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Effect of Scavengers in Acidolytic Cleavage of Cys(Acm)-Containing Peptides From Solid Support: Isolation of an Ethanedithiol Disulfide Adduct.

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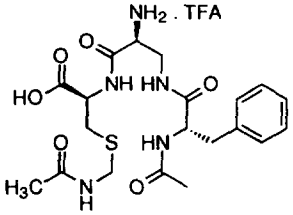
Abstract: Acidolytic cleavage of C-terminal Cys(Acm)-containing tripeptides from the Wang (HMP) resin can lead to partial deprotection of the Acm group and subsequent disulfide formation. The presence of water and the type of scavenger used in the cocktail mixture were found to play a role in the extent of these impurities formed.

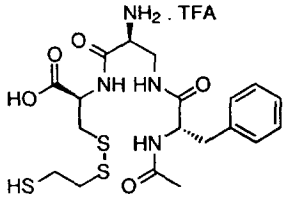
The occurrence of side reactions during the synthesis of peptides is not uncommon. These can occur during coupling/deprotection[1] reactions or during cleavage of peptides from the resin. Suppression of side reactions during acidolytic cleavage of peptides from resins is desirable in order to obtain better yields and fewer impurities. This may be accomplished by the use of judicious protecting groups and appropriate cleavage reagents.[2]

In connection with our program to design new bifunctional chelators to form group VII B metal (eg. Tc and Re) complexes for the development of new radiopharmaceuticals, one of our targets was the C-terminus cysteine tripeptide Ac-Phe-Dap-Cys(Acm)-OH (**1**). The acetamidomethyl (Acm) protecting group was selected because it, reportedly,[3] is stable under the acidic and basic conditions commonly used in solution and solid phase peptide synthesis. Furthermore, the deprotection of the Acm group is achieved using metal (Hg, Ag) salts.[3] Acm is the preferred S-protecting group in the synthesis of cysteine containing Tc or Re chelators since it can be selectively removed by the metal during the chelation process.[4]

We report here the deprotection of the Cys(Acm) group in **1** during cleavage of the peptide from resin, and identification of major side products resulting from reaction with various cleavage reagent mixtures.



1



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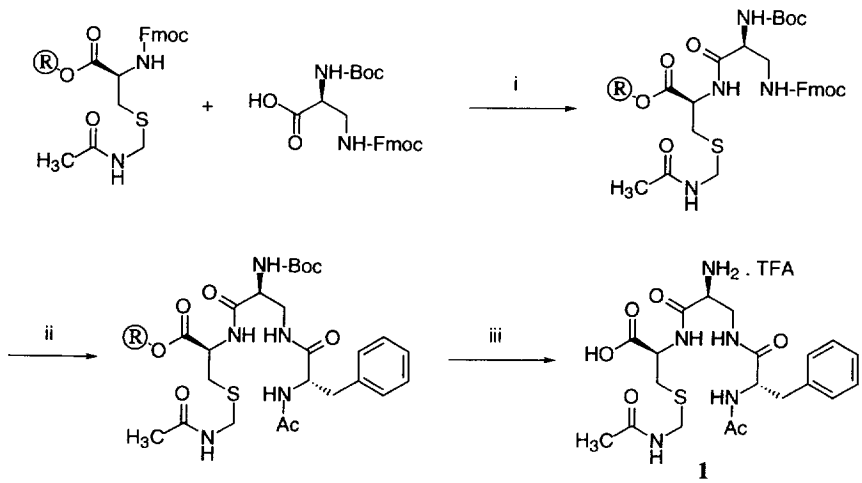
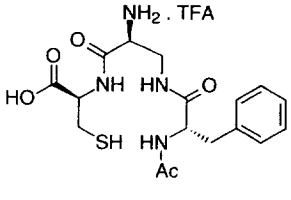
Scheme 1



® = Wang Resin; (i) HOBT/HBTU, Hunig's Base, DMF; (ii) N-Ac-Phe-OH, HOBT/HBTU, Hunig's Base, DMF; (iii) cleavage reagent.

Solid phase peptide synthesis (Scheme 1) was carried out manually using commercially available Fmoc-Cys(Acm) attached to Wang resin (substitution 0.61 mmol/g, mesh size 200-400). Stepwise coupling (6-9 hours) of Fmoc amino acids using HOBT/HBTU was performed. Deblocking of the Fmoc protecting group with 20% piperidine in DMF (v/v) was typically carried out for 30 min. Cleavage of peptide from the Wang resin with simultaneous deprotection of the Boc group was accomplished using a cocktail comprising TFA and scavengers (Table 1).[5] Treatment of the peptide-resin with Cocktail # 1[6] yielded a mixture of products, as indicated by analytical HPLC (see Table 2 below). ESMS of the mixture indicated the presence of **1** (m/z [M+1] 468.2), **2** (m/z [M+1] 489.1) and **3** (m/z [M+1] 397.2). The products **1**[7] and the novel ethanedithiol adduct **2**[7] were separated and isolated by preparative HPLC.[8] A literature search has revealed that isolation and characterization of a disulfide adduct (such as **2**) of the potent scavenger ethanedithiol with Cys(Acm), to the best of our knowledge, has not been reported.



