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Polystyrylsulfonyl-3-nitro-1*H*-1,2,4-triazolide-resin: a new solid-supported reagent for the esterification of amino acids

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Abstract—We describe the application of the polystyrylsulfonyl-3-nitro-1*H*-1,2,4-triazolide-resin, readily available from the corresponding commercially available polystyryl sulfonyl chloride resin, to the solution-phase synthesis of esters from protected α-amino acids and alcohols in high yields and purity with a low level of racemisation of the amino acids. All by-products can be removed by filtration and extraction.

Although solid-supported reagents and scavengers have been used in organic synthesis for decades, it was the development of combinatorial and parallel high throughput synthesis techniques that brought this class of reagents to a wider attention. The application of solid-supported reagents and scavengers for the solution-phase organic synthesis combines the simple work up by filtration with a fast reaction optimisation. Their use in multi-step organic synthesis has been reviewed recently.[1] We reported earlier a convenient and general procedure for the synthesis of esters from carboxylic acids and alcohols employing the commercially available polystyrylsulfonyl chloride resin as the solid-supported condensation reagent.[2] The efficiency of the reagent prompted us to investigate its use in the esterification of protected amino acids.

A wide variety of chiral amino acid building blocks are commercially available and are applied in combinatorial peptide and small organic molecule synthesis mainly for amide bond formation. The development of a simple and general procedure applicable in the solution phase library synthesis of esters from alcohols and carboxylic acids would broaden the scope of accessible compound classes considerably. Many natural products containing α-amino acid esters of carbohydrates (agropine, chrysopine),[3] polyketides (HA 23),[4] alkaloids (pseudoanchynazine A–C)[5] or hydroxy carboxylic acids (depsipeptides, e.g. valinomycin)[6] are known. In the past the esterification of amino acids was investigated with a strong focus on attaching amino acid building blocks to an hydroxylated solid support for solid phase peptide synthesis. To reduce the racemisation levels of the conventional carbodiimide/DMAP method, other procedures with different dehydration reagents like DEAD, 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT)[7] and 2,6-dichlorobenzoyl chloride (DCBC)[8] were developed. We report herein the high yield esterification in solution of Fmoc-protected amino acids with polystyrylsulfonyl chloride resin (PS-SO_2Cl) or polystyrylsulfonyl-3-nitro-1*H*-1,2,4-triazolide-resin (PS-SO_2NT). The latter resin reduced significantly the racemisation level of the reaction.

Fmoc-protected histidine derivatives are known to racemise easily when the carboxy group is activated.[8]

Table 1. Esterification of Fmoc-His(Trt)-OH with PS-SO_2Cl[a,b]

Entry	Solvent	Purity (%)	% D
1	DCM	98	5.3
2	DMF	91	26.2

[a] 0.025 mmol Fmoc-amino acid-OH, 1.5 equiv. methyl-(4-hydroxymethyl)-benzoate **2**, 3 equiv. *N*-methylimidazole (MeIm); 0.5 ml abs. DCM, 16.5 h.

[b] All products were characterised by HPLC-ESI-MS.

Keywords: polystyrylsulfonyl-3-nitro-1*H*-1,2,4-triazolide-resin; solid-supported reagent; solid-supported scavenger; esterification; racemisation.

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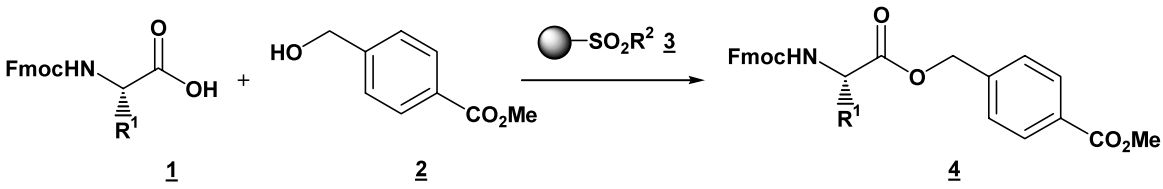

1 2 4

Scheme 1.

