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A new polymer-supported reagent for the Fmoc-protection of amino acids

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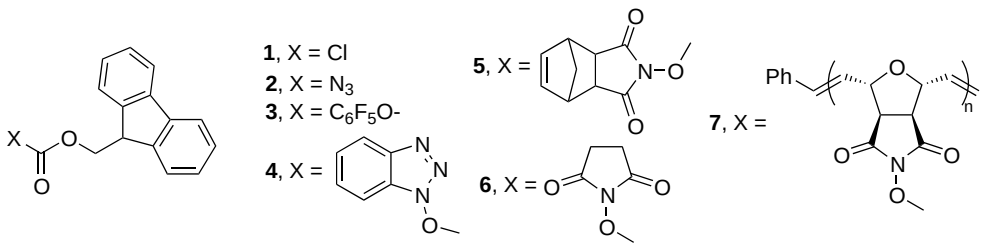
Abstract—A new polymer-supported Fmoc-OSu (Fmoc-P-OSu) has been prepared from polymer-bound *N*-hydroxysuccinimide (P-HOSu), and used as a solid-supported reagent for the Fmoc-protection of amino groups. The residual P-HOSu generated after the protection reaction can be separated by simple filtration and reused. © 2001 Elsevier Science Ltd. All rights reserved.

The 9-fluorenylmethoxycarbonyl (Fmoc) group enjoys a tremendous popularity as a protecting group for primary and secondary amines and especially for amino acids in the synthesis of peptides due to its stability to acid and lability to base.[1,2] In addition, it is readily cleaved nonhydrolytically and the protected amine is liberated as its free base. For the incorporation of the Fmoc group various different reagents with the general structure Fmoc-X have been prepared. The widely used chloroformate ester Fmoc-Cl (**1**) has a series of drawbacks such as its instability and the tendency to promote the formation of undesirable 'Fmoc-dipeptides', presumably via the mixed anhydride intermediate.[3] The use of the azide derivative Fmoc-N_3 (**2**) is claimed to reduce dipeptide formation,[4] although it has obvious problems related to the storage and handling of potentially explosive oxycarbonyl azides. Many workers prefer the use of more shelf-stable carbonates such as Fmoc-pfp[5] (**3**, pfp = perfluorophenoxy), Fmoc-OBt[6] (**4**, Bt = benzotriazol-1-yl), 5-norbonene-2,3-dicarboximido-Fmoc-derivative **5**,[7] and particularly Fmoc-OSu[3,6,8] (**6**, Su = succinimidyl), which also provides *N*-protected amino acids in comparable yields without the formation of the dipeptide impurities.

At the same time, the development of polymer-supported reagents for organic synthesis is also receiving growing attention nowadays because of easy recycling and the simplification of conventional work-up procedures.[9] A recent example on the use of a polymeric reagent for the *N*-Fmoc-protection of amino groups uses an in situ generated reagent from a polystyrene-bound HOBt and Fmoc-Cl (**1**).[10] More recently, a *N*-hydroxysuccinimide ring-opening metathesis polymer obtained from *exo*-*N*-hydroxy-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide using the Grubbs catalyst has been used for the preparation of different supported acyl-transfer reagents such as **7**, although its direct polymerization only was achieved through a HO-involved silylation–polymerization–desilylation sequence.[11] In all this context, and in connection with our project on the development of new solid-supported reagents applicable to peptide chemistry such as P-TBTU,[12] we have recently developed a co-polymer containing the *N*-hydroxysuccinimide moiety (P-HOSu, **8**)[13] which can be easily obtained from an inexpensive commercially available polymer and used as a recoverable racemization-reducing additive for the dicyclohexylcarbodiimide (DCC)-mediated coupling of amino



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acids. We report here the use of P-HOSu (**8**) for the preparation of a polymer-bound Fmoc-OSu (Fmoc-P-OSu, **9**), which can be used as a suitable reagent for the Fmoc-protection of amino acids.[14] This solid-supported reagent would allow the easy separation of the Fmoc-protected amino acid, which could be particularly valuable when working on a small scale.

Starting P-HOSu (**8**) was obtained with a loading of 1.5 mmol g^{-1} by reaction of commercially available styrene/maleic anhydride co-polymer[15] with an 50% aqueous solution of hydroxylamine.[13] Subsequent reaction of a mixture of **8** and K_2CO_3 in water with a solution of 9-fluorenylmethoxycarbonyl chloride (**1**), afforded Fmoc-P-OSu (**9**) as a white solid (Scheme 1).

The obtained Fmoc-P-OSu polymer (**9**) showed identical C=O and C–O stretching bands in the IR spectrum to those of an authentic sample of Fmoc-OSu (**6**).[3] The Fmoc-P-OSu thus prepared was used as a solid-supported Fmoc-protecting reagent for the amino group of various α-amino acids. Thus, free amino acids reacted with **9** in the presence of a base to give the corresponding protected amino acids (Table 1). After precipitation and filtration, the obtained Fmoc-amino acids were obtained from the aqueous solutions upon acidification. The Fmoc-amino acids were pure (^{1}H NMR) and the filtered solid consisted of P-HOSu (**8**), which could be re-used for the preparation of new **9**.

