Base-Induced Glutarimide Formation During Fmoc-Based SPPS

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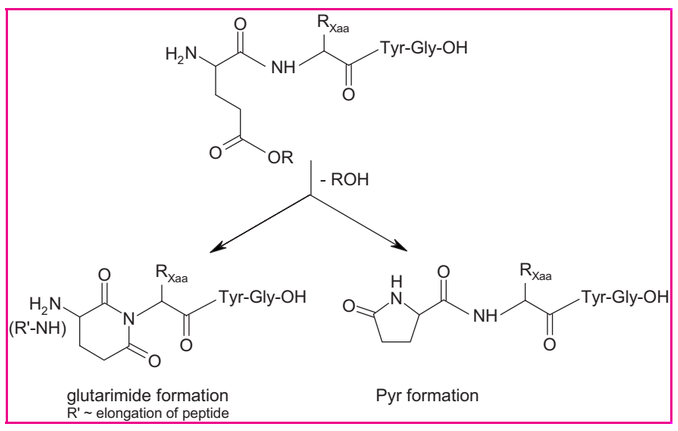
Introduction

The base-catalyzed aspartimide formation of Asp derivatives is one of the most notorious side reactions in Fmoc-based SPPS. The homologous Glu derivatives are far less reactive. Two modes of cyclization could be envisaged for Glu(OR), generating either the glutarimide or a pyrrolidinone. As with aspartimide formation, the extent of cyclization should be determined by the nature of the preceding amino acid, the type of γ-carboxy protecting group, and the conditions of Fmoc cleavage.



Reports describing side reactions of a γ-carboxy group during Fmoc-based SPPS have rarely appeared in the literature [1,2]. Recently, during our work on the aspartimide problem, we also studied the **Glu[3]**-analogues of **H-Val-Lys-Asp[3]-Xaa-Tyr-Ile-OH**, a well-established model peptide for studying aspartimide formation [3,4]. The Asp(OR)-Xaa motifs with Xaa = **Gly, Arg(Pbf)** and **Cys(Acm)** showed a pronounced tendency towards aspartimide formation [4-6]. Moreover, unhindered β-carboxy protecting groups, R =, e.g., allyl or benzyl, allowed rapid and complete Asp cyclization. Thus, the bulky *t*-butyl ester (**OtBu**), the highly acid-labile 2-phenylisopropyl ester (**OPp**) and the unhindered allyl ester (**OAll**) were chosen for Glu γ-carboxy protection. The repetitive Fmoc cleavage steps during SPPS mean frequent treatments and prolonged contact with bases. Two different cocktails and different exposure durations were chosen to mimic these treatments: **piperidine/DMF 1:4 (I)** and **DBU/piperidine/DMF 1:20:79 (II).** Hence, the Glu(OR)-Xaa motifs may also react under these conditions. In case of Glu-HmbGly (Hmb = 2-hydroxy-4-methoxybenzyl), prevention of glutarimide formation may also be expected (as in the Asp-HmbGly motif [5,7]), though the influence of Hmb on Pyr formation cannot be predicted.

Results and Discussion

The model peptides **Val-Lys-Glu-Xaa-Tyr-Ile** and **Pyr-Xaa-Tyr-Ile** were synthesized on Wang resin employing tBu and Boc for side-chain protection. Couplings were performed with TBTU/collidine in DMF. The more reactive TATU/DIPEA had to be used for couplings to *N*-terminal HmbGly. Fmoc was split off with **I** (two treatments, 5 and 10 min) up to the incorporation of Fmoc-Glu(OR). Then Fmoc was removed with either **I** or **II**. Eventually, the peptides were split from the resin by a one-hour treatment with 95% aq TFA (conditions leaving the allyl ester intact) and analyzed by RP-HPLC and ESMS.

