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innovations 1/08

Overcoming aspartimide formation in Fmoc SPPS

Aspartimide formation, which results from a ring-closure between the β-carboxy side-chain of aspartic acid and the nitrogen of the α-carboxamide, is one of the most frequently encountered side reactions affecting Fmoc SPPS [1-4]. Such aspartimides are very susceptible to base-catalyzed epimerization [5] and readily undergo ring-opening reactions giving mixtures of often inseparable α-, β-aspartyl peptides and α- and β-piperidides (Figure 1) [2-4].

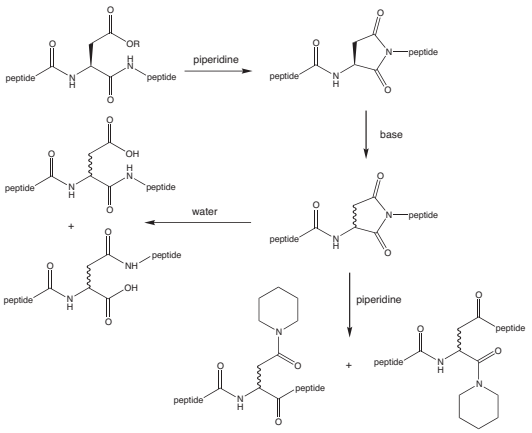

Fig. 1: Mechanism for aspartimide-related by-product formation.

The Asp-Gly sequence is particularly prone to this side reaction, which is estimated to occur to the extent of approximately 0.5% per Fmoc deprotection cycle [6]. The problem is, therefore, most serious in sequences containing more than one site of potential aspartimide formation and in long peptides, as the degree of aspartimide formation is dependent on the total exposure time to piperidine. The only totally effective solution known to date involves reversible protection of the nitrogen of the Asp-Gly amide bond [6, 7, 8], as this completely blocks the ability of the amide nitrogen to attack the β-carboxyl group (Figure 2). In this innovation, we compare the various strategies for the introduction of the backbone amide protection and their efficacy at preventing aspartimide formation.

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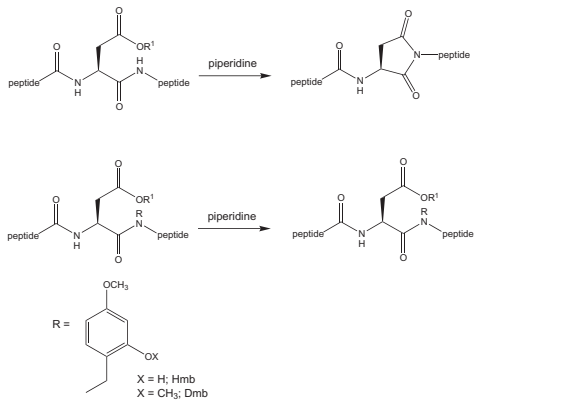

Fig.2: Prevention of aspartimide formation by backbone-amide protection.

Asp-Gly backbone amide protection

Amide bonds in Fmoc SPPS are usually protected with TFA-labile 2,4-dimethoxybenzyl, 2,4,6-trimethoxybenzyl or 2-hydroxy-4-methoxybenzyl groups. Four derivatives are commercially available that can be used to prepare peptide containing a protected Asp-Gly bond (Figure 3). In the case of the amino acid derivatives, Fmoc-(Hmb)Gly-OH **1** [6, 7] & Fmoc-(Dmb)Gly-OH **2**, formation of the Asp-Gly bond requires acylation of a secondary amine on the solid phase, which can be difficult and hard to follow. For this reason, dipeptides Fmoc-Asp(OtBu)-(Hmb)Gly-OH **3** [8] & Fmoc-Asp(OtBu)-(Dmb)Gly-OH **4** have been developed where the problematic tertiary amide bond is preformed.