We therefore used the sensitive Fmoc-His(Trt)-OH as the model substrate. Table 1 summarises the results of the esterification with methyl-(4-hydroxymethyl)-benzoate. The reaction conditions were as described earlier for using polystyrylsulfonyl chloride resin (Scheme 1, $R^2=Cl$) as condensation reagent in DCM and DMF as solvents.[2] Analysis by HPLC showed complete conversion after 2 h in both cases. However, the racemisation level determined via chiral capillary GC–MS was rather high with 5.3% D enantiomer for DCM as solvents and even increased to 26.2% D in DMF as the solvent.[9]

Inspired by the success of MSNT[7] in the esterfication of Fmoc-amino acids with extremely low racemisation levels, we prepared the corresponding 3-nitro-1*H*-1,2,4-triazolide derivative of the polystyrylsulfonyl chloride resin:[10] To a mixture of 5 g of PS-SO_2Cl (1.35 mmol/g) and 1.54 g (2 equiv.) 3-nitro-1*H*-1,2,4-triazole in 50 mL abs. THF 2.0 mL (2.1 equiv.) triethylamine were added at 0°C and the mixture was stirred for 4 h at rt. After filtration through a glass frit, the resin was rinsed with 150 mL abs. THF, 50 mL MTB–ether and dried. Elemental analysis [S(3.49), Cl(3.85), N(6.88), O(8.18)] revealed considerable amounts of triethylammonium chloride by-product. This was removed by washing with 150 mL 1,4-dioxane/water 1:1, 200 mL 1,4-dioxane and 100 mL MTB–ether. Subsequently, the resin is dried in vacuo over night. No chloride could be detected any longer by elemental analysis [S(3.72), Cl(<0.2), N(6.12), O(11.03)]. The loading was calculated based on the sulphur content to 1.16 mmol/g. The thoroughly dried resin still contained some dioxane, indicated by the high oxygen content.

First, we evaluated the reaction conditions of the new condensation reagent. Table 2 demonstrates the influences of the excess of base on the esterification of the model compounds **1** and **2** (Scheme 1, $R^2=$3-nitro-1*H*-1,2,4-triazole), using two equivalents of resin. In contrast to the esterification with PS-SO_2Cl, the excess of base influenced the reaction rate only at the beginning of the reaction. After 240 min, the observed ratio of starting material to product was almost the same for entries 1–3. Complete conversion was observed in all three cases after a reaction overnight.

The minimal amount of resin required was determined as shown in Table 3. For a complete conversion in an overnight reaction 2 equiv. of resin were necessary (entry 3). Independent of the reaction conditions, the ester **4** is formed without any trace of by-products. For all the following experiments, 2 equiv. of MeIm and resin were used. No cleavage of either the Fmoc- or the Trt-protecting group was observed by HPLC.

Table 2. Influence of the excess of base on the esterification reaction[a]

Entry	Equivalents of MeIm[b]	Ratio **1**/**4**[c,d] $t=30$ min	Ratio **1**/**4**[c,d] $t=60$ min	Ratio **1**/**4**[c,d] $t=140$ min	Ratio **1**/**4**[c,d] $t=240$ min	Ratio **1**/**4**[c,d] $t=505$ min
1	1	1.4:1	1:1.6	1:6.4	1:15.2	1:31.3
2	2	1:2.2	1:4.6	1:11.1	1:18.6	1:48.5
3	3	1:4.1	1:7.1	1:11.4	1:18.6	1:48.5

[a] 0.025 mmol Fmoc-His(Trt)-OH **1**, 1.5 equiv. methyl-(4-hydroxymethyl)-benzoate **2**, 2 equiv. resin, 0.5 ml abs. DCM.
[b] Based on Fmoc-His(Trt)-OH.
[c] Ratio determined by HPLC at 220 nm.
[d] **4** was characterised by HPLC-ESI-MS.

Table 3. Influence of the excess of resin on the esterification reaction[a]

Entry	Equivalents of resin[b]	Ratio **1**/**4**[c,d] $t=60$ min	Ratio **1**/**4**[c,d] $t=150$ min	Ratio **1**/**4**[c,d] $t=270$ min	Ratio **1**/**4**[c,d] $t=420$ min	Ratio **1**/**4**[c,d] $t=22$ h
1	1.2	1:1	?	1:2.4	1:2.4	1:3.3
2	1.5	1.1.6	1:7.3	1:7.8	1:7.8	1:12.9
3	2	1:4.2	1:16.4	1:23.5	1:32.3	Complete conversion

[a] 0.025 mmol Fmoc-His(Trt)-OH **1**, 1.5 equiv. methyl-(4-hydroxymethyl)-benzoate **2**, 2 equiv. MeIm; 0.5 ml abs. DCM.
[b] Based on Fmoc-His(Trt)-OH.
[c] Ratio determined by HPLC at 220 nm.
[d] **4** was characterised by HPLC-ESI-MS.

