yield at each coupling step. In the final step, the protecting groups were removed by catalytic hydrogenation in route A, and MSA containing anisole in route B, to give the desired tetrapeptides. The final products prepared by route A and B exhibited a single peak at the same retention time on h.p.l.c. ($5C_{18}$ column). However, this peptide did not have an effect on insulin release in rats at the same concentration as described previously,[15] presumably owing to the different assay methods used in the two experiments.

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