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Formation of Fmoc–β-alanine during Fmoc-protections with Fmoc–OSu

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Abstract: During the Fmoc-protection of H–α-Me–Val–OH, an unknown side product was found and isolated. The characterization using various analytical methods led unambiguously to the result that Fmoc–β-Ala–OH was formed during the reaction. The reagent Fmoc–OSu was proven to be the source of Fmoc–β-Ala–OH, following a mechanism that involved many deprotonation and elimination steps and a Lossen-type rearrangement as key sequence. The impurity Fmoc–β-Ala-OH was found in a variety of reactions in which Fmoc–OSu was applied, either in the reaction mixture or as a contamination of the crude product. Purification of the Fmoc–amino acid derivatives from this impurity incurred high costs and significant reductions in yield. Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Fmoc–β-Alanine impurity; Fmoc–OSu; Lossen-rearrangement; amino acid derivative; peptide synthesis

INTRODUCTION

During the *N*-protection of H–α-Me–Val–OH with Fmoc–OSu, an unexpected side-product was formed. All facile separation methods (like extraction or crystallization) to purify the product failed, thus Craig's counter-current distribution was applied for purification. While the desired product was obtained in 85% yield, the unknown compound was isolated, submitted to various analyses and finally identified as Fmoc–β-Ala–OH. Herein, we report on the origin of this side product, on impacts on Fmocylations with Fmoc–OSu in general and on measures to avoid its formation.

RESULTS AND DISCUSSION

From a theoretical point of view the reagent Fmoc–OSu, or rather its excess applied, is the only possible source for the formation of Fmoc–β-Ala. According to the literature, we envision a Lossen type mechanism [1] to change the C4-chain in the succinimide cycle to the C3-chain in H–β-Ala–OH (Scheme 1 for a general formulation of the Lossen rearrangement).

A putative mechanism under basic conditions can be proposed starting with an opening of the succinimide and subsequent Lossen rearrangement. The resulting isocyanate forms an unstable carbaminate and decarboxylates to H–β-Ala–OH, which subsequently is Fmoc-protected by an additional equivalent Fmoc–OSu. The proposed pathway is shown in Scheme 2.

To prove the concept of the Lossen rearrangement, Fmoc–OSu was stirred with sodium carbonate in acetonitrile and water, simulating the reaction conditions without the presence of H–α-Me–Val–OH. After extractive work-up, crystallization and purification, Fmoc–β-Ala–OH was obtained in 42% yield. The identity of the formed product was confirmed by TLC, HPLC, NMR, and mass spectroscopy and compared with authentic reference material.

As a negative control, the same test reaction was carried out in a neutral environment. Under these conditions no Fmoc–β-Ala–OH could be detected by TLC in the reaction mixture. The same result was observed when Fmoc–OSu was exchanged by Fmoc–Cl. These three experiments prove that Fmoc–β-Ala–OH is unambiguously derived from Fmoc–OSu and that a basic environment is mandatory for the rearrangement reaction to occur (results summarized in Table 1).

Similar problems, compared to the synthesis of Fmoc–α-Me–Val–OH, were encountered when Fmoc-protection of H–tBu–Gly–OH and H–β-Cyclopropyl–Ala–OH was carried out (Table 2).

In the first case, though only 2% Fmoc–β-Ala–OH was identified in the crude product after isolation, a recrystallization that cut the yield in half had to be performed to get rid of the impurity. In the second case, 9% of the contaminating Fmoc–β-Ala–OH was found in the crude product. In order to reduce the amount of this impurity to 0.5% two recrystallizations were necessary, which led to an enormous decrease in the yield from 99 to 43%. Since we were not able to remove Fmoc–β-Ala–OH entirely from the product, we tested two batches from commercial suppliers with respect to their content of impurity and found that both samples contained about 1% of Fmoc–β-Ala–OH.

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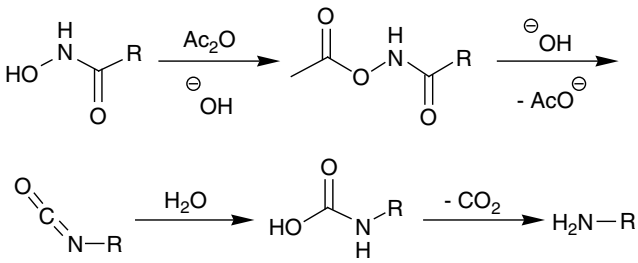


