

Microwave assisted synthesis of N-protected amino acid esters

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Introduction

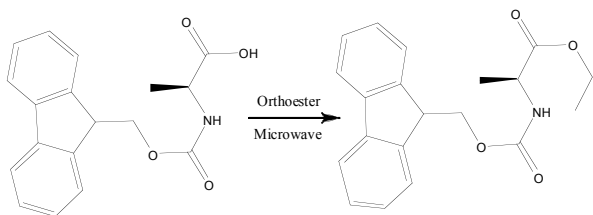
In the past few years, microwave assisted organic synthesis has received great attention from research chemists due to its various advantages, such as dramatic reduction of reaction time, higher yields and more choice for solvent free reactions.

N-protected amino acid esters have found wide applications in peptide synthesis. Methyl and ethyl esters, in particular, are excellent blocking groups and are not affected by the conditions applied for the removal of the widely used Fmoc group. They can also serve as intermediates for synthesis of some enantiopure compounds such as N-protected amino alcohols [1] which are key building blocks for synthesis of oligopeptides [2] and oligopeptidylsulfonamides [3]. For this reason, we launched a project for methodology studies of Microwave assisted synthesis of N-protected amino acid esters.

Results and Discussion

All reagents were purchased from commercial suppliers and were used without purification. RP-HPLC analysis was carried out on a Gilson system, equipped with UV absorbance detector and a Finnigan AQA ESI mass spectrometer. The products were analyzed on C18 columns (Phenomenex 75x4.6 mm, particle size: 5µm), eluted in 0.1 % TFA in acetonitrile/water using a linear gradient (A: 0.1 % TFA in water. B: 0.1 TFA in acetonitrile), optimized for every compound, at flow rate of 1ml/min. Microwave reactions were carried on a Biotage Initiator Sixty scientific microwave system.

In the search of good experimental procedures to synthesize N-protected amino acid esters, Fmoc-L-Ala-OC₂H₅ formation reaction was studied employing different reaction conditions (scheme 1). Fmoc-L-Ala-OH and orthoesters were added to a heavy-walled Emrys process glass vial and sealed with an aluminum crimp cap fitted with a septum. The reaction mixture was subjected to microwave irradiation for the time and temperature indicated. The reactions were monitored by analytical reversed-phased HPLC-MS and the conversions determined by integration of the UV-trace (Table 1).

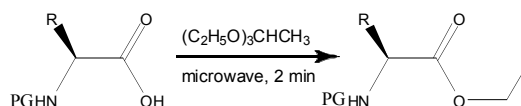


Scheme 1. Microwave assisted synthesis of Fmoc-L-Ala-OC₂H₅

Table 1. Microwave assisted synthesis of Fmoc-L-Ala-OC₂H₅ under various reaction conditions.

time	Temp	Orthoester	Equiv of orthoester	Purity
60min	200°C	triethyl orthoformate	5.0	86%
60min	200°C	triethyl orthoformate	2.5	85%
5min	200°C	triethyl orthoacetate	2.5	91%
2min	200°C	triethyl orthoacetate	2.5	95%
2min	150°C	triethyl orthoacetate	2.5	78%
4min	150°C	triethyl orthoacetate	2.5	84%
10min	150°C	triethyl orthoacetate	2.5	89%

As can be seen from the above table, we firstly employed triethyl orthoformate in the synthesis of Fmoc-L-Ala-OC₂H₅. It took one hour for completion of the reaction. However, as triethyl orthoacetate has been reported to be superior to triethyl orthoformate [4] for similar reactions, we tried this reagent as well. It was found that triethyl orthoacetate gave far better result. We also tried different reaction temperatures (150°C and 200°C). It was found that the reaction was complete in 2 min at 200°C. The optimized reaction conditions were further applied on different protected amino acids (scheme 2). In most cases, the purity of the resulting protected amino acid ester was above 90% (table 2).



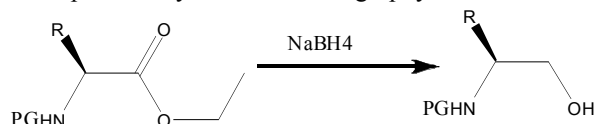
Scheme 2. Microwave assisted synthesis of N-protected amino acid esters.

Table 2. Microwave assisted synthesis of N-protected amino acid esters

Entry	Protected amino acid	Purity
1	Fmoc-L-Ala-OH	95%
2	Fmoc-L-Phe-OH	95 %
3	Fmoc-L-Glu(OtBu)-OH	93 %
4	Fmoc-L-Asp(OtBu)-OH	87 %
5	Fmoc-L-Thr(tBu)-OH	97 %
6	Fmoc-gly-OH	84 %
7	Fmoc-L-Leu-OH	96 %
8	Fmoc-L-Val-OH	95 %

9	Fmoc-L-Cys(tBu)-OH	92 %
10	BOC-L-Phe-OH	92 %
11	Fmoc-L-Pro-OH	91 %
12	Tos-Gly-OH	86 %
13	Tos-L-Ala-OH	84 %
14	Tos-L-Phe-OH	95 %
15	Z-L-Ala-OH	93 %
16	Z-L-Val-OH	92 %
17	Z-Gly-OH	89 %
18	Z-L-Phe-OH	95 %

We further reduced three crude N-protected amino acid ester to N-protected amino alcohol (scheme3, table3). Crude N-protected amino acid ester (2 mmol), obtained from the above step, was put in a 100 mL flask, 20 mmol NaBH₄ and 50 ml 20% H₂O/THF was added and stirred for 12 h. The reaction was monitored by TLC. After completion of the reaction, THF was evaporated and ethyl acetate added. The EtOAc phase was washed with water, dried with magnesium sulfate and evaporated. The residue was purified by flash chromatography.



Scheme 3. Reduction of N-protected amino acid ester to N-protected amino alcohol

Table 3. Reduction of N-protected amino acid ester to N-protected amino alcohol

Entry	N-protected amino acid ester	Reaction time	Yield
1	Fmoc-L-Ala-OC ₂ H ₅	12 h	93%
2	Fmoc-L-Phe-OC ₂ H ₅	12 h	89%
3	Fmoc-L-Cys(tBu)-OC ₂ H ₅	16 h	92%

In summary, we have developed a rapid and convenient microwave assisted synthesis of N-protected amino acid esters, which works with a variety of N-protecting groups, and thus provides facile access to these valuable building blocks.

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