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# Saponification of Esters of Chiral α-Amino Acids Anchored through their Amine Function on Solid Support

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Abstract: Anchoring an α-amino acid residue by its amine function onto a solid support is an alternative to develop chemistry on its carboxylic function. This strategy can involve the use of amino-acid esters as precursors of the carboxylic function. A complete study on the Wang-resin was performed to determine the non-racemizing saponification conditions of anchored α-amino esters. The use of LiOH, NaOH, $NaOSi(Me)_3$, various solvents and temperatures were tested for this reaction. After saponification and cleavage from the support, samples were examined through their Marfey's derivatives by reversed phase HPLC to evaluate the percentage of racemization. Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: reverse anchoring; saponification; racemization; solid phase synthesis; Marfey's derivatives

## INTRODUCTION

Gem-diamino derivative syntheses on a solid support via the Hofmann rearrangement was described recently [1]. For this purpose α-amino amide derivatives were anchored by their amino function onto a carbonate activated Wang-resin. To our knowledge, only a few publications have explored the possibility of anchoring amino acids by their amine function so as to modify the carboxylic function. Redemann *et al.* [2] described α-hydroxy-β-amino aldehyde syntheses using Dondoni's homologation reaction sequence [3]. Garcia *et al.* anchored Weinreb amide derivatives of α-amino acids to a solid support for the synthesis of α,α-disubstituted-α-acylaminoketones [4]. Gosselin *et al.* generated anchored α-amino aldehydes for the synthesis of norephedrines [5]. To open the chemistry on the free carboxylic acid function, a reliable method for obtaining α-amino acids bonded to a solid support by their amino function was needed. This paper describes the on-support saponification of α-amino esters without racemization and in quantitative yields. This strategy should be useful for preparing amide bond surrogates easily using solid support organic chemistry.

The Wang-resin was converted into its p-nitrophenyl carbonate linker to permit anchorage of amines was chosen [6]. The resulting urethane derivatives could be quantitatively cleaved from the support by exposure to trifluoroacetic acid to yield free amines. All attempts to link free amino acids [7] to the carbonate Wang resin led in our hands to a mixture of anchored amino acid plus dimer and trimer peptides. To avoid harsh saponification conditions, it was decided to anchor α-amino acid esters as their OBg esters [8] which can be saponified in solution under smooth conditions. Surprisingly, bonded α-amino acid OBg esters were found to be stable in 10 eq. aqueous 2 N $K_2CO_3$ and needed in fact the same experimental conditions with methyl esters to be saponified. It was then decided to anchor the residues as their commercially available methyl ester derivatives (Scheme 1). This paper describes

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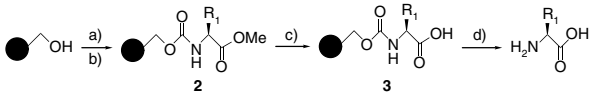


a: Cl-COO-pNP, NMM, DCM, 0°C;
b: $H_2N$-$CHR_1$-COOMe, DIEA, HOBt.$H_2O$, DCM/DMF, RT, 12 h;
c: saponification conditions; d: TFA

Scheme 1

the optimal conditions for saponification of methyl esters on Wang-resin solid support and reports the possible racemization of the anchored carbamate amino acid residue which can occur during the saponification step.

## METHODS AND RESULTS

Various conditions were tested for the saponification step. The reaction kinetics were studied with the bulky valine methyl ester linked to the support. As the valine residue is transparent in terms of UV, monitoring of the saponification step was performed by reverse phase HPLC analysis after cleavage from the support by acidolysis and derivatization of the obtained amines with the Marfey's reagent. These conditions allowed the quantification of (1) possible racemization and (2) the conversion of methyl ester to acid. They were used for all experimental conditions and all studied α-amino acid residues. First, aqueous DMF or DMSO solutions were found to be unsuitable solvents for saponification on a solid support, whereas aqueous THF was more effective (for example, 10% of linked Val-OMe was saponified in DMSO compared with 97% in THF in the same experimental conditions). So THF was selected as the solvent for this reaction. As shown in Figure 1, aqueous 2 M LiOH was found to be more efficient in THF than aqueous 2 M NaOH with 97% conversion for 10 eq. LiOH instead of 77% conversion for 10 eq. NaOH after 8 h. Optimization of the reaction was then undertaken (number of eq. of lithium hydroxide, temperature and reaction time). The results obtained for saponification of five amino acid residues are shown in Table 1.

