Fmoc*: A More Soluble Analogue of the 9-Fluorenylmethoxycarbonyl Protecting Group

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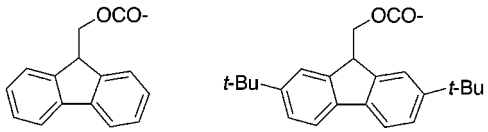
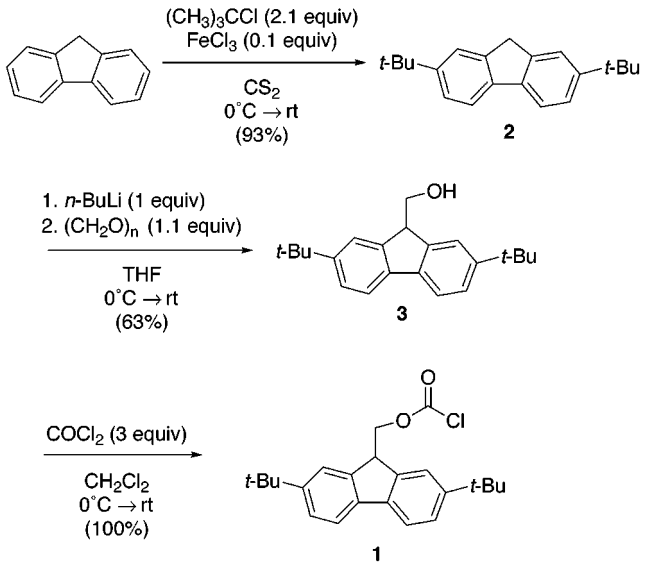
The 9-fluorenylmethoxycarbonyl (Fmoc) group has enjoyed tremendous popularity as a protecting group for amines in the synthesis of peptides and related compounds because of its stability to acid and lability to base.[1] The poor solubilities of many Fmoc-protected amino acids and other compounds in organic solvents offset its merits, as do difficulties in removing the byproduct of its deprotection.[2] We became painfully cognizant of the notorious solubility problems of the Fmoc group when we tried to use it in the synthesis of a series of peptidomimetic compounds. To solve this problem, we have developed the 2,7-di-*tert*-butyl-Fmoc group as a more soluble analogue of the Fmoc group. We have nicknamed this group *Fmoc** to reflect its relationship to the Fmoc group. This paper describes its preparation and use.



Fmoc Fmoc*

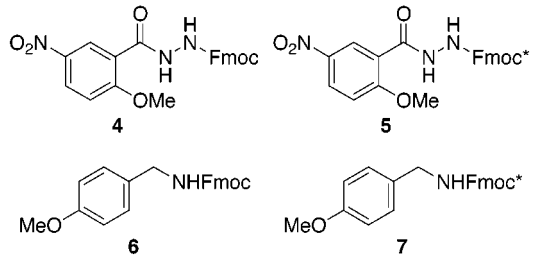
Like Fmoc, the Fmoc* protecting group is readily introduced as its chloroformate. The chloroformate (Fmoc*-Cl, **1**) is prepared in three steps from fluorene, as shown in Scheme 1. In the first step, the two *tert*-butyl groups are added by Friedel−Crafts alkylation. The di-*tert*-butylation of fluorene had been previously reported by Buu-Hoi and Cagniant. Their procedure, which involved treating a neat mixture of fluorene and *tert*-butyl chloride with $AlCl_3$, proved capricious.[3] We have found that using carbon disulfide as solvent and the milder catalyst $FeCl_3$ renders the reaction reproducible and affords di-*tert*-butylfluorene **2** in high yield. The hydroxymethyl group is added in the second step. This step involves the lithiation of **2** and its reaction with paraformaldehyde by a procedure analogous to Chong, Lajoie, and Tjepkema's conversion of fluorene to 9-fluorenylmethanol.[4] This reaction is the most difficult of the three. Only 1.0 equiv of *n*-BuLi should be used; when we used an excess of 0.1 equiv, we obtained substantially diminished yields of hydroxymethyl product **3**, along with the fulvene elimination product and recovered di-*tert*-butylfluorene **2**. The reaction mixture sometimes undergoes a color change

Scheme 1



roughly 25 min after the paraformaldehyde is added, but this color change does not appear to indicate completion of the reaction (in contrast to that reported for the conversion of fluorene to 9-fluorenylmethanol). By ^1H NMR spectroscopic monitoring of this reaction, we found that the optimal time for quenching the reaction is after 50 min. In the third step, the hydroxymethylated compound (**3**) is converted to Fmoc*-Cl by treatment with phosgene following the procedure of Carpino and Han.[1] We have found the reaction of **3** to be considerably slower than that reported by Carpino and Han for the reaction of 9-fluorenylmethanol (3 days at 24 °C vs 4 h at 0 °C).

