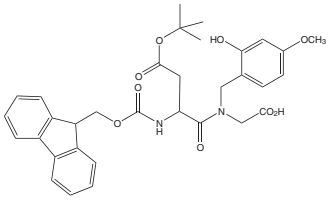
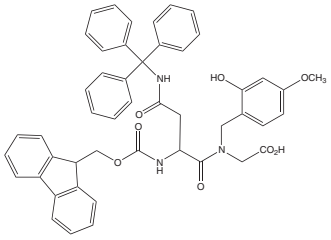
novabiochem
innovations 1/03

NEW Derivatives for overcoming aspartimide formation

Fmoc-Asp(OtBu)-(Hmb)Gly-OH



Fmoc-Asn(Trt)-(Hmb)Gly-OH



The most frequently encountered side reaction affecting Asp residues during solid phase synthesis is aspartimide formation, resulting from a ring-closure between the nitrogen of the α-carboxy amide bond and the β-carboxy side-chain, with loss of the ester protecting group (Figure 1) [1, 2]. It is a particularly serious problem in Fmoc SPPS as cyclization is promoted by strong bases such as piperidine and DBU used to effect Fmoc group removal [3 - 6].

Aspartimides are very susceptible to base-catalyzed epimerization [7] and readily undergo ring-opening reactions, leading to the formation of a variety of by-products. Attack by water yields predominantly the β-aspartyl peptide, the presence of which can often be overlooked as it has an identical mass to the desired α-isomer and frequently co-elutes on HPLC. Ring-opening by piperidine gives a mixture of α- and β-piperidides, which are characterized in MS as peaks at 67u greater than that of the expected peptide.

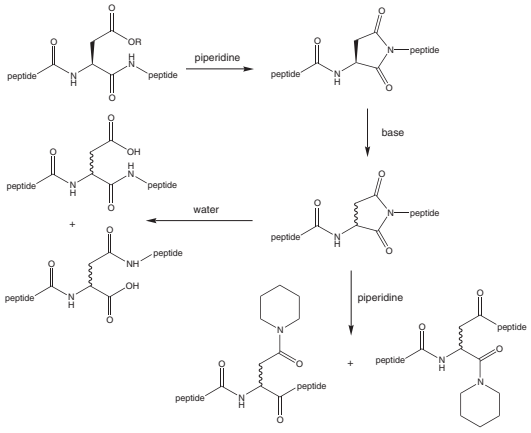


Fig. 1: Mechanism for aspartimide-related by-product formation.

The Asp-Gly sequence is particularly prone to aspartimide formation, which is estimated to occur to the extent of approximately 0.5% per Fmoc deprotection cycle [8]. 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. It is also exacerbated by the use of DBU as this base has been shown to more effectively promote aspartimide formation than piperidine.

Of the various approaches that have been advocated to overcome this problem, such as the addition of dinitrophenol or HOBt to the piperidine solution [6], only masking of the Asp-Gly amide bond with Hmb offers complete protection [8, 9]. However, the acylation of Hmb derivatives can be problematic, and the reaction is difficult to follow and often requires nonstandard

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acylation conditions. The solution is to use Fmoc-Asp(OtBu)-(Hmb)Gly-OH [10]. This derivative is introduced using standard coupling methods, extending the peptide chain by two residues in one step. Its use has also the additional advantage of overcoming aggregation during chain extension.
Coupling can be a little sluggish, however, owing to lactone formation between the phenolic hydroxyl and carboxyl group. Addition of excess HOBt and HOSu accelerates the reaction by ring-opening the lactone to the more reactive OBt or OSu ester: for example, 1.2 eq. of Fmoc-Asp(OtBu)-(Hmb)Gly-OH activated with PyBOP/DIPEA in the presence of 5 eq. HOSu and 2 eq. of HOBt effected complete coupling to Phe-Wang resin in 3 hours.
The analogous asparagine derivative, Fmoc-Asn(Trt)-(Hmb)Gly-OH, is also available. This derivative can be used in the same manner as a pseudoproline dipeptide to prevent aggregation.