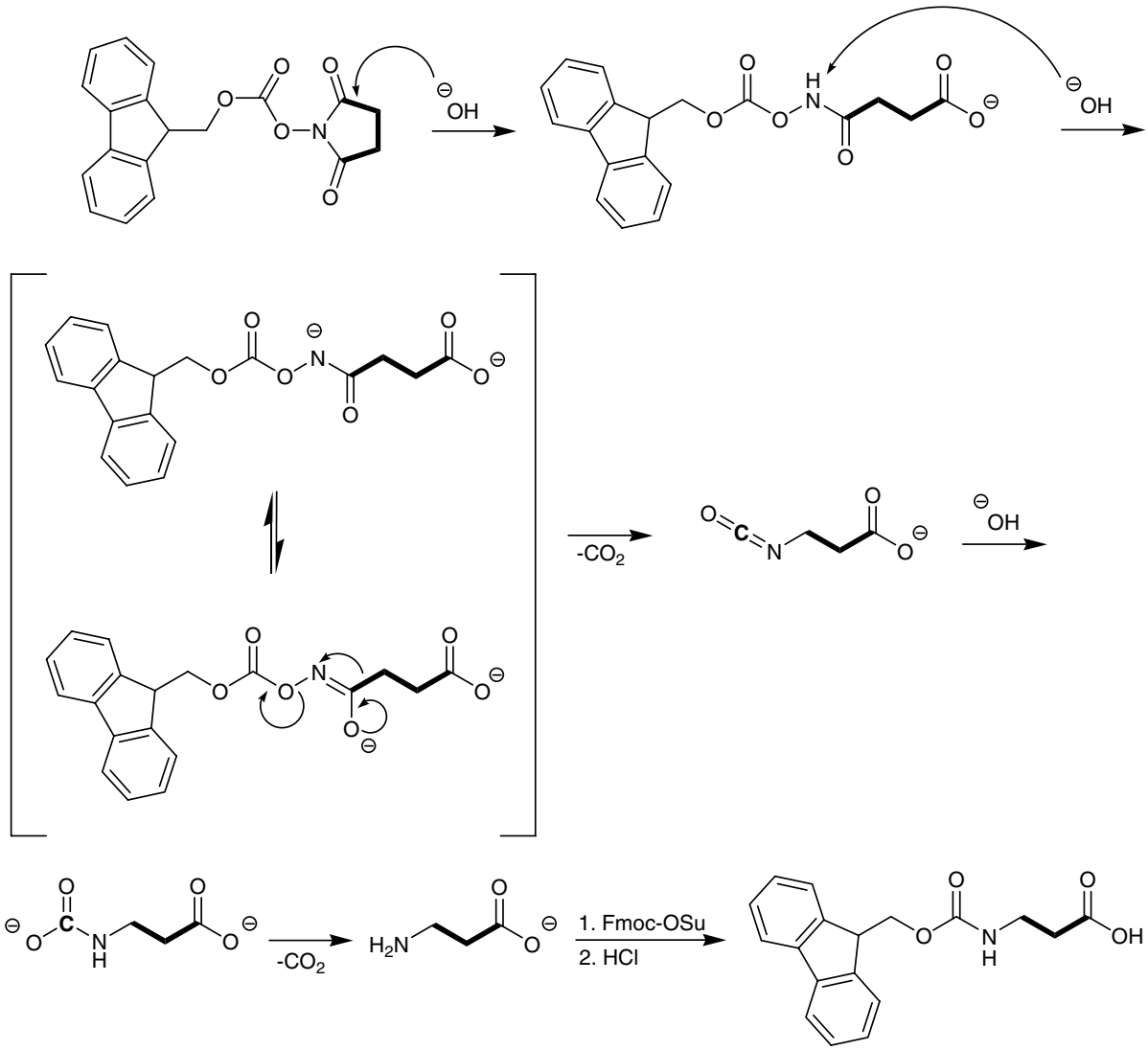
Scheme 1 Lossen rearrangement in an alkaline environment.

These three examples show that due to the formation of Fmoc–β-Ala–OH, very often additional purification steps are required, which can reduce drastically the yield of the desired product with concomitant loss of time and increase of costs. Therefore, we highly recommend that whenever possible Fmoc–OSu should be replaced by Fmoc–Cl to avoid Fmoc–β-Ala–OH formation.

Table 1 Formation of Fmoc–β-Ala–OH in the absence of an amino acid

Reagent	Base	Solvents	Formation of Fmoc–β-Ala–OH
Fmoc—OSu	2 eq K_2CO_3	Acetonitrile/H_2O	Yes (42% isolated yield)
Fmoc—OSu	—	Acetonitrile/H_2O	No
Fmoc—Cl	2 eq K_2CO_3	Acetonitrile/H_2O	No

Since in numerous Fmoc-protection steps, the use of Fmoc–OSu cannot be avoided for other reasons, we studied the influence of the Fmoc–OSu amount during the protection. For this purpose, we reacted the fairly hindered amino acid H–Asn(Trt)–OH with 2 eq sodium carbonate in acetonitrile/water in the presence of various amounts of Fmoc–OSu (Table 3).



Scheme 2 Putative mechanism for the formation of Fmoc–β-Ala–OH from Fmoc–OSu via Lossen rearrangement.

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As expected, the reaction mixture of the experiment with 1.25 eq Fmoc–OSu showed the highest ratio of Fmoc–β-Ala–OH to Fmoc–Asn(Trt)–OH (6 : 94). In contrast, the HPLC of the reaction mixtures containing 1.0 and 0.9 eq Fmoc–OSu showed only 0.2% or traces of Fmoc–β-Ala–OH, respectively.

