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Fmoc-OASUD: A new reagent for the preparation of Fmoc-amino acids free from impurities resulting from Lossen rearrangement

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ABSTRACT

A new reagent, 9-fluorenylmethoxy-carbonyl-*N*-hydroxy-3-azaspiro[5,5]undecane-2,4-dione ester (Fmoc-OASUD) for the preparation of Fmoc-amino acids is described. The Fmoc-OASUD reacts with amino acids at room temperature in the presence of a base and gives Fmoc-amino acids in high yields and purity. The Fmoc-amino acids prepared using Fmoc-OASUD are free from impurities due to Lossen rearrangement, which are generally present when Fmoc-OSu is used. This is mainly because of the higher stability of Fmoc-OASUD compared to Fmoc-OSu.

Introduction

The 9-fluorenylmethyloxycarbonyl (Fmoc) group was first introduced by Carpino, as a base labile amino protecting group.[1,2] The advantage of Fmoc is that it is cleaved under very mild basic conditions, but stable under acidic conditions.[3] Because of this orthogonal advantage, Fmoc group is used widely in peptide synthesis, especially in solid phase synthesis.

The initial method for the introduction of Fmoc group was based on the use of the 9-fluorenylmethoxyl chloroformate.[4] However, Fmoc-amino acids prepared using Fmoc-Cl were found to be contaminated with significant levels of corresponding Fmoc-dipeptides and tripeptides.[5,6] As an alternative, Fmoc-N-Hydroxy-succinimide ester (Fmoc-OSu) was developed as a reagent for the preparation of Fmoc-amino acids.[7] The Fmoc-amino acids prepared using Fmoc-OSu were free from peptide impurities. Presently, Fmoc-OSu is considered as the reagent of choice for the preparation of Fmoc-amino acids.[8] It is also preferred for the large scale preparation of Fmoc-amino acids.[9]

However, Fmoc-amino acids prepared using Fmoc-OSu were found to contain Fmoc-β-alanine, and Fmoc-β-alanyl dipeptide as major impurities.[10,11] Since these impurities can take part further in peptide synthesis, such contaminated Fmoc-amino acids pose serious problems in manufacturing peptide drugs.

The origin of such β-alanine related impurities, is attributed to the decomposition of Fmoc-OSu followed by Lossen type rearrangement (Scheme 1).[8] Zalipsky also reported the formation of β-Alanine impurities from succinimidyl carbonates through Lossen rearrangement.[12]

Our earlier studies showed that use of dicyclohexylamine, results in the suppression of β-Alanine and dipeptide impurities from Fmoc-amino acids prepared from Fmoc-OSu.[13] Recently, we reported *N*-hydroxy-3-azaspiro[5,5]undecane-2,4-dione (OH-ASUD **1**), as an alternative to N-hydroxysuccinimide, for preparing peptides. The **1**, reacts with N-protected amino acids and gives ASUD activated esters of amino acids, which are useful in peptide synthesis.[14,15]

Herein, we report that the 9-fluorenylmethoxy-carbonyl-*N*-hydroxy-3-azaspiro[5,5]undecane-2,4-dione ester (Fmoc-OASUD, **2**), is a novel reagent for preparing Fmoc-amino acids in high yields and purity. The **2** reacts with amino acids, at room temperature, in the presence of a base, to give Fmoc-amino acids **3** (Scheme 2).

Results and discussion

The reagent **2** is conveniently prepared in high yields by reacting **1** with Fmoc-Cl in presence of a base in aqueous acetone (Scheme 3).

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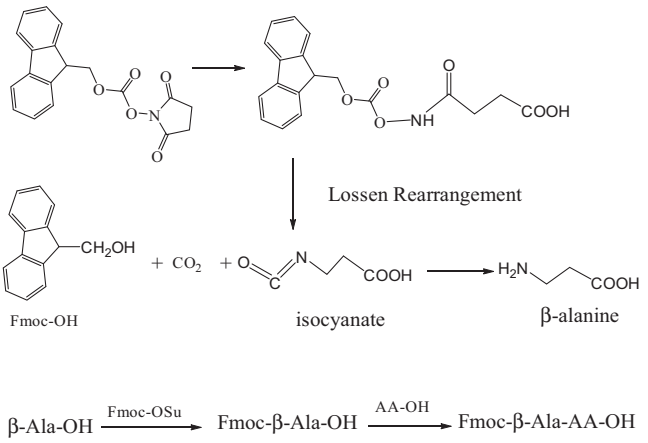


Scheme 1. Formation of β-alanine and related impurities from Fmoc-OSu through Lossen rearrangement.

The **2** is a crystalline solid which is stable and can be stored at ambient temperature in air-tight container for several months without any degradation.

