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2,7-Di-*tert*-butyl-Fmoc-P-OSu: A New Polymer-Supported Reagent for the Protection of the Amino Group

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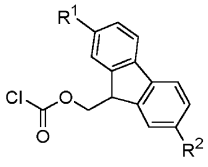
Abstract—The polymer-supported (2,7-di-*tert*-butyl-9-fluorenyl)methyl succinimidyl carbonate (Dtb-Fmoc-P-OSu), derived from (2,7-di-*tert*-butyl-9-fluorenyl)methyl chloroformate (Fmoc-Cl) and a polymeric *N*-hydroxysuccinimide (P-HOSu), has been used for the preparation of Dtb-Fmoc-protected amines and amino acids. After the *N*-protection reaction, the liberated P-HOSu can be recovered and reused. This Dtb-Fmoc-protection improves the solubility of the Fmoc-protected analogues.

The 9-fluorenylmethoxycarbonyl (Fmoc) group has grown enormously in popularity since its introduction as a protecting group for primary and secondary amines and especially for amino acids. It is employed widely in peptide synthesis due to its stability to acid and lability to base, the chloroformate ester Fmoc-Cl **1** being traditionally employed for its incorporation.[1,2] However, in spite of its properties as a protecting group, very few modifications to the basic structure of the Fmoc group have been reported in order to increase its versatility.[3] Thus, 9-(2-sulfo)fluorenylmethoxycarbonyl chloride (**2**, Sulfmoc-Cl) has been prepared by sulfonation of Fmoc-Cl **1** and used for the incorporation of the Sulfmoc group on the free amine of a growing peptide chain during a solid-phase synthesis,[4a] allowing the easy ion-exchange chromatographic purification of the final cleaved peptide. In addition, 9-(2,7-dibromo)-fluorenylmethoxy carbonyl chloride **3** has been prepared by bromination of **1**. The presence of the two electron-withdrawing bromine groups favors an easier final removing of the *N*-protection using less basic amines.[4b] However, the use of reagents **2** and **3** has not been very extensive.

Recently, other 2,7-disubstituted Fmoc-derived reagents have been prepared in order to overcome the problem of the poor solubility of many Fmoc-protected amino acids in organic solvents. Thus, 2,7-di-*tert*-butyl-9-fluorenylmethoxycarbonyl chloride **4** (Fmoc*-Cl or Dtb-Fmoc-Cl) has been reported and used as a protecting group reagent, enhancing solubilities of the corresponding Dtb-Fmoc-protected derivatives by up to 2 orders of magnitude compared to the Fmoc-protected counterparts.[5] The deprotection can be carried out with 20% solution of piperidine in DMF, and the piperidine adduct formed upon deprotection can be easily removed by hexane extraction.[5] In addition, 2,7-bis(trimethylsilyl)-9-fluorenylmethoxycarbonyl chloride (**5**, Bts-Fmoc-Cl) has been obtained and used as a protecting reagent showing also a noticeable enhancement in the solubility of the final systems, its preparation is, however, rather cumbersome.[6]



1, $R^1 = R^2 = H$
2, $R^1 = SO_3H$, $R^2 = H$
3, $R^1 = R^2 = Br$
4, $R^1 = R^2 = t\text{-Bu}$
5, $R^1 = R^2 = SiMe_3$

In spite of the wide use of chloroformates, mainly Fmoc-Cl **1**, it is somewhat unstable and toxic and tends to form 'Fmoc-dipeptide' by-products.[7] For these reasons, efforts have been devoted to the preparation of Fmoc-protecting reagents which presents a different leaving group instead of the chlorine.[8] Amongst the reagents developed, the 9-fluorenylmethyl succinimidyl carbonate Fmoc-OSu **6** (Su = succinimidyl) is probably the reagent that has shown more applicability due to its shelf-stability and high performance.[1,7,8d,9] In respect to this compound, our group has recently reported the

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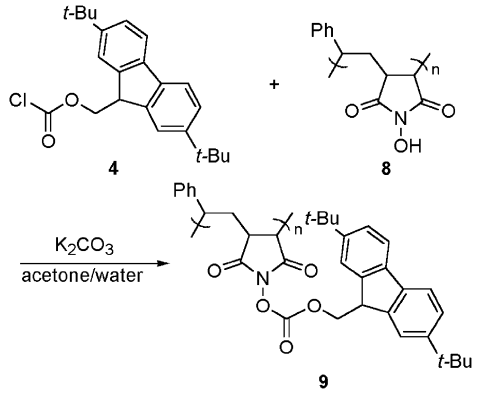
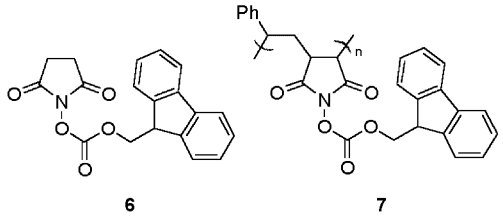