The separation of Fmoc–Xaa–OH from unreacted H–Xaa–OH is in general much easier than the purification with regard to Fmoc–β-Ala–OH or its deriving dipeptides. Therefore, if the purification of the crude products is difficult, and it is not possible to replace Fmoc–OSu, its quantity should be limited to an equimolar amount because any excess favors the formation of Fmoc–β-Ala–OH.

The presence of Fmoc–β-Ala–OH in commercially available compounds plays a very important role in Fmoc–amino acid derivatives, which are widely used as building blocks in solid phase peptide synthesis. Since Fmoc–β-Ala–OH was already shown to represent a severe problem in SPPS [2], we examined a range of compounds we used in SPPS for their Fmoc–β-Ala–OH content. In most of the studied derivatives, the amount of Fmoc–β-Ala–OH was less than 0.1%, but for some batches of Fmoc–Lys(Boc)–OH, Fmoc–Trp–OH, Fmoc–Ala–OH, and Fmoc–Cys(Trt)–OH, analysis showed percentages of the impurity Fmoc–β-Ala–OH in the range of 0.1–0.5%. Improved synthetic procedures for the preparation of these Fmoc–amino acid derivatives are currently under development.

CONCLUSION

We would like to caution a broader chemical community that the presence of Fmoc–β-Ala–OH contamination in Fmoc–amino acid derivatives has been a hitherto underestimated problem in peptide chemistry. We were able to prove unambiguously that the source of this impurity is Fmoc–OSu that is first ring-opened and then rearranged under basic conditions leading initially to H–β-Ala–OH. In the course of the reaction, this intermediate is converted to Fmoc–β-Ala–OH. Since the literature already describes the formation of Fmoc–Xaa–Xaa–OH during the Fmoc-protection of H–Xaa–OH [3], it is not astonishing that the two possible dipeptides Fmoc–Xaa–β-Ala–OH or Fmoc–β-Ala–Xaa–OH are sometimes found in traces in reaction mixtures and crude products after Fmoc-protections with Fmoc–OSu.

Table 2 Fmoc–β-Ala–OH as impurity during the syntheses of three at Bachem commercially available Fmoc–amino acid derivatives. Reaction conditions: **(A)** 1.25 eq Fmoc–OSu, 2.0 eq K_2CO_3, acetonitrile/H_2O; **(B)** 1.25 eq Fmoc–OSu, 2.0 eq K_2CO_3, dioxane/H_2O

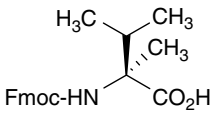
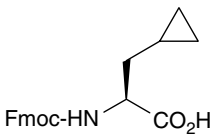
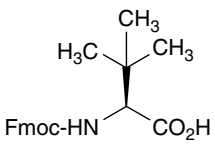
Amino acid derivative	Reaction conditions	Purification method	Yield (%) Fmoc–Xaa–OH	Content (%) of Fmoc–β-Ala–OH
Fmoc–α-Me–Val–OH 	A	Counter-current distribution	85	1.8 (isolated)
Fmoc–β-cyclopropyl–Ala–OH 	B	Crude product Two recrystallizations	99 43	9 0.5
Fmoc–tBu–Gly–OH 	B	Crude product Recrystallization	89 56	2 ≪0.1

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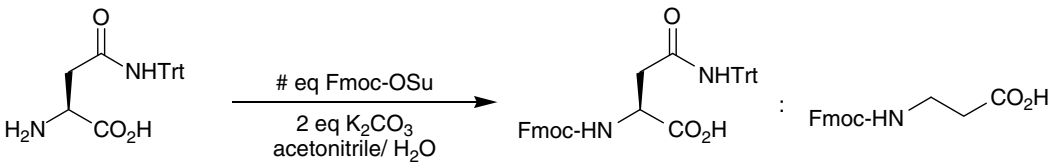
Table 3 Formation of Fmoc-β-Ala-OH during Fmoc-protection of H-Asn(Trt)-OH: influence of the equivalents of Fmoc-OSu



H-Asn(Trt)-OH **Fmoc-Asn(Trt)-OH** **Fmoc-β-Ala-OH**

# eq Fmoc-OSu	Ratio	
1.25 eq	94.0	6.0
1.0 eq	99.8	0.2
0.9 eq	>99.9	Not detected

In several cases, the removal of any of these impurities is accompanied with loss of material and time. Therefore, we recommend to reconsider the use of Fmoc–OSu in critical cases and to apply Fmoc–Cl as an alternative, which obviously was shown not to be converted to Fmoc–β-Ala–OH. In reactions where the use of Fmoc–OSu is inevitable, the formation of Fmoc–β-Ala–OH may be reduced by the use of an equimolar amount of the reagent as has been demonstrated in this contribution.

Finally, we want to encourage all suppliers in the field of amino acid derivatives to carefully analyze their products not only for Fmoc–dipeptides as impurities but also for Fmoc–β-Ala–OH, Fmoc–Xaa–β-Ala–OH and Fmoc–β-Ala–Xaa–OH. We hope these vendors are able to set the acceptable limits as low as possible.

Note added in proof: After submission of this manuscript, we became aware of a publication [4], which proved the concept of our findings.

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