Systematic Investigation of the Aspartimide Problem

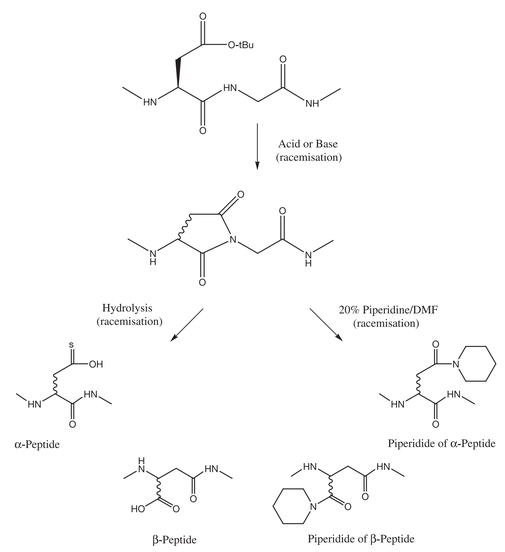
M. Mergler, F. Dick, B. Sax, P. Weiler and T. Vorherr

BACHEM AG, Hauptstr. 144, CH-4416 Bubendorf, Switzerland

Introduction

Aspartimide formation, catalysed by acids and by bases, represents one of the best-documented side reactions in peptide synthesis. Even bulky β-carboxy side-chain protecting groups such as OtBu do not prevent this undesired reaction (see Scheme 1). Since the combination Asp-Gly represents the worst-case scenario, the hexapeptide fragment Val-Lys-Asp-Gly-Tyr-Ile (**I**) has been used to study parameters influencing aspartimide formation [1]. We have also decided to use this simple model peptide **I** to study the effects of different Fmoc-cleavage conditions and alternative Asp side chain protection. Based on the recently developed backbone-protection [2], the derivative Fmoc-Asp(OtBu)-(Hmb)-Gly-OH was also synthesised and included in this study.

Scheme 1: Aspartimide and Related Reaction Products



Results and Discussion

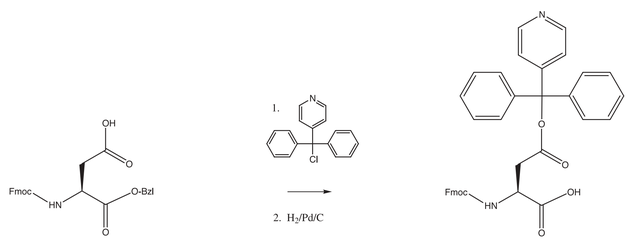
For Asp side chain protection the following protecting groups were applied for synthesis of **I** as their Fmoc-derivatives: *OtBu*, β-*3-methylpent-3-yl ester (OMpe)* [3], *4-pyridyl-diphenyl-methyl ester (OPyBzh*) (synthesis see Scheme 2), the bicyclic orthoester *4-methyl-2,6,7-trioxabicyclo-[2,2,2]-octane (OBO)* (synthesis see Scheme 3) [4] and as already mentioned the combination OtBu side chain protection plus Hmb-backbone protection.

The new OPyBzh-protecting group had ideal properties with respect to lability, e.g. it is cleaved by 2% TFA in DCM. Unfortunately, high levels of aspartimide and piperidides were detected following synthesis of **I**. Detailed analysis, performed on the Fmoc-cleavage reaction of the fully protected fragment Fmoc-Asp(OPyBzh)-Gly-Tyr(tBu)-Ile-OH, revealed that aspartimide was formed instantaneously to a large extent upon treatment with 20% piperidine/DMF.

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.

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 (see Figure 1).

