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New Methods for Side-Chain Protection of Cysteine

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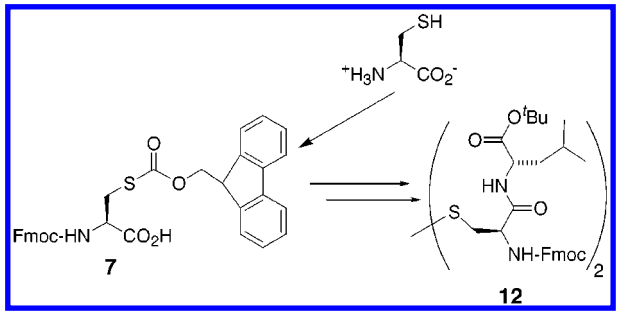
ABSTRACT



Cysteine sulfhydryl protection with either the Fmoc or the Fm group was accomplished in one step and in high yield using commercially available FmocCl or FmocOSu, respectively. Mechanisms for the Fmoc to Fm transformations are discussed. Additionally, Fmoc-Cys(Fmoc)-OH (7) was synthesized and used in amide bond forming reactions. The *S*-Fmoc group is cleaved selectively from peptides containing the *N*-Fmoc group.

The 9-fluorenylmethyloxycarbonyl (Fmoc) group, introduced by Carpino and Han in 1970,[1] has become one of the most widely used protecting groups in peptide chemistry and is gaining popularity in sugar[2] and nucleotide[3] chemistry. While Fmoc is used to protect nitrogen and alcohols,[4] Fmoc-protected thiols have not been reported. Instead, the related 9-fluorenylmethyl (Fm) group is used for thiol protection.[5] The Fm group is prepared in a two-step process that usually begins with 9-fluorenylmethanol.[6] Here, we report the synthesis of di-Fmoc-protected cysteine **7** and its use in peptide coupling reactions. The *S*-Fmoc protecting group can be removed preferentially over the *N*-Fmoc group. Additionally, we developed an efficient, one-step synthesis of the Fm-protected thiol from either the Fmoc-protected or free thiol.

Reaction of Boc-Cys-OMe[7] (**1**) with FmocCl and TEA in CH_2Cl_2 led to the formation of the Fmoc derivative **2** in 98% yield (Scheme 1). The Fmoc-protected thiol **2** is a stable and easily handled white solid having a shelf life of at least 6 months. However, when thiol **1** was reacted with FmocOSu and TEA in CH_2Cl_2, only Boc-Cys(Fm)-OMe (**3**) was isolated in 91% yield. Compound **2** was not detected in the reaction mixture.

Since the only difference between the reaction conditions for formation of **2** or **3** is the Fmoc reactant, the reaction mechanism was investigated in detail. Reaction of **2** with a 1:1 mixture of HOSu and TEA for 4 h led to the loss of CO_2 and the formation of **3** in 92% yield. Additional

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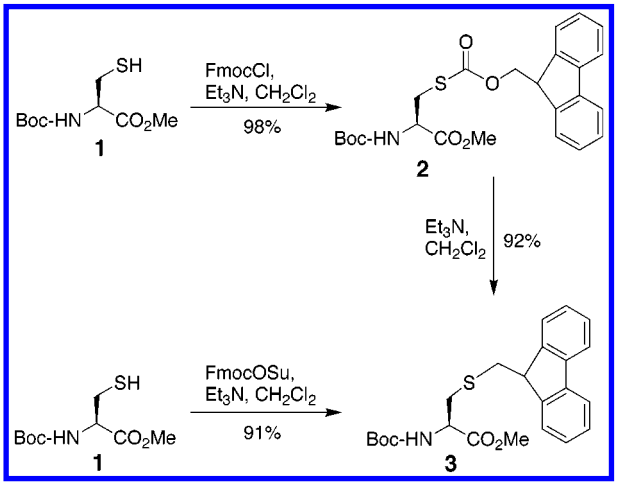
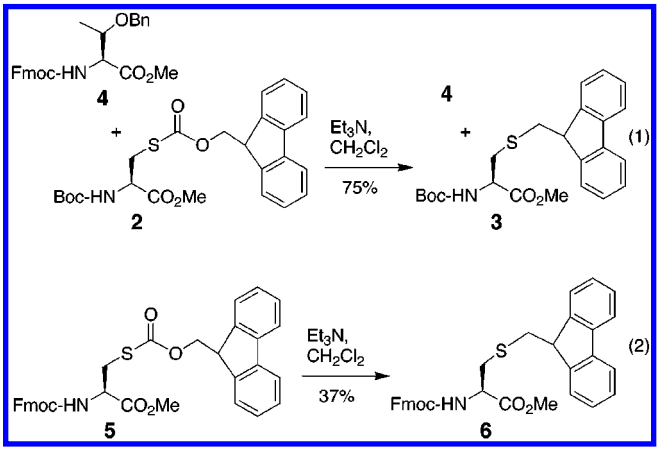
Scheme 1