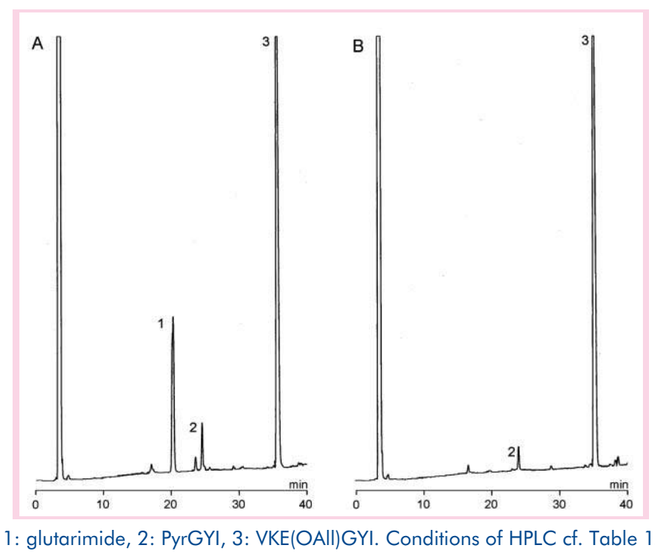
Analytical HPLC-results of the **Gly**-containing peptides are compiled in **Table 1**, results of the **Cys(Acm)-**containing products are listed in **Table 2. Table 3** contains data of the series with Xaa = **Arg.** Our results show that Glu(OtBu) withstands even prolonged exposition to DBU. Glu(OPp) is only slightly less stable, whereas Glu(OAll) turns out to be labile, especially towards DBU. The cyclization of Glu(OAll) yielding glutarimide is favored in the vicinity of Gly, whereas Arg(Pbf) promotes the formation of Pyr. Both products are generated from the Cys(Acm)-containing peptide in the presence of DBU. Moreover, the data show the stabilizing effect of Hmb backbone protection, even during prolonged contact with DBU. As expected, glutarimide formation is suppressed. Additionally, less Pyr is generated.
The HPLC-chromatograms of VKE(OAll)GYI obtained after prolonged treatment with piperidine shown in **Figure 1** clearly demonstrate this beneficial effect of Hmb backbone protection. The HPLC-profiles in **Figure 2** show that in case of Xaa = Arg OPp is the selectively cleavable γ-carboxy protecting group of choice due to its superior stability against bases.

Table 1: Formation of glutarimide and pyrrolidinone during the synthesis of VKEGYI. HPLC-analysis of crude products (Bakerbond C_{18} 300 Å; A: 5% CH_3CN, B: 60% CH_3CN in triethylammonium phosphate buffer pH 2.3; 5 to 35%B in 45 min; flow 1 ml/min; detection at 220 nm).

E(OR) R =	base[b]	time (min)	VKEGYI (%)	PyrGYI (%)	glutarimide (%)
tBu	I	45	92.8	trace	trace
	I	225	92.1	"	0.5
	II	45	90.2	"	0.2
	II	225	89.2	0.2	0.9
	II	405	88.8	0.3	1.6
Pp	I	45	90.5	0.3	trace
	I	225	89.5	0.3	0.2
	II	45	87.3	0.4	0.3
	II	225	87.1	0.6	1.6
All[a]	I	45	89.1	2.7	3.1
	I	225	72.5	3.2	17.0
	I	405	65.0	3.3	23.5
	II	45	50.8	8.1	31.7
	II	225	trace	8.4	73.0
All/	I	45	91.7	1.7	trace
HmbGly[a]	I	225	90.0	1.8	0.2
	II	45	88.1	4.1	0.3
	II	225	86.9	4.4	0.9

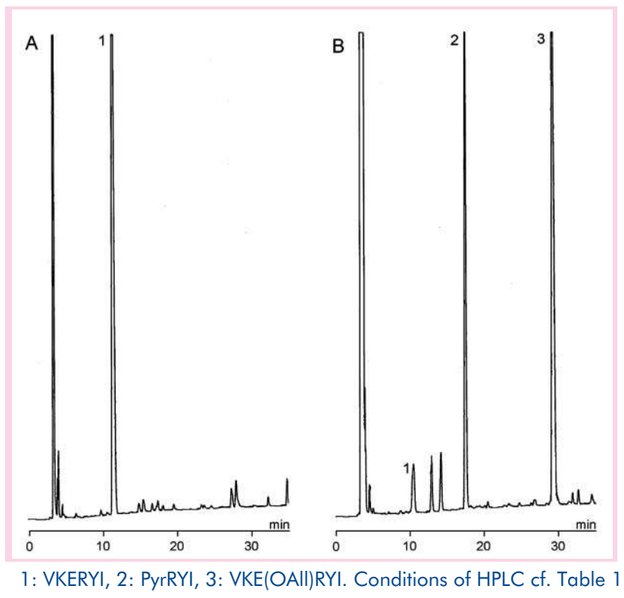
[a] product: VKE(OAll)GYI.
[b] I: piperidine/DMF 1:4, II: DBU/piperidine/DMF 1:20:79.

