Peptides

The Wave of the Future

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Systematic Investigation of the Aspartimide Problem

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Introduction

Aspartimide formation is one of the best-documented side reactions in peptide synthesis. Even bulky side-chain protecting groups such as OtBu do not prevent this undesired reaction. In Fmoc/tBu-based SPPS, the repetitive piperidine treatments needed for Fmoc removal lead to aspartimide formation and further by-products. Since the combination Asp-Gly represents the worst-case scenario, the hexapeptide Val-Lys-Asp-Gly-Tyr-Ile (**I**) [1] was used in the present work to investigate parameters influencing aspartimide formation (Fmoc-cleavage conditions, nature of Asp side chain protection). Based on the idea of backbone-protection [2], Fmoc-Asp(OtBu)-(Hmb)-Gly-OH was also synthesised and included in this study.

Results and Discussion

For Asp side chain protection, the following groups were applied for synthesis of **I** as their Fmoc-derivatives: OtBu, β-3-methylpent-3-yl ester (OMpe) [3], 4-pyridyl-diphenyl-methyl ester (OPyBzh) [4], the bicyclic ortho-ester 4-methyl-2,6,7-trioxa-bicyclo-[2,2,2]-octane (OBO) [4] and, as already mentioned, the combination OtBu side chain protection plus Hmb-backbone protection. In the first step, several potential by-products, VKdGYI, VKd(GYI), VKD(GYI), VKD(piperidide)GYI and VKD(GYI)-piperidide, resulting from the opening of the aspartimide cycle, were independently synthesized. Furthermore, RP-HPLC-optimization of these potential contaminants was carried out to properly resolve and quantify the different side products.

The OPyBzh-protecting group had ideal TFA lability. Unfortunately, high levels of aspartimide and piperidides were detected following synthesis of **I**. Another new derivative, the orthoester protected Asp derivative (OBO-protection), was designed to completely suppress nucleophilic attack at the β-carboxy group. Surprisingly, α-piperidide was generated during synthesis and, in addition, large quantities of aspartimide were observed during the second stage of OBO removal, which consists of the saponification under basic conditions. Therefore, the disappointing results obtained on OPyBzh- and OBO-protection were not included in Table 1.

Table 1. HPLC-analysis of crude products (Bakerbond C18 300 Å, phosphate buffer pH 2.3, CH_3CN as modifier).

Protection	Base[a]	Product (%)	D/L-Aspartimide (%)	L-α-Piperidide (%)	L-β-Piperidide (%)
OtBu	Pip.	89.1	3.0	1.5	<0.3[b]
OMpe	Pip.	93.9	0.7	<0.3[b]	<0.3[b]
OtBu/Hmb	Pip.	94.0	<0.3[b]	<0.3[b]	<0.3[b]
OtBu	DBU	52.1	21.8	9.4	0.6
OMpe	DBU	83.0	7.8	1.9	<0.3[b]
OtBu/Hmb	DBU	94.1	<0.3[b]	<0.3[b]	<0.3[b]

[a] Pip.: Piperidine/DMF 1 : 4, DBU: DBU/Piperidine/DMF 1 : 20 : 79; [b] below detection limit.

Two Fmoc-cleavage procedures, the standard protocol piperidine/DMF (1 : 4) and 1% DBU in piperidine/DMF (1 : 4), and different protecting groups for the Asp residue in model peptide **I** (see Table 1) were employed to study aspartimide formation. Peptide **I**, synthesized according to the various strategies indicated above, was obtained after TFA assisted cleavage, and the crude products were subsequently analysed by HPLC.

In fact, Fmoc-removal in the presence of DBU worsened the effects already observed in the case of the standard piperidine treatment. However, no significant amounts of β-peptide were detected. In the case of OtBu-protection, upon DBU treatment an as yet undefined component (5.8%) was observed in addition to the known compounds. The OMpe-protecting group showed a significant improvement with respect to aspartimide formation when compared to regular OtBu-protection. Most interestingly, if the Hmb-backbone protection approach was followed, neither under standard conditions nor in the presence of DBU, aspartimide or related side product was observed (detection limit 0.3%).

Conclusions

This systematic investigation clearly showed that in our test system, no detectable amounts of aspartimide were formed if Hmb-backbone protection was applied in addition to standard OtBu-protection of the Asp side chain. However, the synthesis of all different Hmb protected amino acid derivatives followed by their incorporation into dipeptides would be quite labourious. Therefore, taking into account the markedly improved properties of Mpe-protection compared to the standard OtBu-group, this recently described variant should be considered for sequences prone to aspartimide formation.

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