Scheme 2



experiments showed that when **2** was exposed to stoichiometric TEA, conversion of Fmoc to Fm was complete in 4 h, but when **2** was exposed only to HOSu for 24 h, no reaction occurred. These results show that the conversion from Fmoc to Fm is dependent upon the tertiary amine base.

Both the pK_a and the steric bulk of the amine affect the transformation of the *S*-Fmoc group (**2** or **5**) to the *S*-Fm derivative (**3** or **6**) as shown by the data in Table 1.[8] Weaker

Table 1. Effect of Base and N-Protecting Group on *S*-Fmoc Cleavage of R-Cys(Fmoc)-OMe for **2** to **3** (R = Boc) or **5** to **6** (R = Fmoc)

		% yield	
base[a]	pK_a	**3**	**6**
N-hydroxysuccinimide	4	0	0
pyridine	5.25	0	0
N-methylmorpholine	7.38	5[b]	<5[c]
triethylamine (1.0 equiv)	10.75	92	37
triethylamine (0.1 equiv)	10.75	11[b]	nd[d]
piperidine	11.12	69[c]	nd
diisopropylethylamine	11.44	<2	<2

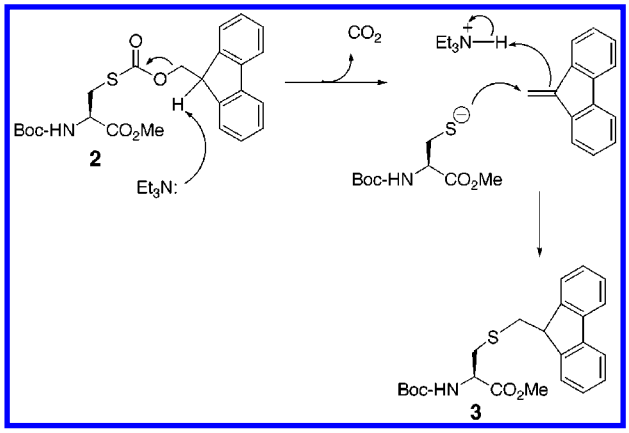
[a] pK_a values of the conjugate acid. [b] Mass balance was starting material and free thiol. [c] Mass balance was disulfide formation. [d] nd = not determined.

tertiary bases such as pyridine and *N*-methylmorpholine do not efficiently effect *S*-Fmoc cleavage, and deprotection with the more hindered diisopropylethylamine is much slower.[9]

The stability of the *S*-Fmoc group relative to the *N*-Fmoc group was determined in a pair of experiments (Scheme 2). Reaction of Boc-Cys(Fmoc)-OMe (**2**) and Fmoc-Thr(OBn)-OMe (**4**) with TEA in a competition experiment afforded conversion of *S*-Fmoc to *S*-Fm without any *N*-Fmoc removal (reaction 1). In addition, reaction of Fmoc-Cys(Fmoc)-OMe (**5**) with TEA afforded complete *S*-Fmoc removal without loss of the *N*-Fmoc group (reaction 2), illustrating a remarkable difference in the chemical reactivity of these two groups. The yield of Fm **6** could be improved to 82% by changing the solvent to CH_3CN; however, under these conditions a trace of *N*-Fmoc cleavage was observed by TLC.