Fmoc*-protected compounds exhibit up to 2 orders of magnitude greater solubility than their Fmoc analogues.[5] Fmoc-protected peptidomimetic building block **4** has low solubility in common volatile organic solvents, such as chloroform, tetrahydrofuran, methanol, and ether, while Fmoc*-protected analogue **5** is 1−2 orders of magnitude more soluble. Table 1 illustrates the solubilities of these compounds in these solvents. Fmoc*-protected *p*-methoxybenzylamine **7** is also more soluble than its Fmoc analogue **6**; however, the improvements in solubility are less dramatic (Table 2).



The protection and deprotection of amines and related nitrogen bases with Fmoc* is just like their protection and deprotection with Fmoc. Amines can be protected by

(1) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *22*, 3404−3409.
(2) Kocienski, P. J. *Protecting Groups*; Thieme: New York, 1994; p 202.
(3) Buu-Hoi, N. P.; Cagniant, P. *Chem. Ber.* **1944**, *77*, 121−126.
(4) Chong, J. M.; Lajoie, G.; Tjepkema, M. W. *Synthesis* **1992**, 819−820.

(5) Previous experience has shown that di-*tert*-butylation can dramatically increase the solubilities of aromatic compounds with poor solubilities. Nowick, J. S.; Ballester, P.; Ebmeyer, F.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 8902−8906.

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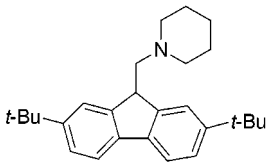
Table 1. Solubility of Fmoc and Fmoc* Derivatives 4 and 5 in Common Organic Solvents

solvent	solubility of **4** (mg/mL)	solubility of **5** (mg/mL)
$CHCl_3$	1	>100
THF	8	>110
methanol	0.2	2
ether	<0.2	1

Table 2. Solubility of Fmoc and Fmoc* Derivatives 6 and 7 in Common Organic Solvents

solvent	solubility of **6** (mg/mL)	solubility of **7** (mg/mL)
$CHCl_3$	>110	>270
THF	>130	>180
methanol	4.5	6
ether	6	50

treatment with Fmoc*-Cl in a biphasic mixture of dichloromethane and aqueous sodium carbonate[6] and can be deprotected with a 20% solution of piperidine in DMF.[7] An added benefit of Fmoc* is that the piperidine adduct that forms upon deprotection (**8**) is highly lipophilic, allowing its easy removal. This adduct can be removed from a DMF solution of the deprotected amine by extraction several times with hexanes.[8,9] Alternatively, it can be removed by dissolving the deprotected amine in dimethyl sulfoxide, a solvent in which **8** is insoluble.



8

In summary, Fmoc* alleviates solubility and byproduct-removal problems associated with the Fmoc protecting group. Fmoc*-Cl is easy to synthesize and can readily be used in place of Fmoc-Cl. We anticipate that Fmoc* will prove popular as an alternative to Fmoc.

Experimental Section

Materials and Methods. Commercial-grade reagents and solvents were used without further purification except as indicated. CH_2Cl_2 and THF were dried prior to use by percolation through anhydrous Al_2O_3 as described by Grubbs and coworkers.[10] Fluorene was recrystallized from ethanol. *n*-Butyllithium was titrated with 2-butanol using 1,10-phenanthroline as indicator and toluene as solvent.[11] *p*-Methoxybenzylamine was distilled prior to use. All reactions were performed under nitrogen; in the Friedel–Crafts and phosgenation reactions, the nitrogen line was vented to a bubbler to prevent contaminating the nitrogen manifold with HCl. Reactions were stirred magnetically, unless otherwise indicated. Solvents were removed by rotary evaporation under reduced pressure.

