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A convenient and scaleable procedure for removing the Fmoc group in solution

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Abstract

An improved and simple reagent system was developed to facilitate solution-phase deprotection of Fmoc-protected amines. Catalytic DBU in the presence of an aliphatic or polymer-supported thiol rapidly removed the Fmoc group to provide the amine free base in excellent yields and purity on multi-gram scale.

Keywords: protecting groups; thiols; polymer support; supported reagents/reaction.

Despite the multitude of primary and secondary amine protecting groups published in the literature,[1] the preferential use of Fmoc,[2] Boc,[3] and CBZ[4] moieties appears well entrenched. The popularity of these carbamates is not surprising since they are easily removed (using base, acid, and hydrogenolysis respectively), provide good orthogonality, are inexpensive, and many amines are sold commercially so protected. Unlike the catalytic methods available for unmasking the Boc and CBZ groups, however, the classical means of Fmoc deprotection employs a large excess of a secondary amine such as piperidine in DMF.[2d,e] Piperidine functions both as a base to fragment the Fmoc group and as a scavenger to trap the liberated dibenzofulvene (DBF) via a Michael-type addition thereby outcompeting reaction with the product amine (Scheme 1). Use of piperidine/DMF is better suited to Fmoc deprotections on solid-phase than those in solution due to the low volatility of these solvents, the solvent-dependent reversible scavenging of dibenzofulvene by piperidine, and DBF polymerization at higher concentrations.[2c]

Our research required the preparation of fluorescent 7-amino-4-methylcoumarin (AMC) amino acid derivatives for use as protease substrates.[5] These compounds are typically difficult to make without using special N^{α}-amino-protecting groups because of the acidic and hydrogenolytic lability of the coumarin or protected peptidal side chain—precluding the use of CBZ due to a violation of orthogonality.[6] Rather than investigate more esoteric or expensive amine-protecting groups, we sought to develop improved conditions for Fmoc removal in solution.

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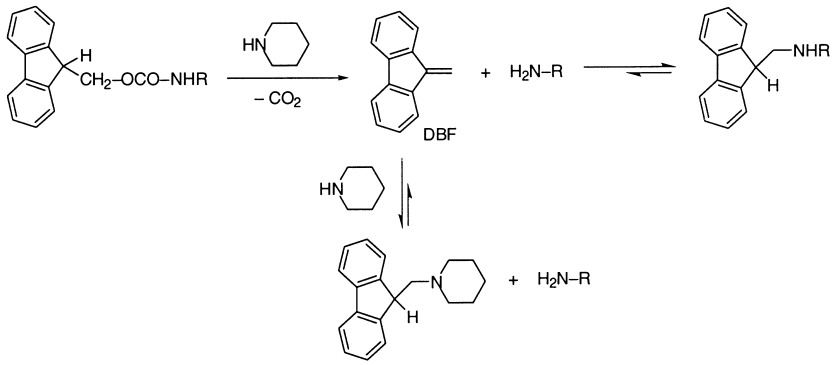


Scheme 1. Deprotection of Fmoc by piperidine

We reasoned that dissociating the base and scavenger functions of piperidine into separate reagents in a volatile solvent might facilitate the isolation of the deprotected amine product. Thus, use of a non-nucleophilic, stronger base than piperidine should allow the stoichiometry to be reduced to catalytic quantities and use of a better Michael donor than either piperidine or the product amine should lead to a concomitant reduction in scavenger stoichiometry.

A variety of soluble and polymer-supported bases were studied to assess their ability to deprotect Fmoc-Phe-OMe at catalytic levels (Fig. 1). DBU emerged as the best catalyst since 25 mol% in THF gave rapid deprotection to H-Phe-OMe and DBF within 1 h in the absence of scavenger—consistent with earlier reports describing its use in excess.[7] Unfortunately, we had less success using polystyrene-supported DBU or TBD,[8] even when using stoichiometrically, due to unfavorable kinetics resulting from the high dilution required to swell the resin.