04-12-1235	**Fmoc-Asp(OtBu)-(Hmb)Gly-OH**	1 g
NEW		5 g
04-12-1234	**Fmoc-Asn(Trt)-(Hmb)Gly-OH**	1 g
NEW		5 g

Applications

The benefits of using Fmoc-Asp(OtBu)-(Hmb)Gly-OH are exemplified through the synthesis of the following peptide.

Application 1: Synthesis of H-Val-Lys-Asp-Gly-Tyr-Leu-NH_2

H-Val-Lys(Boc)-Asp(OtBu)-Gly-Tyr(tBu)-Leu-NovaSyn® TGR resin was prepared automatically using a NovaSyn® Crystal peptide synthesizer on NovaSyn® TGR resin. All acylation reactions were carried out using a 5-fold excess of Fmoc-amino acid activated with 1 eq. of PyBOP® in the presence of 1 eq. of HOBt and 2 eq. of DIPEA. A coupling time of 60 min was used throughout. The synthesis of H-Val-Lys(Boc)-Asp(OtBu)-(Hmb)Gly-Tyr(tBu)-Leu-NovaSyn® TGR resin was also carried out in an identical manner except Fmoc-Asp(OtBu)-(Hmb)Gly-OH was used to introduce the Asp-Gly dipeptide. Prior to cleavage, both peptidyl resins were treated with 20% piperidine in DMF for 24 h. After this time, the peptidyl resins were treated with TFA/ TIS /water 95:2.5:2.5 (5 ml) for 3 h. after which time the peptide was isolated in the usual manner by evaporation and ether precipitation.
The product obtained using standard conditions was characterized by LC/ESI-MS (Figure 2) as a complex mixture of aspartimide and piperidide-modified by-products, whereas the product from the synthesis using Fmoc-Asp(OtBu)-(Hmb)Gly-OH was essentially free of these impurities.

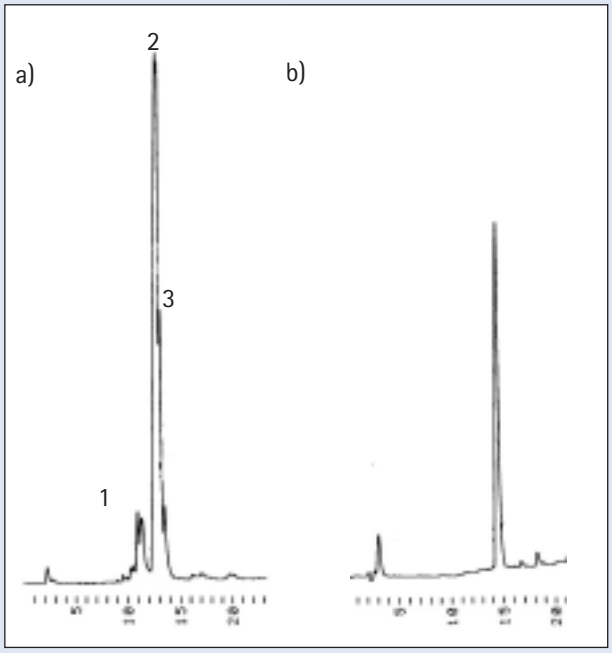

Figure 2: .a) HPLC profile of crude H-Val-Lys-Asp-Gly-Tyr-Leu-NH_2 prepared using standard amino acid building blocks after treatment with 20% piperidine in DMF for 24 h. 1: α- and β-aspartylpeptides; 2: aspartimide peptide; 3: piperidinylalaninyl-peptides; b) peptide prepared using Fmoc-Asp(OtBu)-(Hmb)Gly-OH after treatment with 20% piperidine in DMF for 24 h.

References

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Merck Biosciences AG · Switzerland
Weidenmattweg 4
4448 Läufelfingen
Phone +41 (62) 285 2525
Fax +41 (62) 285 2520

www.novabiochem.com

Merck Biosciences AG · Switzerland
Weidenmattweg 4
4448 Läufelfingen
Phone +41 (62) 285 2525
Fax +41 (62) 285 2520

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