The data obtained are consistent with a mechanism for conversion of *S*-Fmoc to *S*-Fm via the expected elimination/addition reaction pathway shown in Scheme 3. When

Scheme 3



FmocOSu is used in the synthesis of **2**, free TEA exists in the reaction mixture, and the reaction proceeds via the expected elimination−decarboxylation pathway to form the free thiolate in the presence of the reactive dibenzofulvene. The thiolate then adds to the dibenzofulvene in a Michael-type reaction to form the Fm-protected cysteinyl derivative.[10] Additional supporting evidence for this pathway includes

(8) The conversion of **2** to **3** took place in CH_2Cl_2 with only 1 equiv of the designated base and the cysteine derivative in the reaction mixture. The products were identified by ^{1}H NMR after a reaction time of 4 h.

(9) At higher concentrations of reactants and temperatures above 40 °C during workup, the deprotection of the *S*-Fmoc group is faster.

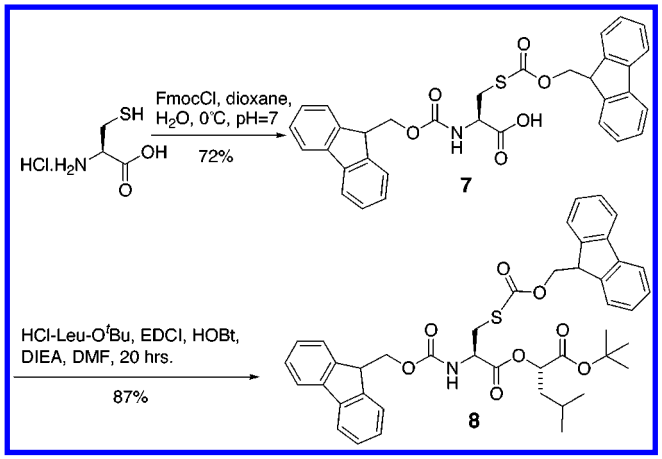
(10) Addition of nucleophiles to dibenzofulvene: Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.

detection of dibenzofulvene in the reaction mixture by ^{1}H NMR and isolation of dibenzofulvene and Boc-Cys-OMe (**1**) from the reaction mixture after shorter reaction times.

The surprising result is obtained with FmocCl, which forms the *S*-Fmoc derivative exclusively. We believe this results from the weaker base strength of chloride vs the anion of *N*-hydroxysuccinimide formed during the reaction. Reaction of the two Fmoc reagents with the thiol produces two leaving groups with very different pK_a values. The anion of HOSu is 11 orders of magnitude more basic than that of chloride (HOSu pK_a = 4 vs HCl pK_a = −7 in water). During the course of the reaction using FmocCl, the HCl produced fully protonates TEA, thus preventing abstraction of the Fmoc β-hydrogen by the amine base. When FmocOSu is used in the reaction, the HOSu does not fully protonate TEA, allowing proton abstraction and transformation of **2** to **3** as demonstrated previously (vide supra). The anion of *N*-hydroxysuccinimide also can deprotonate the *S*-Fmoc group under specific reaction conditions: DIEA/HOSu slowly cleaves the *S*-Fmoc group from esters **2** and **5** but not from amide **8**.[11]

The usefulness of the *S*-Fmoc derivative was demonstrated with the synthesis and peptide coupling of *N*- and *S*-Fmoc-protected cysteine derivative **7**. Reaction of Fmoc-Cl with commercially available HCl−Cys-OH by using a protocol described for di-Boc protection of cysteine (Scheme 4)[12] gave

Scheme 4



di-Fmoc acid **7**. Alternate synthetic routes to **7** from Fmoc-Cys-OH (under various conditions) or by saponification of Fmoc-Cys(Fmoc)-OMe (**5**) led to complex mixtures of products. Incorporation of protected amino acid **7** into peptides can be readily achieved. Reaction of **7** and HCl−Leu-O^{t}Bu under standard conditions afforded dipeptide **8** in 87% yield.[13]