As a model reaction, synthesis of Fmoc-L-alanine from L-alanine and **2** in the presence of a base was studied at various experimental conditions. L-alanine was reacted with **2** in the presence of sodium carbonate as base in water–dioxane (1:2) system at room temperature. The reaction was completed in about an hour. Acidification of the reaction mixture gives Fmoc-L-alanine in good yields. However it contained **2** as a minor impurity which is eliminated by methyl *tert*-butyl ether wash before acidification. Sometimes, traces of **1** were also observed as impurity. Although most of **1** remains in aqueous phase, slight amount gets extracted into ethylacetate layer. However, this also gets eliminated in acetone–water recrystallization.

Various solvents were studied (Table 1). Apart from solubilizing **2** partially or completely, solvents played an important role in the reaction. Best results were obtained from 1,4-dioxane and acetonitrile. Other solvents such as acetone, THF, and DMF gave low yields. Various bases were also studied. Best results were obtained from sodium carbonate and triethylamine. Sodium carbonate gave better yields when used with dioxane, compared to acetonitrile, while triethylamine with acetonitrile gave higher yields. Reaction was faster and completed within 0.5 h when triethylamine was used either with acetonitrile or dioxane. Use of other bases such as sodium hydroxide, sodium bicarbonate, diisopropylamine, and diisopropylethylamine resulted in lower yields.

Table 1
Synthesis of Fmoc-L-alanine using **2**[*]

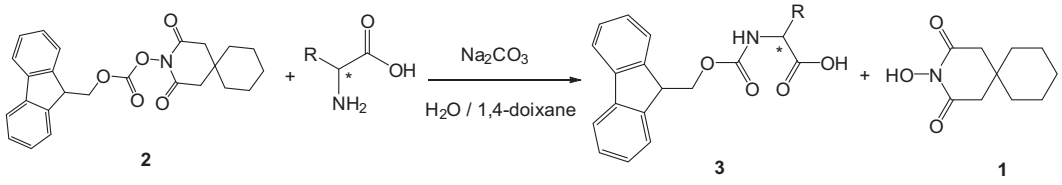
Entry	Base	Solvent	Time (h)	Yield [a] (%)	Purity [b] (%)	Fmoc-Ala-Ala-OH [b]
1	Na_2CO_3	1,4-Dioxane	1	94	99.63	0.04
2	Na_2CO_3	Acetonitrile	1	89	99.98	ND [c]
3	Na_2CO_3	Acetone	1	69	98.68	ND [c]
4	Na_2CO_3	THF	2	66	97.38	0.03
5	Na_2CO_3	DMF	3	57	99.02	0.06
6	Et_3N	Acetonitrile	0.50	91	99.58	0.02
7	Et_3N	1,4-Dioxane	0.75	72	98.92	ND [c]
8	NaOH	Acetonitrile	0.50	84	99.37	0.06
9	NaOH	1,4-Dioxane	0.50	80	99.46	0.04
10	$NaHCO_3$	Acetonitrile	3	78	98.79	ND [c]
11	$(i\text{-}Pr)_2NH$	Acetonitrile	0.75	65	99.06	0.04
12	$(i\text{-}Pr)_2EtN$	Acetonitrile	0.50	73	99.21	0.02

[*] L-aminoacid (11.2 mmol) in the presence of base (12.3 mmol) in water (10 mL) and solvent (20 mL) mixture, was reacted with **2** (4.71 g, 11.2 mmol) at room temperature.
[a] Isolated yield.
[b] Symmetry C18 (250 × 4.6 mm, 5 μm) column, Elution: Gradient program 30–80% of 0.1% TFA in H_2O/0.1% TFA in CH_3CN over 35 min; flow rate = 1.0 ml/min; PDA detection at 254 nm.
[c] ND: not detectable.

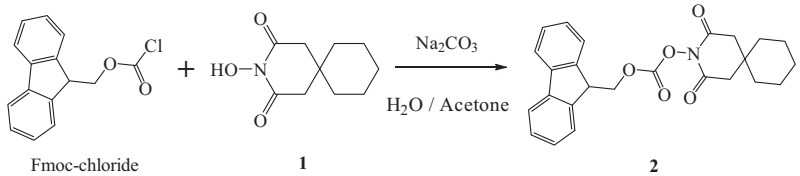
In all the reactions, the HPLC analysis of the product showed high purity with no detectable formation of impurities that could result from Lossen type rearrangement.

This is mainly because of higher stability of **2** compared to Fmoc-OSu. No hydrolysis of **2** was observed in the reaction medium up to 3 h, which is the maximum reaction time studied. When **2** was stirred in aqueous alkaline medium for 5 h, about 0.12% of an impurity was observed. The impurity was found to be Fmoc-gabapentin, resulting from Lossen rearrangement (Scheme 4).

The Fmoc-gabapentin was confirmed by its LCMS and by comparing with the standard sample prepared as described by Wu et al. earlier.[16] Some of the samples of Fmoc-L-alanine, were contaminated with the dipeptide, Fmoc-Ala-Ala-OH in less than 0.1% level. Its presence was also confirmed by comparing with the standard sample. If necessary, a second recrystallization with



Scheme 2. Synthesis of Fmoc-amino acids using Fmoc-OASUD reagent.



Scheme 3. Synthesis of **2**.