Scheme 1. Synthesis of Dtb-Fmoc-P-OSu **9**.

preparation of a reagent that incorporates the succinimide moiety into a polymer.[10] This polymer-supported Fmoc-OSu (Fmoc-P-OSu) **7** is partially soluble in acetone and is as efficient as the non-polymeric **6**, allowing the easy separation and recovery of the polymer-supported *N*-hydroxysuccinimide (P-HOSu) once the protection reaction has finished, something especially valuable when working on a small scale. From these antecedents we proposed the development of other polymer-supported Fmoc-OSu-related reagents useful for the protection of the amino group but affording more soluble protected compounds. The 2,7-di-*tert*-butyl substitution on the fluorenyl system employed in reagent **4** seemed the appropriate choice due to the easy direct introduction of the *tert*-butyl groups to the fluorene using a Friedel–Crafts alkylation reaction.[11]



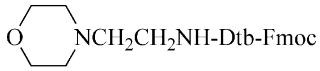
The synthesis of polymer-supported di-*tert*-butyl-Fmoc-OSu **9** (Dtb-Fmoc-P-OSu) was accomplished following the synthetic methodology outlined in Scheme 1. Thus, starting 2,7-di-*tert*-butyl-9-fluorenylmethoxycarbonyl chloride **4** was prepared from 2,7-di-*tert*-butyl-9-fluorenylmethanol[5] in 95% yield by reaction with triphosgene, instead of using the toxic phosgene.[5] The reaction of a solution of chloroformate **4** in acetone with polymeric *N*-hydroxysuccinimide **8** (P-HOSu),[12] in the presence of K_2CO_3 in water afforded Dtb-Fmoc-P-OSu **9** as a stable white solid, which could be kept for days in an open flask at ambient temperature without appreciable decomposition.

This obtained Dtb-Fmoc-P-OSu polymer **9** showed identical C=O and C–O stretching bands in the IR spectrum to those of Fmoc-P-OSu **7**[10] and additional C–H bending doublet bands at 1382 and 1363 cm^{-1}, the long wavelength band more intense, typical of *tert*-butyl groups.

The Dtb-Fmoc-P-OSu **9** prepared was used as a solid-supported *N*-Dtb-Fmoc-protecting reagent. Thus, the amino group of amines (Table 1, entries 1–3) and different free α-amino acids (Table 1, entries 4–8) were protected after reaction with **9** in the presence of K_2CO_3 as base in acetone/water as solvent and at room temperature, to give the corresponding Dtb-Fmoc-protected systems after acidification and filtration. The filtrate contained pure Dtb-Fmoc-derivatives (^1H NMR), whereas the solid consisted of P-HOSu **8** which could be easily recovered and re-used for the preparation of new **9**.

Following this protocol, Dtb-Fmoc-protected amines such as *p*-methoxybenzylamine were obtained in 82% yield in 7 h reaction time, a yield comparable to that reported when the chloroformate **4** was used (88%).[5] It is also interesting to compare the obtained yields of *N*-Dtb-Fmoc-systems with the corresponding less-bulky Fmoc-derivatives. Thus, when the *N*-protection reaction was performed on isopropylamine, the obtained final *N*-Dtb-Fmoc-protected derivative was obtained in 94% yield (Table 1, entry 2), which is higher than when the corresponding Fmoc-protected amine was obtained using a reported Fmoc-protecting reagent such as polystyrene-supported in situ generated Fmoc-OBt (Bt = benzotriazol-1-yl) (78%),[13] and identical to when using Fmoc-P-OSu **7**. Moreover, the reaction of **9** with 2-morpholinoethylamine afforded the corresponding

Table 1. *N*-Protection of amines and amino acids with reagent **9**

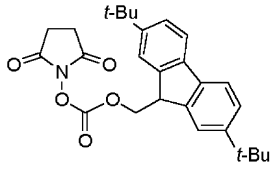
Entry	*N*-Protected compound[a]	*t* (h)	Yield (%)[b]	Mp (°C)[c]	$[\alpha]_D^{25}$,[d]
1	*p*-$MeOC_6H_4CH_2NH$-Dtb-Fmoc	7	82	142–143	
2	*i*-PrNH-Dtb-Fmoc	7	94	143–144	
3		7	96	189–190	
4	Dtb-Fmoc-Gly	24	87	188–189	
5	Dtb-Fmoc-Ala	24	83	97–98	−25.7
6	Dtb-Fmoc-Val	24	87	102–103	−26.1
7	Dtb-Fmoc-Phe	24	83	97–98	−20.3
8	Dtb-Fmoc-Aib	24	76	116–117	

[a]All compounds gave consistent spectroscopic (IR, ^1H NMR, ^{13}C NMR) data.
[b]Isolated pure crude yield (^1H NMR).
[c]For the crude products.
[d]Measured in DMF ($c = 1$).