2,7-Di-*tert*-butylfluorene (2).[3] An ice-cooled, 2-L, three-necked, round-bottomed flask equipped with a nitrogen inlet adapter, a rubber septum, and a mechanical stirrer was charged with fluorene (25.00 g, 150.4 mmol), CS_2 (100 mL), and $FeCl_3$ (2.44 g, 15.0 mmol). 2-Chloro-2-methylpropane (34.4 mL, 316 mmol) was added via syringe over 5 min. After 10 min, the ice bath was removed, and the reaction mixture was stirred for 3.5 h. Water (100 mL) was then added, and the resulting mixture was partitioned between CH_2Cl_2 (200 mL) and 1 M aqueous HCl (100 mL). The aqueous layer was extracted with additional CH_2Cl_2 (50 mL), and the combined organic layers were washed successively with saturated aqueous $NaHCO_3$ (100 mL) and saturated aqueous NaCl (75 mL), dried over $MgSO_4$, and concentrated to yield 40.31 g of a yellow solid. The residue was dissolved in 200 mL of hexanes, and the solution was filtered through a column of silica gel (9 cm h × 8 cm d) with the aid of an additional 1 L of hexanes. The filtrate was concentrated to yield 38.82 g (93%) of 2,7-di-*tert*-butylfluorene (**2**) as a white solid, which was used in the next reaction without further purification. An analytical sample was recrystallized from ethanol: mp 120–122 °C (lit.[3] mp 122 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, J = 8.0 Hz, 2 H), 7.56 (s, 2 H), 7.38 (d, J = 8.1 Hz, 2 H), 3.86 (s, 2 H), 1.37 (s, 18 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 149.4, 143.3, 139.1, 123.8, 121.9, 119.1, 37.1, 34.8, 31.6.

2,7-Di-*tert*-butyl-9-fluorenylmethanol (3).[4] An ice-cooled, 250-mL, three-necked, round-bottomed flask equipped with a nitrogen inlet adapter, a glass stopper, a rubber septum, and a magnetic stirring bar was charged with 2,7-di-*tert*-butylfluorene (**2**) (10.00 g, 35.9 mmol) and THF (150 mL). A solution of *n*-butyllithium in hexanes (24.4 mL, 1.47 M, 35.9 mmol) was injected over 5 min via syringe. After an additional 3 min, finely pulverized paraformaldehyde (1.19 g, 39.6 mmol) was added in a single portion, the ice bath was removed, and the reaction mixture was stirred for 50 min. The reaction was then quenched by adding saturated aqueous $NaHCO_3$ (120 mL) and extracted with ether (120 mL + 3 × 50 mL). The combined organic layers were washed with saturated aqueous NaCl (100 mL), dried over $MgSO_4$, filtered, and concentrated to yield 10.87 g of white crystals. Recrystallization from hexanes afforded 6.96 g (63%) of 2,7-di-*tert*-butyl-9-fluorenylmethanol (**3**) as white prisms: mp 119–120 °C; IR (KBr) 3350 (br) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, J = 8.0 Hz, 2 H), 7.61 (s, 2 H), 7.41 (dd, J = 8.0, 1.6 Hz, 2 H), 4.07 (appar s, 3 H), 1.52 (br s, 1 H), 1.38 (s, 18 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 149.9, 144.3, 138.9, 124.7, 121.4, 119.3, 65.3, 50.5, 34.9, 31.6; HRMS (CI) m/z for $C_{22}H_{28}O$ (M^+) calcd 308.2140, found 308.2144. Anal. Calcd for $C_{22}H_{28}O$: C, 85.66; H, 9.15. Found: C, 85.71; H, 9.22.