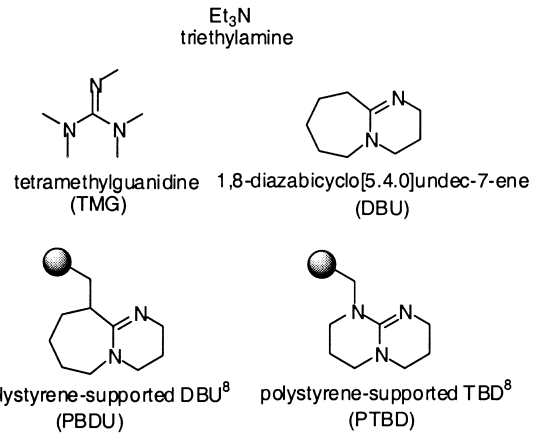


Figure 1. Amine catalysts used to deprotect the Fmoc group

We next turned to thiol-based Michael donors that might replace piperidine as superior DBF scavengers capable of outcompeting the product amine nucleophile. Ueki and co-workers showed octanethiol to be an efficient DBF scavenger[9] and we expanded the list to include several soluble and solid supported thiols (Fig. 2).

The DBF scavenging efficiency of the soluble thiols in Fig. 2[10] was assessed in two ways. First, the deprotection of Fmoc-Phe-AMC using DBU and 10 equivalents of either thiophenol, DTT,

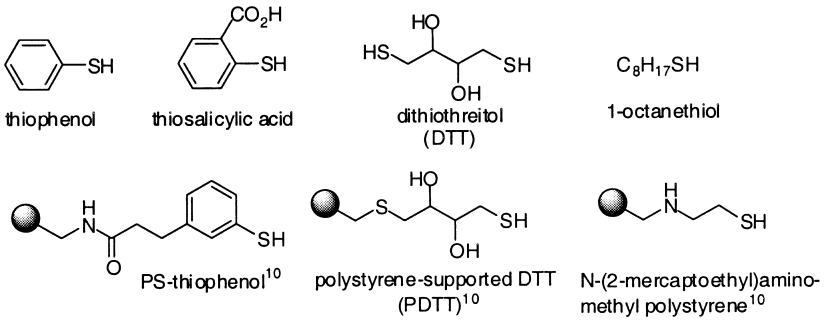
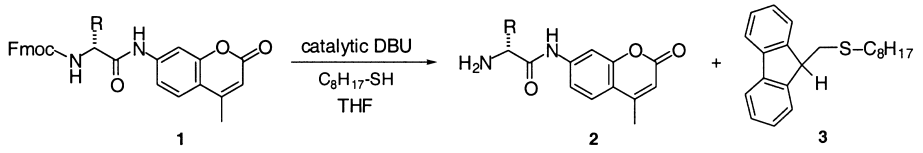


Figure 2. Thiols used to scavenge dibenzofulvene

and octanethiol was monitored for unreacted DBF by HPLC. Second, fluorenylmethyl octylthioether and fluorenylmethyl phenylthioether were independently synthesized and treated with 25 mol% DBU in THF to determine which adduct was more prone to the formation of DBF and thiol under equilibrating conditions. In both experiments, octanethiol was a faster and less reversible DBF scavenger than thiophenol and its derivatives which also tended to slow the Fmoc cleavage by quenching the catalytic DBU due to their increased acidity. Because the Michael addition to form the thioether is a reversible process, 10 equivalents of 1-octanethiol were required to give >99% DBF scavenging.

Using a catalytic DBU/octanethiol cocktail in THF proved to be the optimal reagent system for preparing the representative set of compounds listed in Table 1.[11] No reaction occurred without added DBU and the reaction times could be dramatically reduced using additional DBU catalyst. Since octanethiol, DBU, and fluorenylmethyl octylthioether (**3**) are all diethylether miscible, the product amine is easily isolated by trituration, recrystallization, column chromatography, or extraction.

Table 1
Deprotection of Fmoc by DBU/1-octanethiol



Entry	Starting Material (**1**)	Product (**2**)	DBU (mol%)	Time (h)	% Yield
1	Fmoc-Lys(Boc)-AMC	H-Lys(Boc)-AMC	3	4	100[a]
2	Fmoc-Ser(tBu)-AMC	H-Ser(tBu)-AMC	10	3	70[b]
3	Fmoc-Glu(OtBu)-AMC	H-Glu(OtBu)-AMC	10	48	90[b]
4	Fmoc-Asn(Trt)-AMC	H-Asn(Trt)-AMC	10	23	81[b]
5	Fmoc-Cys(Trt)-AMC	H-Cys(Trt)-AMC	10	30	88[b]

All reactions were run on 25-76 mmol scale in THF at rt for the indicated time using 10 eq octanethiol.[11] Purities were determined to be >95% AUC @ 214 nm by analytical HPLC.

[a] Yield after trituration with Et_2O.