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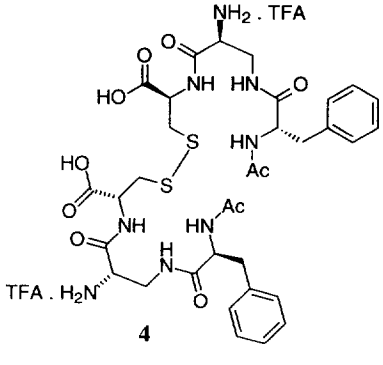

4

Acidolytic cleavage of the peptide off the resin in the presence of a "milder" scavenger such as phenol (Cocktail #2), gave **1** and significant amounts of **3** and **4**. The formation of these side products may be attributed to the hydrolysis and oxidation on the cysteine sulfur. Cocktail # 3, containing triisopropylsilane (TIPS) instead of phenol, gave similar results. Absence of water in the cleavage reagent (Cocktail #4) decreases the amount of the "quenched" monomer **3**, and gives the disulfide-dimer **4**[9] as the predominant impurity, with a higher yield of **1**. When Cocktail # 8 was used wherein water comprised 20% of the mixture, the crude analytical HPLC of the product showed the presence of high amounts of the unprotected Cys and its disulfide form along with other impurities. The yield of **1** with the use of this cleavage mixture dropped significantly. Among the three scavengers analyzed (TIPS, phenol and anisole), the best yields were repeatedly obtained with TIPS.

Table 1. Composition of reagents used for cleavage from solid support.

Cocktail No.	TFA %	Water† %	TIPS %	EDT %	Phenol %	Anisole %
1	92.5	2.5	2.5	2.5	-	-
2	88	5	2	-	5	-
3	95	2.5	2.5	-	-	-
4	95	-	5	-	-	-
5	95	2.5	-	-	2.5	-
6	95	2.5	-	-	-	2.5
7	95	-	-	-	-	5
8	75	20	-	-	-	5

† distilled water.

Table 2. Comparison of composition (% peak area by HPLC) of product formed using cocktails 1-8.

Cocktail No.	**1** (%)	**2** (%)	**3** (%)	**4** (%)
1	54	16	13	3
2	59	-	17	12
3	65	-	15	11
4	70	-	4	20
5	52	-	12	19
6	58	-	12	22
7	55	-	9	23
8	47	-	22	14

In summary, use of ethanedithiol as a scavenger during the cleavage and deprotection of the cysteine (Acm)-containing tripeptide Ac-Phe-Dap(Boc)-Cys(Acm)-OH from the Wang (HMP) resin results in the formation of an ethanedithiol disulfide adduct (**2**) as a major reaction side product. Formation of the disulfide dimer is observed in the absence of ethanedithiol. Absence of water gives better overall yield of the desired product. The best results were obtained in the absence of water and with the use of triisopropylsilane as the scavenging agent.

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5. Typical procedure used for the cleavage of the peptide from the resin involved incubation of 500 mg of the peptide-resin with 10 ml of the cocktail mixture at room temperature with occasional swirling. After two hours the resin was filtered and washed with TFA (2x4 ml). The filtrate was then concentrated to ~ 1-2 ml *in vacuo* and triturated with cold diethyl ether (10 ml). The product was filtered, washed with cold ether (2x8 ml) and dried overnight before analysis.
6. Novabiochem catalogue 1994/95, Technical Notes pp S34-S35.
7. HRMS-FAB of **1**, m/z calcd. for $C_{20}H_{29}N_5O_6S$ + H: 468.1917; Found: 468.1895. Analytical data of **2**; Calculated for $C_{19}H_{28}N_4O_5S_3$ + CF_3COOH: C, 41.85; H, 4.85; N, 9.30; S, 15.96. Found: C, 41.14; H, 4.94; N, 9.35; S, 15.36. HRMS-FAB of **2**, m/z calcd. for $C_{19}H_{28}N_4O_5S_3$ + H: 489.1300; Found: 489.1295.
8. HPLC method used a Hewlett Packard Model 1050 instrument and a Vydac C18 column (4.6 mm x 25 cm) at a flow rate of 1 ml/min with a gradient mobile phase from 2% B to 100% B over 45 min (A = 100% water with 0.1% TFA and B = 90% aqueous acetonitrile with 0.1 % TFA) monitored at 220 nm. Ret. time (min): **1**, 11.33; **2**, 14.01; **3**, 7.65; **4**, 15.85.
9. HRMS-FAB of **4**, m/z calcd. for $C_{34}H_{47}N_8O_{10}S_2$ + H: 791.2856; Found: 791.2859.

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