One-Pot Synthesis of *S*-Acetamidomethyl-*N*-fluorenylmethoxycarbonyl-L-cysteine (Fmoc-Cys(Acm)-OH)

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A new strategy for the preparation of *S*-acetamidomethyl-*N*-fluorenylmethoxycarbonyl-L-cysteine is reported. Inexpensive, readily available reagents are used, and no special equipment is required. A very homogeneous product is obtained after a simple crystallization at the end of the process, without purification of intermediates.

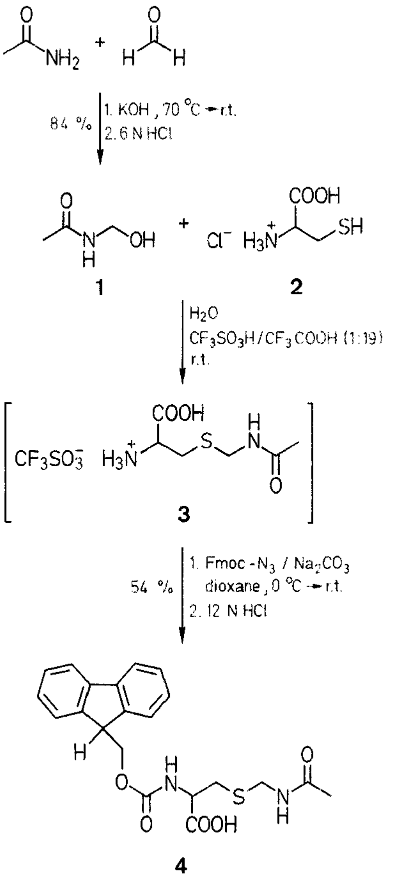
It is well known that the side chain of cysteine always has to be protected during the coupling step of a peptide synthesis. As a result, thiol protection has been the object of many conscientious studies.[1,2,3,4] One of the most used cysteine protecting groups in solid phase peptide synthesis is acetamidomethyl (Acm),[5] which has the great advantage of being stable to all the usual reagents in the synthesis of either free peptides or protected segments in a convergent strategy.[6] Thus, it can be deprotected independently at the end of the synthesis, once all other protecting groups have been removed.[2]

Amino acids are very often introduced as *N*-*t*-butoxycarbonyl (Boc) or *N*-fluorenylmethoxycarbonyl (Fmoc) derivatives. Two syntheses of Fmoc-Cys(Acm)-OH are described in the literature. The preparation of an amorphous compound from Fmoc-chloride and H-Cys(Acm)-OH has been reported.[7] No experimental details or melting points were given. More recently, a further synthesis by the same method has been described.[3] It is indicated that, in some preparations, the compound failed to crystallize in a pure form and had to be purified by silica-gel chromatography.

We have undertaken the synthesis of Fmoc-Cys(Acm)-OH using Fmoc-azide for the introduction of the Fmoc-group. Formation of small amounts of Fmoc-dipeptides when Fmoc-amino acids are synthesized *via* Fmoc-chloride has been reported,[8-13] and we have shown that the use of Fmoc-azide circumvents this side reaction.[8] In addition, we have found that the reaction of pure H-Cys(Acm)-OH with Fmoc-chloride affords a complex reaction mixture, that in our hands always requires silica-gel chromatography to give an acceptable product. All these problems have been suppressed using the above mentioned Fmoc-azide. After the reaction, an oil is obtained, which readily crystallizes to give a pure white solid.

We have likewise studied the preparation of H-Cys(Acm)-OH. So far, the only method described[5] involves the reaction of cysteine with *N*-hydroxymethylacetamide in the presence of anhydrous hydrogen fluoride or hydrogen chloride. Hydrogen fluoride is recommended by the authors,[5] even though its use is hazardous and special equipment is necessary. Here, we propose to replace it by trifluoromethanesulfonic acid. The reaction carried out under these conditions leads to *S*-acetamidomethyl-L-cysteine trifluoromethanesulfonate, which can be reacted with Fmoc-azide without further purification or isolation of the free amine. Finally, *N*-hydroxymethylacetamide can be prepared from inexpensive acetamide and formaldehyde following a known procedure.[14]

The main advantages of the present one-pot procedure are: i) use of inexpensive, readily available reagents, ii) no special equipment required, iii) good yields in all steps with no purification of intermediates, and iv) excellent quality of final product.