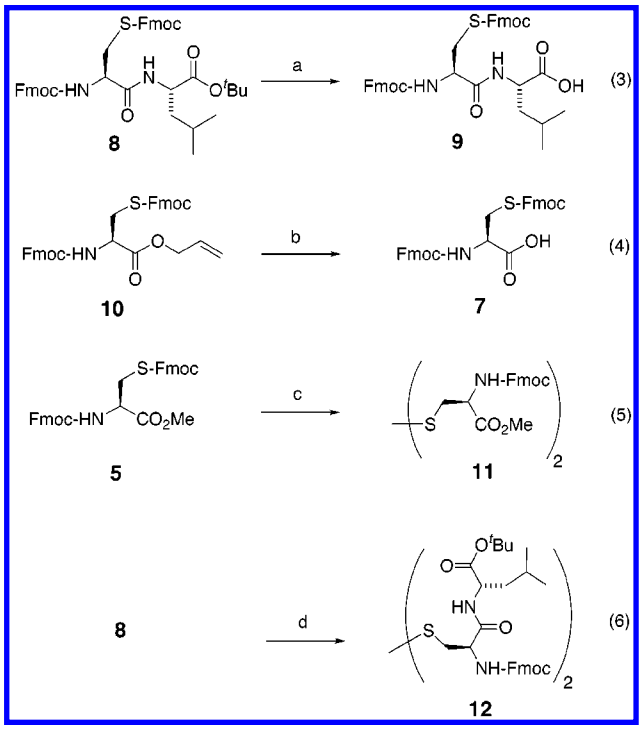
(11) In an additional experiment compound **3** was treated with a 1:1 mixture of DIEA:HOSu, yielding a mixture of starting material and disulfide in a 1:1 ratio.

(12) Schnabel, E.; Stoltefuss, J.; Offe, H. A.; Klaude, E. *Justus Liebigs Ann. Chem.* **1971**, *743*, 57.

(13) The *S*-Fmoc group in amide derivative **8** is more stable under basic conditions than esters **2** or **5**.

The protecting groups on dipeptide **8** can be removed selectively from either the carboxylic acid or sulfur functionality (Scheme 5). For the carboxylic acid, both *tert*-butyl

Scheme 5[a]



[a] (a) 4 N HCl in dioxane, 77%; (b) Pd(PPh$_3$)$_4$, dimedone, THF, 94%; (c) Et$_3$N, I$_2$, MeOH, CH$_2$Cl$_2$, 75%; (d) Et$_3$N, C$_6$H$_5$CH$_2$SH, CH$_3$CN, CH$_2$CL$_2$ 45%.

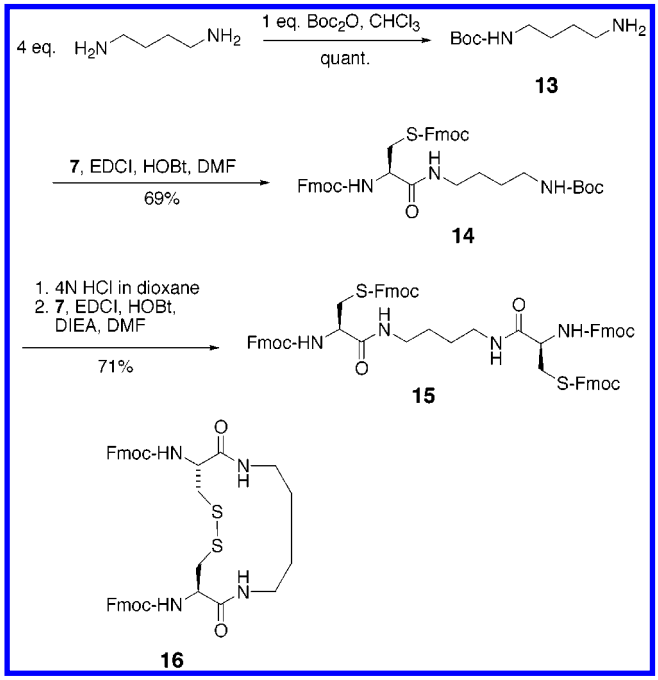
and allyl were used successfully as orthogonal protecting groups. Removal of the *tert*-butyl ester in dipeptide **8** with 4 N HCl in dioxane provided the free acid **9** without any detectable loss of the *S*-Fmoc group (reaction 3). Selective allyl removal was accomplished by reacting fully protected amino acid Fmoc-Cys(Fmoc)-OAllyl (**10**) with Pd(PPh$_3$)$_4$ and dimedone in THF to give free acid **7** in 94% yield (reaction 4).[14] Furthermore, the reactivity differences between *N*- and *S*-Fmoc allow selective removal of the *S*-Fmoc group with Et$_3$N in the presence of iodine or benzenethiol, to form disulfides **11** and **12**, respectively, without any detectable loss of the *N*-Fmoc group (reactions 5 and 6).