The Fmoc-protection reaction was performed in a mixture of acetone/water as solvent and in the presence of K_2CO_3 as base. Changes in solvent to dichloromethane/water mixtures did not reported appreciable differences (Table 1, compare entries 1 and 4). In addition, changing the base to $NaHCO_3$ afforded similar results (Table 1, compare entries 1 and 2), whereas the use of NaOAc lowered slightly the final yield (Table 1, entry 3). The isolated yields of the obtained Fmoc-amino acids were generally good and in some cases not very different compare to those reported when Fmoc-OSu has been used. Thus, Fmoc-Ala was isolated in 95% yield when **9** was used (Table 1, entry 1), whereas a 97% yield of Fmoc-Ala has been reported when using Fmoc-OSu.[3] In addition, a 87% reported yield of the corresponding Fmoc-protected amino acid has been obtained using Fmoc-OSu with the more sterically hindered Val,[3] whereas Fmoc-P-OSu afforded 75% yield of Fmoc-Val (Table 1, entry 6). It is interesting to notice that this latter yield is considerably higher than the 43% reported when polystyrene-supported in situ generated Fmoc-OBt was employed.[10] The isolated yield of Fmoc-Phe and Fmoc-Pro using **9** was moderate (Table 1, entries 9 and 11) and low in the case of Fmoc-Asn (Table 1, entry 13). When the *N*-protection reaction was performed with an alkylamine such as isopropylamine, the obtained final yield was 82%, which is slightly higher than to that reported when using in situ generated Fmoc-OBt (78%).[10] In addition, the reaction of **9** with 2-morpholinoethylamine afforded the corresponding Fmoc-protected amine in 94% yield, which is similar to the 93% yield reported when the reagent from polymer **7** was employed.[11]

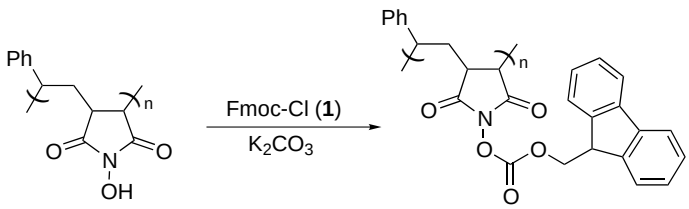
We conclude that Fmoc-P-OSu can be a promising new solid-supported amino *N*-Fmoc-protecting reagent which can be prepared from inexpensive P-HOSu (**8**) allowing its easy separation from the final Fmoc-amino acid, and its recovery once the protection reaction is finished. Further studies on the application of this styrene/maleic anhydride co-polymer (P-HOSu, **8**) for the synthesis of other solid-supported reagents useful in peptide synthesis are now underway.

In a *typical Fmoc-protection reaction of amino acids using Fmoc-P-OSu*, to a suspension of **9** (270 mg, 0.4 mmol) in acetone (20 mL) was added a solution of the

Table 1. *N*-Fmoc-amino acids prepared using Fmoc-P-OSu (**9**)

Entry	Fmoc-amino acid	Solvent	Base	Yield (%)[a,b]
1	Fmoc-Ala	Acetone/water	K_2CO_3	95
2	Fmoc-Ala	Acetone/water	$NaHCO_3$	95
3	Fmoc-Ala	Acetone/water	NaOAc	89
4	Fmoc-Ala	CH_2Cl_2/water	K_2CO_3	94
5	Fmoc-Gly	Acetone/water	K_2CO_3	96
6	Fmoc-Val	Acetone/water	K_2CO_3	75
7	Fmoc-Leu	Acetone/water	K_2CO_3	70
8	Fmoc-Ile	Acetone/water	K_2CO_3	68
9	Fmoc-Phe	Acetone/water	K_2CO_3	46
10	Fmoc-Pro	Acetone/water	K_2CO_3	43
11	Fmoc-Ser	Acetone/water	K_2CO_3	77
12	Fmoc-Thr	Acetone/water	K_2CO_3	65
13	Fmoc-Asn	Acetone/water	K_2CO_3	34

[a] Isolated pure compounds (^{1}H NMR).
[b] All compounds gave satisfactory NMR (^{1}H and ^{13}C), IR and MS data.



8 **9**

Scheme 1.

corresponding amino acid (0.4 mmol) and K_2CO_3 (39 mg, 0.4 mmol) in water (15 mL). The suspension was stirred at rt for 1 day and the solvents were evaporated in vacuo (15 Torr). Toluene was added (20 mL) and the resulting solid was filtered. The solid was suspended in water (20 mL) and the P-HOSu was filtered off. The filtrate was acidified with HCl(c) (2 mL) and extracted with AcOEt (3×20 mL). The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo (15 Torr) affording the pure Fmoc-amino acids.

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