N-Hydroxymethylacetamide (1):

Acetamide (10 g, 0.17 mol) and potassium hydroxide (1 g, 0.018 mol) are dissolved in a solution of formaldehyde/water (7:13) (12 ml, 0.12 mol). The mixture is stirred for 5 min at 70 °C, then overnight at room temperature and is then acidified with 6 normal hydrochloric acid (to pH 7). The solution is concentrated by rotary evaporation, and the residue is diluted in acetone and dried over magnesium sulfate. *N*-hydroxymethylacetamide is obtained as an oil (12.7 g, 84 %) by filtering and evaporating the solvent and shown to be 85 % pure by NMR (br s at δ = 8.5 ppm corresponding to NH from *N*-hydroxymethylacetamide and two br s at δ = 7.4 and δ = 6.8 ppm corresponding to NH from acetamide). No further purification is required.

S-Acetamidomethyl-N-fluorenylmethoxycarbonyl-L-cysteine (4):

N-hydroxymethylacetamide (**1**; 3.0 g, 29 mmol) and cysteine hydrochloride (**2**; 3.8 g, 24 mmol) are dissolved in water (5.6 ml). The mixture is cooled in an ice-bath and trifluoromethanesulfonic acid/trifluoroacetic acid (1:19; 37 ml) is added. After 90 min stirring under argon, TLC (*n*-butanol/acetic acid/H_2O, 10:2:3) indicates that all cysteine has reacted. The solvent is removed by rotary evaporation, including chasing with ether (5 × 20 ml) to give an oil. The oil is dissolved in a 10 % aqueous sodium carbonate solution (50 ml), and the resulting solution is brought to pH 10 by adding more 10 % aqueous sodium carbonate. The solution is cooled in an ice bath and Fmoc-azide (5.8 g, 21 mmol) in dioxane (62 ml) is added. After 2 h of stirring in the ice bath, the reaction is continued at room temperature for 2 more days and kept at pH 10. The mixture is then poured into water (300 ml), extracted with ether, acidified with 12 normal hydrochloric acid (to pH 2), and extracted with ethyl acetate. The organic layer is dried over magnesium sulfate and after filtration the solvent is evaporated. Product **4** is recrystallized by dissolving in hot dichloromethane, adding hexane to incipient turbidity and allowing to stand at room temperature overnight to give a white solid; yield = 5.35 g (62 % yield from Fmoc-azide and 54 % from initial cysteine hydrochloride); m.p. 147–148 °C; $[\alpha]_D$ = −44 (c 1, dimethylformamide).

$C_{21}H_{22}N_2O_5S$	calc.	C 60.85	H 5.35	N 6.76	S 7.73
(414.5)		61.01	5.28	6.84	7.59

IR (KBr): ν = 3320, 3060, 2940, 1730, 1700, 1530, 1370 cm^{-1}.

^{1}H-NMR (DMSO-d_6): $\delta = 1.85$ (s, 3H); 2.81 (dd, 1H, $J = 9.8$ Hz, 13.7 Hz); 3.03 (dd, 1H, $J = 4.5$ Hz, 13.7 Hz); 4.1–4.4 (m, 6H); 7.34 (t, 2H, $J = 7.2$ Hz); 7.44 (t, 2H, $J = 7.2$); 7.7 (br, 1H); 7.74 (d, 2H, $J = 7.6$ Hz); 7.91 (d, 2H, $J = 7.4$ Hz), 8.5 (br, 1H).

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