Dtb-Fmoc-protected amine in 96% yield (Table 1, entry 3), which is similar to the yield reported when a metathesis-generated polymeric HOSu-derived Fmoc-reagent (93%)[14] or Fmoc-P-OSu **7** (94%)[10a] were employed.

When the protection reaction was carried out on different α-amino acids, the corresponding *N*-Dtb-Fmoc-protected derivatives were obtained after 1 day (Table 1, entries 4–8). In almost all cases, yields were higher than 80%, something remarkable considering, for instance, that polystyrene-supported in situ generated Fmoc-OBt afforded only 43% of Fmoc-Val,[13] and Fmoc-P-OSu **7** gave a 75% yield.[10a] In addition, the protection reaction of a sterically hindered amino acid such as the aminoisobutyric acid (Aib) (Table 1, entry 8) afforded a 76% of the corresponding protected amino acid. These Dtb-Fmoc-protected amino acids presented a much higher solubility than the corresponding Fmoc-derivatives, being soluble for instance in toluene.

We also prepared the analogous new non-polymeric reagent Dtb-Fmoc-OSu **10**,[15] in 90% yield from **4**, using the same methodology used for the preparation of **9**. This reagent presented identical characteristic IR bands than **9** and was used for performing some of the protection reactions carried out using its polymeric counterpart **9**, under the same reaction conditions. Yields obtained using **10** were rather higher than when using **9**. Thus, the reaction of *p*-methoxybenzylamine with Dtb-Fmoc-OSu afforded the corresponding *N*-protected derivative in 97% yield, whereas the *N*-protection reaction carried out on alanine gave a 93% yield.



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We conclude that Dtb-Fmoc-P-OSu **9** is a new, safe, stable, and efficient solid supported reagent for the Dtb-Fmoc-protection of the amino group. The use of this reagent affords results comparable to those obtained from the non-polymeric counterpart Dtb-Fmoc-OSu **10**, but with the advantage of the easy separation and recycling of the P-HOSu **8** liberated after the protection reaction.

In a typical Dtb-Fmoc-protection reaction of amino acids using Dtb-Fmoc-P-OSu, to a suspension of **9** (270 mg, 0.4 mmol) in acetone (20 mL) was added a solution of the 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). The solid was suspended in a mixture of AcOEt (15 mL) and water (15 mL) and acidified with HCl(c) (2 mL). The suspension was filtered and the solid, consisting in P-HOSu, was washed with AcOEt (2×10 mL) and water (2×10 mL). The combined filtrates were decanted and the organics were washed with 5% $NaHCO_3$ (3×20 mL), dried (Na_2SO_4) and evaporated affording pure protected amino acids (see Table 1).

Acknowledgements

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11. 2,7-Di-*tert*-butylfluorene is now commercially available (Aldrich).
12. Obtained with a loading of 1.5 mmol g^{-1} by reaction of inexpensive commercially available styrene/maleic anhydride co-polymer with an 50% aqueous solution of hydroxylamine (Chinchilla, R.; Dodsworth, D. J.; Nájera, C.; Soriano, J. M. *Tetrahedron Lett.* **2001**, *42*, 4487). This copolymer was easy to handle in different solvents and presented good mechanical stability, the cross-linking with diamines, as in the described case of poly(ethylene-co-*N*-hydroxymaleimide) (Fridkin, M.; Patchornik A.; Katchalski, E. *Biochem.* **1972**, *11*, 466. Andreev, S. M.; Tsiryapkin, V. A.; Samoilova, N. A.; Mironova, N. V.; Davidovich, Y. A.; Rogozhin, S. V. *Synthesis* **1977**, 303) was not found to be necessary.

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15. 2,7-Di-*tert*-butyl-9-fluorenylmethyl succinimidyl carbonate **10** (Dtb-Fmoc-OSu): mp 170–171 °C; H NMR IR (KBr) ν 1803, 1786, 1736, 1384, 1364, 1262, 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.38 (s, 18H), 2.81 (s, 4H), 4.30 (t, $J = 7.3$ Hz, 1H), 4.56 (d, $J = 7.3$ Hz, 2H), 7.44 (m, 2H), 7.64 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.4, 31.5, 34.9, 46.3, 73.3, 119.3, 122.0, 125.2, 138.7, 142.5, 150.3, 151.6, 168.5.