Table 4. Racemisation of Fmoc-amino acids after esterification[a,b]

Entry	Fmoc-amino acid	Solvent	% D (PS-SO_2NT)	% D (MSNT)[d]	% D (DCBC)[e]
1	Fmoc-His(Trt)-OH	DCM	1.50	–	27
2	Fmoc-His(Trt)-OH	DMF	14.2	–	–
3	Fmoc-Cys(Trt)-OH	DCM	0.36	–	4
4	Fmoc-Phe-OH	DCM	0.12	0.1	0.3
5	Fmoc-Ile-OH	DCM	0.22[c]	0.2	0.4
6	Fmoc-Asp(*t*Bu)-OH	DCM	0.45	1.4	0.5

[a] 0.025 mmol Fmoc-amino acid-OH **1**, 1.5 equiv. methyl-(4-hydroxymethyl)-benzoate **2**, 2 equiv. MeIm; 0.5 ml abs. DCM, 16 h.
[b] All products were characterised by HPLC-ESI-MS.
[c] D-Allo-Ile.
[d] Data from Ref. 7.
[e] Data from Ref. 8.

We then compared the racemisation levels of five representative Fmoc amino acid derivatives esterified with methyl-(4-hydroxymethyl)-benzoate **2** by PS-SO_2NT under the optimised reaction conditions to results reported for MSNT and the Sieber method on solid supported alcohols. Table 4 summarises the racemisation data of the new esterification method and data from literature. The amino acids His, Cys and Asp were included in this selection in their standard side chain protected form, because these amino acids showed racemisation of more then 1% when esterified with either MSNT or 2,6-dichlorobenzoyl chloride as soluble reagents.[7,8] With the exception of Fmoc-His(Trt)-OH, the racemisation of all amino acids was lower then 0.5% when esterified with PS-SO_2NT in DCM (entries 1, 3–6). These values were quite similar to the reported data for Phe, Ile and Asp (entries 4–6) while the racemisation levels of Cys and especially His could be remarkably improved (entries 1 and 3). As the side chain protection of these two amino acids were different in the investigation of the MSNT promoted esterification, a direct comparison with literature data is not possible. As in the case of PS-SO_2Cl the racemisation level increased heavily in DMF (entry 2).

To establish a simple work-up procedure suitable for parallel high throughput performance, we used the inherent ability of PS-SO_2NT to act also as a scavenger resin for alcohols. We applied an excess of alcohol over the carboxylic acid to drive the esterification reaction to completion together with an excess of PS-SO_2NT as scavenger for the surplus alcohol. *N*-Methylimidazole and the resin supported reagents could be removed by simple filtration of the reaction mixture through Amberlyst-15, an acidic ion exchange resin. Finally, the 3-nitro-1*H*-1,2,4-triazole by-product was separated by precipitation of the expected ester in water. ^{1}H NMR showed no signs of remaining *N*-methylimidazole and 3-nitro-1*H*-1,2,4-triazole.

A typical procedure comprises: To a mixture of 15.5 mg (0.025 mmol) Fmoc-His(Trt)-OH, 7.8 mg (1.1 equiv.) Fmoc-glycinol and 46.8 mg (3 equiv.) of PS-SO_2NT (1.60 mmol/g) in 0.5 mL abs. DCM, 4.0 μL MeIm were added. After 5 h, additional 2.0 μL of MeIm were added and the reaction was shaken overnight. The slurry was filtered through 120 mg Amberlyst 15 in a syringe equipped with a polypropylene frit. The resin was rinsed thoroughly with 7 mL DCM and the combined eluents were concentrated, redissolved in 600 μL acetonitrile and precipitated with 20 mL of water. After centrifugation, decantation and drying in vacuo the expected ester was obtained in 90% yield (21.0 mg) and 100% purity determined by HPLC.

In summary, we have demonstrated the use of polystyrylsulfonyl-3-nitro-1*H*-1,2,4-triazolide-resin as an efficient dehydration reagent for the formation of esters from Fmoc-protected amino acids. When compared to polystyryl sulfonyl chloride resin, the new solid-supported reagent reduces considerably the amount of racemisation. The method uses only commercially available supported reagents and scavengers and allows compounds to be obtained in excellent yields and high purity by a simple filtration and extraction work-up without the need for chromatography.

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