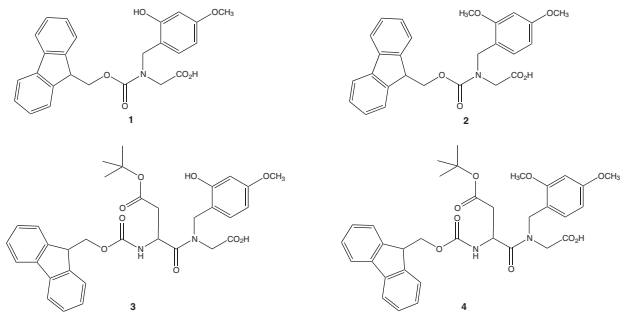

Fig.3: Available derivatives for Asp-Gly amide protection.

In our study [9], derivatives **2**, **3** and **4** were selected and compared in the synthesis of the known aspartimide-prone sequence H-Val-Lys-Asp-Gly-Tyr-Leu-NH_2.

Synthesis of H-Val-Lys-Asp-Gly-Tyr-Leu-NH_2

Peptidyl resins **5-8** (Table 1) were prepared manually on Rink Amide MBHA resin using the conditions shown in Table 2. Completeness of coupling reactions was monitored using TNBS and chloranil tests where appropriate.

Table 1: Peptidyl resins prepared in this study.

	Peptidyl resin	Reagents used for introduction of Asp-Gly
5	H-Val-Lys(Boc)-Asp(OtBu)-Gly-Tyr(tBu)-Leu-Rink Amide MBHA	Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH
6	H-Val-Lys(Boc)-Asp(OtBu)-(Dmb)Gly-Tyr(tBu)-Leu-Rink Amide MBHA	Fmoc-Asp(OtBu)-OH, Fmoc-(Dmb)Gly-OH
7	H-Val-Lys(Boc)-Asp(OtBu)-(Hmb)Gly-Tyr(tBu)-Leu-Rink Amide MBHA	Fmoc-Asp(OtBu)-(Hmb)Gly-OH
8	H-Val-Lys(Boc)-Asp(OtBu)-(Dmb)Gly-Tyr(tBu)-Leu-Rink Amide MBHA	Fmoc-Asp(OtBu)-(Dmb)Gly-OH

Notable differences were observed in the ease of assembly of the different peptides, depending on which derivatives were used for the incorporation of the Asp-Gly sequence. In the synthesis of peptide **6**, double coupling was required to achieve complete addition of the Asp residue. Incorporation of Fmoc-Asp(OtBu)-(Hmb)Gly-OH into peptide **7** took 1.5 hours compared to 30 minutes for Fmoc-Asp(OtBu)-(Dmb)Gly-OH in peptide **8**. This difference could be attributed to the propensity of the former to form a lactone from the carboxyl and phenolic hydroxyl groups. Fmoc-Asp(OtBu)-(Dmb)Gly-OH, therefore, seems the most efficient tool for the incorporation of Asp-Gly backbone amide protection.

*Table 2: General reaction conditions used to prepare resins **5-8**.*

	Conditions
Resin	RinkAmide MBHA resin
Coupling	Fmoc-Aaa-OH/TBTU/DIPEA (2:2:4), 30 min
Deblock	20% Piperidine in NMP (2 x 15 min)
Cleavage	TFA/water/TIS (95:2.5:2.5) for 3 h

Aspartimide formation

The crude cleaved products obtained from peptidyl resins **5-8** were analyzed and characterized by LC-MS. HPLC profiles of the crude peptides are shown in Figures 4-7. As expected, the product from resin **5**, which was prepared using standard Fmoc-amino acid derivatives, contained a considerable quantity of aspartimide by-products. Resins **6** & **8** made utilizing Dmb-backbone protection gave products of excellent quality, with negligible aspartimide formation. In the case of resin **7**, which was prepared using Hmb-backbone protection, little aspartimide formation was observed, but the product did contain a significant quantiy of a yet unidentified material (Table 3).