In all experiments, a 2 M aqueous solution of lithium hydroxide was used and a constant percentage of 70/30 was kept between THF and the aqueous solution. As shown in Table 1, the experimental conditions used for column 2 (5 eq. LiOH, RT, 8 h) are a good alternative except for the bulky valine residue. Furthermore, in these conditions, little racemization (<0.5%) could be observed. For bulky amino acids, the reaction time could be lengthened without significant racemization. The use of 25 eq. of LiOH for 2 h at 50 °C allowed quantitative saponification with low racemization levels (column 5) except for the

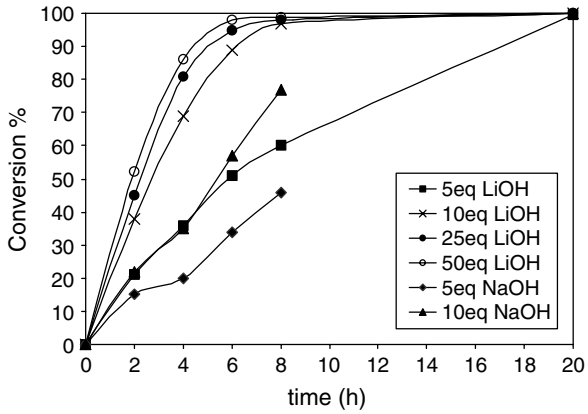


Figure 1 Saponification kinetics of Val-OMe linked to the resin in THF at room temperature.

Table 1 Conversion and Racemization Percentage of Linked Amino Acids in Various Saponification Conditions in Aqueous THF Solution

| a.a. | 2 M LiOH, 5 eq., RT, 8 h | 2 M LiOH, 10 eq., RT, 8 h | 2 M LiOH, 10 eq., RT, o.n. | 2 M LiOH, 25 eq., 50 °C, 2 h | 1 M $NaOSiMe_3$, 15–20 eq. RT, 4 h |
|---|---|---|---|---|---|
| **Val** | 60[b]/<0.5[c] | 99.5[b]/<0.5[c] | 99.7[b]/<0.5[c] | 99.5[b]/0.7[c] | 100[b]/5[c] |
| **Phe** | 100[b]/<0.5[c] | 100[b]/<0.5[c] | 100[b]/<0.5[c] | 99,3[b]/0.6[c] | 100[b]/22[c] |
| **Leu** | 100[b]/<0.5[c] | 100[b]/<0.5[c] | 100[b]/<0.5[c] | 100[b]/0.9[c] | 100[b]/10[c] |
| **Trp** | 100[b]/<0.5[c] | 100[b]/2.5[c] | 100[b]/3.4 | 100[b]/1.6[c] | 100[b]/17[c] |
| **Ser**[a] | 100[b]/nd | 100[b]/8[c] | 100[b]/7[c] | 100[b]/10[c] | 100[b]/32[c] |

[a] Serine residue was used as its *t*Bu ether.
[b] Percentage of conversion to carboxylic acid.
[c] Percentage of racemization determined by RP HPLC of Marfey's derivatives.

Ser residue. These conditions were found suitable for further parallel combinatorial chemistry, keeping in mind that racemization of some residues can occur in these conditions as exemplified by the serine residue. Also studied was the use of $NaOSiMe_3$ (commercially available as a 1 M solution in THF) which was earlier reported suitable for saponification [2,9] and a high percentage of racemization was found (column 6 in Table 1). Racemization rates were measured by reversed phase HPLC analysis of crude released compounds derivatized with the Marfey's reagent in standard conditions. All compounds were analysed by mass spectrometry to confirm their structures. On the other hand, anchoring of $\alpha$-amino acid methyl esters through their amino function to trityl resin was performed. In our hands and in the above conditions, saponification of the ester function was unsuccessful on the trityl resin.

In a typical experiment, 1 g of Wang-resin-Phe-OMe (0.73 mmol/g) was saponified in a mixture of aqueous 2 M LiOH (3.65 ml) and THF (8.50 ml) at room temperature. After 10 h, the resin was drained and successively washed with water, a 1 M aqueous solution of potassium hydrogenosulphate, water, methanol and dichloromethane. It was dried under vaccum to a constant weight. The carboxylic function was then activated with BOP reagent (5 eq.) in the presence of DIEA (5 eq.) and phenethylamine (5eq.) for 1 h. Cleavage of the $\alpha$-amino derivative was performed in 10 ml of TFA/DCM (75/25 : v/v) for 90 min at room temperature to recover after lyophilization the desired compound: TFA, H-Phe-phenethylamide (270 mg, yield 96%).

## DISCUSSION

In conclusion, the anchoring of $\alpha$-amino esters by their amine function on Wang derivatized resin followed by saponification of the ester function on this support was explored. This study permitted LiOH and THF to be selected as the best conditions for this last reaction. The use of $NaOSiMe_3$ should be avoided, at least on a solid support, as it leads to high levels of racemization. The application of this strategy to the synthesis of different amino acid derivatives is currently under investigation to furnish a variety of enantiomerically pure products bearing an amino function.

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