Figure 1. HPLC-profiles of VKE(OAll)GYI obtained by standard methods *(A)* as well as by applying Hmb backbone protection *(B)*. Contact time with piperidine/DMF 225 min.



1: glutarimide, 2: PyrGYI, 3: VKE(OAll)GYI. Conditions of HPLC cf. Table 1.

Figure 2. HPLC-profiles of VKERYI obtained by SPPS applying OPp for γ-carboxy protection *(A)* and VKE(OAll)RYI *(B)*. Contact time with DBU/piperidine/DMF 225 min.



1: VKERYI, 2: PyrRYI, 3: VKE(OAll)RYI. Conditions of HPLC cf. Table 1.

Table 2: Formation of by-products during synthesis of VKEC(Acm)YI. HPLC-analysis of crude products (conditions s. Table 1)

E(OR) R =	base[b]	time (min)	VKEC/Am)YI (%)	PyrC(Acm)YI (%)	glutarimide (%)	unidentified by-prod.(%)
tBu	I	45	80.8	trace	trace	8.6
	I	225	78.7	"	"	9.0
	II	45	77.8	"	"	9.2
	II	225	75.4	"	"	9.9
Pp	I	45	84.6	"	"	6.2
	I	225	80.1	"	"	7.9
	II	45	80.9	0.2	"	5.6
	II	225	80.7	0.3	0.6	6.1
	II	405	79.3	0.3	1.0	5.8
All[a]	I	45	80.6	2.2	0.2	8.1[c]
	I	225	76.6	2.1	2.0	9.5
	II	45	68.2	10.1	13.0	7.1
	II	225	34.9	13.2	24.3	6.3

[a] product: VKE(OAll)C(Acm)YI.
[b] I: piperidine/DMF 1:4, II: DBU/piperidine/DMF 1:20:79.
[c] probably the allyl derivative of the by-product.

Table 3: Formation of pyrrolidinone during synthesis of VKERYI. HPLC-analysis of crude products (conditions s. Table 1)

E(OR) R =	base[b]	time (min)	VKERYI (%)	PyrRYI (%)
tBu	I	45	91.6	trace
	I	225	91.6	"
	II	45	83.6	"
	II	225	81.7	"
Pp	I	45	91.6	"
	I	225	90.8	"
	II	45	81.4	"
	II	225	76.5	0.4
All[a]	I	45	84.3	6.3
	I	225	84.2	6.9
	II	45	62.5	23.7
	II	225	49.5	28.2
	II	405	42.0	35.0[c]

[a] product: VKE(OAll)RYI.
[b] I: piperidine/DMF 1:4, II: DBU/piperidine/DMF 1:20:79.
[c] additionally, a small amount of glutarimide could be detected.

Conclusion

The tBu ester is a most reliable γ-carboxy protecting group for Glu in Fmoc-based SPPS withstanding -as shown here- even prolonged contact with DBU. When replacing OtBu by the orthogonal OAll, Glu-derived by-products, glutarimide and/or Pyr, were formed in considerable quantities in our model peptides VKEXaaYI. Especially cyclization yielding glutarimide was promoted by prolonged treatment with DBU. These side reactions could be suppressed nearly completely by applying Glu(OPp) or, in case of the Glu-Gly motif, Hmb backbone protection, which did not prevent Pyr formation completely. Because the application of Hmb may create other synthetic problems (except for unhindered amino acids, e.g., Gly, Ala...) [6,7], OPp represents an excellent choice, if the γ-carboxy protecting group has to be removed selectively for postsynthetic modifications of the Glu side chain in a base-sensitive vicinity.
Fmoc-Glu(OPp)-OH (B-2500) is available at Bachem.

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