Di-Fmoc cysteine **7** was used to produce pseudosymmetrical diamide **15** (Scheme 6). Reaction of **7** and mono-Boc-protected putrescene[15] using EDCI and HOBt gave Fmoc-Cys(Fmoc)-DAB-Boc **14** in 69% yield. The Boc group was cleaved with 4 N HCl in dioxane and the acid coupled with **7** to give di-Fmoc-Cys diamide **15** in 71% for both steps. Attempts to prepare diamide **15** directly from unprotected putrescene and di-Fmoc cysteine **7** were unsuccessful; only low yields of **15** were obtained under a variety of reaction conditions.

(14) Zhang, H. X.; Guibe, F.; Balavoine, G. *Tetrahedron Lett.* **1988**, *29*, 623.

(15) Krapcho, A. P.; Kuell, C. S. *Synth. Comm.* **1990**, *20*(16), 2559.

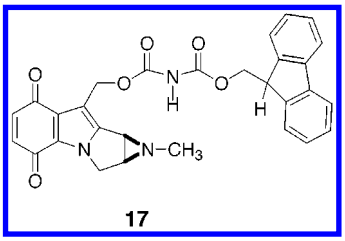
Scheme 6



Compound **15** was used to form the 14-membered ring macrocycle **16** by selective deprotection and oxidation of sulfur. Synthesis and evaluation of this compound as a potential parallel β-sheet initiator will be reported separately.

The differences in rate of cleavage of the *N*- and *S*-Fmoc groups can be rationalized by considering the effect of leaving group pK_a on the reaction mechanism. The mechanism for β-elimination in the fulvene system has been postulated to lie on the borderline between E1cb and E2.[16] The leaving group has a large influence on which mechanism occurs, i.e., the better the leaving group, the more the E2 pathway predominates. When the mechanism is E1cb (presumably as it is for nitrogen), the rate of reaction becomes dependent on removal of the Fmoc β-hydrogen. While typical *N*-Fmoc deprotection takes place using an excess of secondary amine base, experimental evidence shows a tertiary amine base will eventually effect deprotection, but at a much slower rate.[17] However, with the *O*- and *S*-Fmoc cases, better leaving groups are present which shift the reaction mechanism to the E2 pathway and increase the Fmoc cleavage rate in the presence of a tertiary amine base. Interestingly, diisopropylethylamine did not cleave the *S*-Fmoc group (Table 1), but *N*-methylmorpholine did, suggesting that deprotonation with hindered bases is not easily achieved. It is possible that other systems containing the *S*-Fmoc group may be sufficiently hindered to escape removal with triethylamine.

Another example of a leaving group altering the normal *N*-Fmoc cleavage can be found in a reaction reported by Vedejs et al. In their work, an *N*-Fmoc group was rapidly removed from the carbamate nitrogen in mitosene deriviative **17** in 89% yield by reaction with Et_3N in CH_3CN.[18] Since



Et_3N has been shown to be largely ineffective in the removal of *N*-Fmoc from simple amines, the observed cleavage may be due to the enhanced leaving group potential of the carbamate nitrogen.[19]

Our results establish that *S*-Fmoc-protected cysteine is readily synthesized and can be used to prepare *S*-Fm-Cys derivatives in good yield. These reaction conditions give *S*-Fm protection more efficiently and in higher yield than previously reported methods.[5] Fmoc-Cys(Fmoc)-OH (**7**) has also been shown to be a readily available compound amenable to peptide coupling reactions in good yield. Additionally, *S*-Fmoc was found to be more labile than *N*-Fmoc when exposed to a tertiary amine base, and this differential reactivity may be useful in synthetic strategies involving selective deprotection of SR groups in the presence of *N*-Fmoc groups.

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Supporting Information Available: Experimental procedures and full characterization for compounds **1**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Vedejs, E.; Klapars, A.; Naidu, B. N.; Piotrowski, D. W.; Tucci, F. C. *J. Am. Chem. Soc.* **2000**, *122*, 5401.

(19) Alternatively, the reaction might proceed via an imide anion formed six atoms away from the Fmoc proton.