2,7-Di-*tert*-butyl-9-fluorenylmethoxycarbonyl chloride (1). An ice-cooled, 250-mL, three-necked, round-bottomed flask equipped with a nitrogen inlet adapter, a glass stopper, a rubber septum, and a magnetic stirring bar was charged with **3** (6.75 g, 21.9 mmol), CH_2Cl_2 (20 mL), and a solution of phosgene in toluene (33.2 mL, 1.98 M, 65.7 mmol). The ice bath was allowed to melt, and the reaction mixture was stirred for 72 h. Concentration of the reaction mixture yielded 8.13 g (100%) of Fmoc*-Cl (**1**) as a light brown oil of sufficient purity for use. An analytical sample was obtained as a white solid by adding a minimal amount of pentane, chilling to −78 °C under nitrogen until crystals formed, decanting the mother liquor, and removing residual pentane under vacuum: mp 63–65 °C; IR (KBr) 1780 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 8.0 Hz, 2 H), 7.60 (s, 2 H), 7.44 (dd, J = 8.2, 2.0 Hz, 2 H), 4.54 (d, J = 8.0 Hz, 2 H), 4.26 (t, J = 7.6 Hz, 1 H), 1.37 (s, 18 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 150.7, 150.2, 142.4, 138.7, 125.3, 122.0, 119.5, 73.9, 46.1, 34.9, 31.5; HRMS (CI) m/z for $C_{23}H_{27}O_2Cl$ (M^+) calcd 370.1699, found 370.1700. Anal. Calcd for $C_{23}H_{27}O_2Cl$: C, 74.48; H, 7.34. Found: C, 74.87; H, 7.41.

(6) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; John Wiley & Sons: New York, 1999; pp 506–507.

(7) Periodic TLC monitoring of the deprotection of **6** and **7** revealed that the cleavage of the Fmoc* group proceeds at almost the same rate as the Fmoc group. Complete conversion of the Fmoc* derivative **7** to *p*-methoxybenzylamine with 20% v/v piperidine in DMF requires 20 min while the Fmoc derivative **6** requires 15 min. The slightly slower rate of the Fmoc* compound may arise from electron donation by the *tert*-butyl substituents on fluorene.

(8) UV analysis of the DMF and hexane layers demonstrated that approximately 75% of the piperidine adduct **8** is removed with each extraction by an equal volume of hexane. After four hexane washes, <1% of **8** remains in the DMF layer.

(9) The removal of the Fmoc* deprotection byproduct (**8**) is most efficient when the deprotected amine contains highly polar functional groups, such as amides. Lipophilic amines can partition into the hexanes phase resulting in a decreased yield. Deprotection of **7** followed by washing of the DMF layer with four portions of hexanes gave only a 60% yield of *p*-methoxybenzylamine. Some of the *p*-methoxybenzylamine was lost by partitioning into the hexanes layer.

(10) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. A.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518–1520.

(11) Watson, S. C.; Eastham, J. F. *J. Organomet. Chem.* **1967**, *9*, 165–168.

Representative Procedure for the Fmoc*-Protection of an Amine. An ice-cooled, two-necked, round-bottomed flask equipped with a magnetic stirring bar, nitrogen inlet adapter, and septum was charged with *p*-methoxybenzylamine (0.422 g, 3.07 mmol), dichloromethane (1.0 mL), and 10% aqueous Na_2CO_3 (8.3 mL). After 5 min, a solution of Fmoc*-Cl (1.14 g, 3.07 mmol) in CH_2Cl_2 (3.2 mL) was added over 2 min. The ice bath was removed, and the reaction mixture was stirred at room temperature for 2 h.[6] The reaction mixture was diluted with 60 mL of CH_2Cl_2 and washed with 60 mL of 1 M HCl. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to yield 1.38 g of white foam. Purification by column chromatography on silica gel (1:3 EtOAc/hexanes) yielded 1.28 g (88%) of **7** as a white solid.

Representative Procedure for the Fmoc*-Deprotection and Removal of Byproduct 8 by Extraction. A pear-shaped flask equipped with a magnetic stirring bar and nitrogen inlet was charged with 2-MeO-5-NO_2-C_6H_3-CONHNH-Fmoc* (**5**, 147 mg, 0.270 mmol). A solution of 20% (v/v) piperidine in DMF (2.7 mL) was added in one portion, and the mixture was stirred at room temperature for 20 min. The solution was diluted with 5 mL of DMF and extracted 4 × 10 mL with hexanes. The DMF layer was concentrated to yield 53.4 mg (94%) of 2-MeO-5-NO_2-C_6H_3-$CONHNH_2$ as a white solid.

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