Scheme 2: Synthesis of Fmoc-Asp(OPyBzh)-OH



Scheme 3: Synthesis of Fmoc-Asp(OBO)-OH

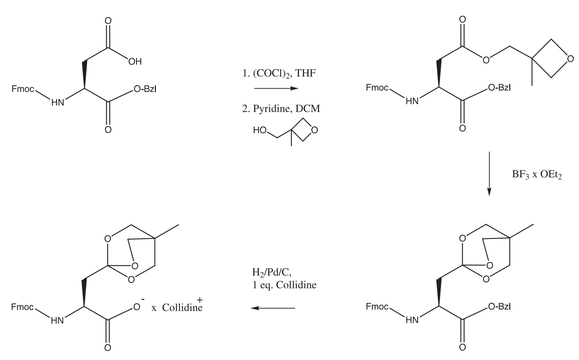
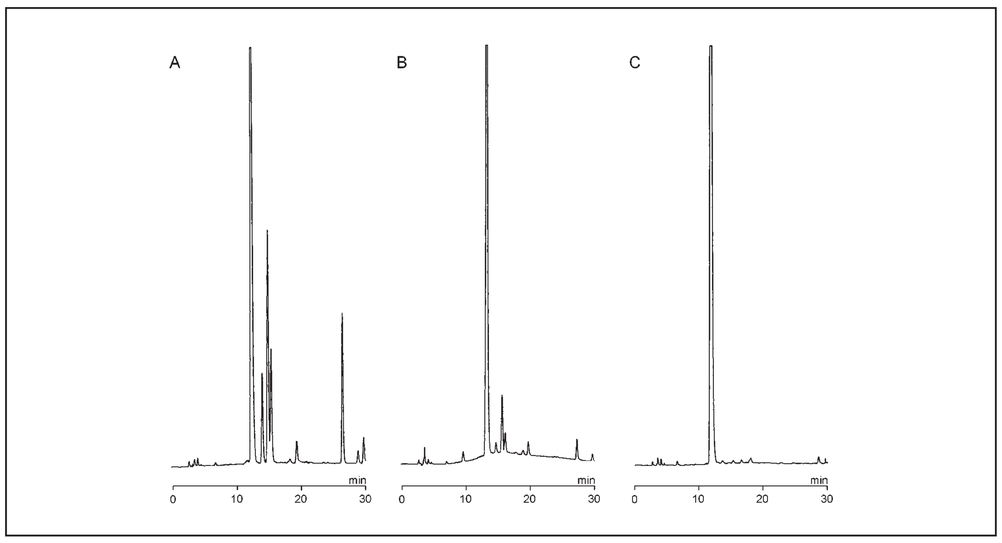


Table 1. HPLC-analysis of crude products (Bakerbond C_{18} 300Å, A: 4% CH_3CN in phosphate buffer pH 2.3, B: 60% CH_3CN, 40% phosphate buffer pH 2.3; 5 to 35% B in 45 min; flow: 1 ml/min; detection: 220 nm)

Protection	Base [a]	Product (%)	D/L-Aspartimide (%)	L-α-Piperidide (%)	L-β-Piperidide (%)
OtBu	Piperidine	89.1	3.0	1.5	< 0.3 [b]
OMpe	Piperidine	93.9	0.7	< 0.3 [b]	< 0.3 [b]
OtBu + Hmb	Piperidine	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] Piperidine: Piperidine/DMF 1:4, DBU: DBU/Piperidine/DMF 1:20:79; [b] below detection limit

Figure 1



Analytical HPLC-chromatograms of crude products obtained after synthesis of peptide **I** using 1% DBU for Fmoc-cleavage (for detailed conditions, please refer to Table 1).
A: Derivative applied for synthesis: Fmoc-Asp(OtBu)-OH
B: Derivative applied for synthesis: Fmoc-Asp(OMpe)-OH
C: Derivative applied for synthesis: Fmoc-Asp(OtBu)-(Hmb)-Gly-OH.

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.

References

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[2] J. Offer, M. Quibell and T. Johnson, J. Chem. Soc. Perkin Trans. I, (**1996**), 175.
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[4] M.A. Blaskovich, G. Evindar, N.G.W. Rose, S. Wilkinson, Y. Lao and G.A. Lajoie, J. Org. Chem. 63, (**1998**), 3631.