[b] Yield after trituration with Et_2O and subsequent recrystallization from EtOH/Et_2O.

Also successful was the use of *N*-(2-mercaptoethyl)aminomethyl polystyrene as a solid-supported scavenger[12] for DBF that could be removed by filtration.[13] This finding is significant since use of solid-supported piperidine derivatives proved unsuccessful in our hands and are known to give inconsistent results and incomplete scavenging.[14] The resin can also be recycled by treating it with 25% piperidine in DMF.[15]

In conclusion, the catalytic DBU/octanethiol reagent system proved to be generally useful for small to large-scale deprotections of the Fmoc group in solution. Use of *N*-(2-mercaptoethyl)-aminomethyl polystyrene resin as the dibenzofulvene scavenger provided a convenient means of deblocking the Fmoc group by simple filtration and evaporation to the free base. These methods are an improvement over conventional methods and should find a broad utility for deblocking Fmoc-protected amines.

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10. PS-thiophenol was purchased from Argonaut Technologies; PDTT was synthesized from Merrifield resin and DTT; *N*-(2-mercaptoethyl)aminomethyl PS was purchased from Novabiochem.
11. **Typical DBU/octanethiol deprotection procedure**: To Fmoc-Lys(Boc)-AMC (15.9 g, 25.5 mmol) magnetically stirring in 250 mL of dry THF was added 1-octanethiol (44.2 mL, 255 mmol) followed by dropwise addition of catalytic 1,8-diazabicyclo[5.4.0]undec-7-ene (114 μL, 0.77 mmol, 3 mol%) over 15 min. The flask was connected to a N_2 inlet/outlet and allowed to stir at rt for a total of 4 h after which analytical HPLC or TLC indicated that the starting material was consumed and the dibenzofulvene had been scavenged. The solvents were removed by rotary evaporator and the residue triturated with Et_2O. The white solid was collected by filtration and dried under vacuum to give 10.3 g (100%) of analytically pure H-Lys(Boc)-AMC; ^{1}H NMR (300 MHz, CD_3OD) δ 7.80 (s, 1H), 7.67 (d, 1H), 7.47 (d, 1H), 6.19 (s, 1H), 3.42 (m, 1H), 2.99 (m, 2H), 2.41 (s, 3H), 1.72 (m, 2H), 1.60 (m, 2H), 1.44 (m, 2H), 1.35 (s, 9H); ES-MS m/z 404 ($M+H^+$), $M_r = 404$ calcd for $C_{21}H_{30}N_3O_5$. In general, products may be optionally recrystallized from $EtOH/Et_2O$.
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13. **Typical DBU/N-(2-mercaptoethyl)aminomethyl PS deprotection procedure**: Fmoc-Ser(*t*Bu)-AMC (345 mg, 0.64 mmol) was dissolved in THF (18 mL) in a 40 mL polypropylene screw cap vial. *N*-(2-Mercaptoethyl)aminomethyl polystyrene (5.0 g, 1.26 mmol/g, Novabiochem) was added followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (48 μL, 0.32 mmol, 50 mol%) and the tube was capped under N_2 and shaken reciprocally on its side for 1.5 h or until reaction monitoring by TLC or analytical HPLC confirmed complete deprotection and DBF scavenging. The

reaction mixture was filtered through a glass frit and the resin washed 2×THF and 2×MeOH, and the combined solvents were concentrated under vacuum. Residual DBU was removed by passing the oil through a silica gel plug with 10% MeOH in DCM followed by concentration to give 218 mg (90%) of pure H-Ser(tBu)-AMC; ^{1}H NMR (300 MHz, CD_3OD) δ 7.78 (s, 1H), 7.65 (d, 1H), 7.45 (d, 1H), 6.18 (s, 1H), 3.56 (m, 2H), 3.26 (m, 1H), 2.40 (s, 3H), 1.13 (s, 9H); ES-MS m/z 319 ($M+H^+$), $M_r = 319$ calcd for $C_{17}H_{23}N_2O_4$.

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15. *N*-(2-Mercaptoethyl)aminomethyl polystyrene resin could be regenerated by agitating it with three 25 mL portions of 25% piperidine in DMF on a glass fritted funnel for 10 min per cycle followed by filtration. Minimal fluorescence was released after the third wash as seen by TLC under a handheld long wavelength UV lamp. The resin was rinsed 2×DMF, 3×MeOH, and dried under vacuum.