*Table 3: Purities of products obtained from resins **5-8**.*

Peptidyl resin	Purity (%)	Aspartimide (%)
5 (Fig. 4)	74	14
6 (Fig. 5)	91	0.5
7 (Fig. 6)	82	1.7 + 6 (unidentified)
8 (Fig. 7)	84	0.8

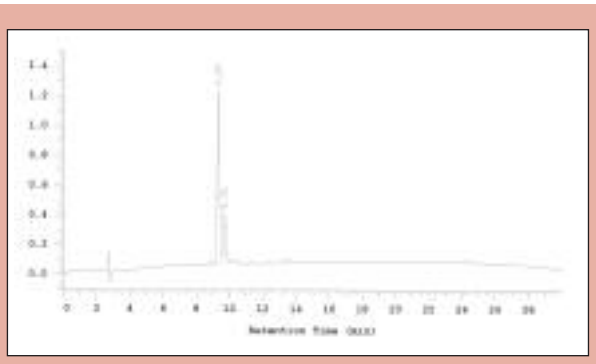

*Fig. 4: HPLC profile of crude peptide from resin **5** prepared using standard Fmoc amino acid building blocks.*

Conclusions

Dmb(Gly) appears to offer excellent protection against aspartimide formation. Thus, Fmoc-Asp(OtBu)-(Dmb)Gly-OH, with its ease of introduction under standard coupling methods, is the best choice of preventing aspartimide formation in peptides containing the Asp-Gly sequence.

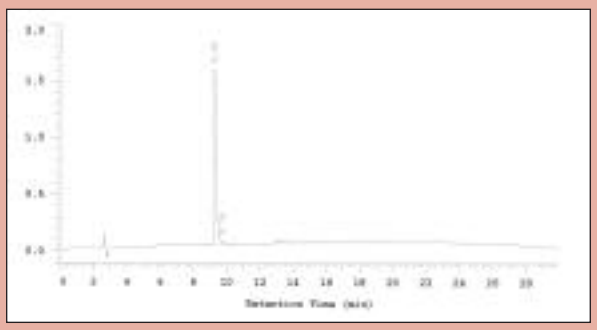

*Fig. 5: HPLC profile of crude peptide from resin **6** prepared using Fmoc-(Dmb)Gly-OH.*

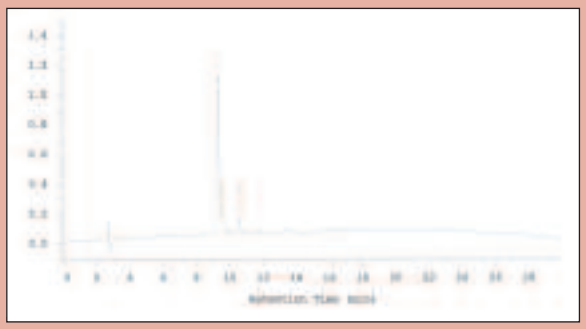

*Fig. 6: HPLC profile of crude peptide from resin **7** prepared using Fmoc-Asp(OtBu)-(Hmb)Gly-OH.*

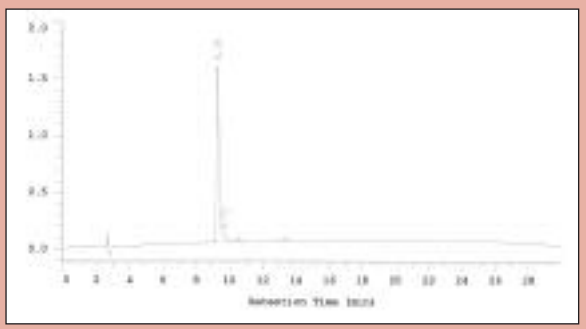

*Fig. 7: HPLC profile of crude peptide from resin **8** prepared using Fmoc-Asp(OtBu)-(Dmb)Gly-OH.*

Ordering information

04-12-1282	Fmoc-Asp(OtBu)-(Dmb)Gly-OH	1 g 5 g
04-12-1268	Fmoc-(Dmb)Gly-OH	1 g 5 g
01-64-0037	Rink Amide MBHA resin (100-200 mesh)	1 g 5 g 25 g

Other Dmb-dipeptides

04-12-1265	Fmoc-Ala-(Dmb)Gly-OH	1 g 5 g
04-12-1266	Fmoc-Gly-(Dmb)Gly-OH	1 g 5 g
04-12-1280	Fmoc-Ile-(Dmb)Gly-OH	1 g 5 g
04-12-1294	Fmoc-Leu-(Dmb)Gly-OH	1 g 5 g
04-12-1283	Fmoc-Val-(Dmb)Gly-OH	1 g 5 g

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