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MODIFICATION OF TRYPTOPHAN RESIDUES DURING ACIDOLYSIS OF 4-METHOXY-2,3,6-TRIMETHYLBENZENESULFONYL GROUPS. EFFECTS OF SCAVENGERS

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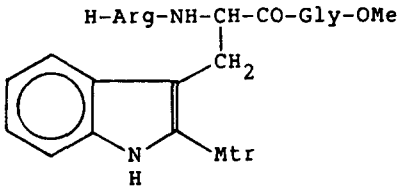
Summary: The formation of by-product 1 of tryptophan during the cleavage of Mtr from arginine can be minimized by addition of 1,2-ethanedithiol. Under too drastic conditions, however, a dithioketal product 2 is formed.

For the protection of arginine side chains during the solid-phase peptide synthesis by the 9-fluorenylmethoxycarbonyl (Fmoc) method, the 4-methoxy-2,3,6-trimethylbenzenesulfonyl[1] (Mtr) group is frequently used[2]. After deprotection of a 14-amino-acid peptide containing three arginines and one tryptophan with 95% trifluoroacetic acid (TFA) at 50°C, we obtained a by-product which according to the FAB-MS still contained one Mtr group. This group could not be cleaved by longer treatment with TFA, and the UV spectrum indicated that the tryptophan had been altered (λmax 287 nm, ε ca 11000). A similar by-product has recently been found by Eberle et al.[3] during the synthesis of melanin-concentrating hormone. It is obviously difficult to split off an Mtr group in the presence of tryptophan. We decided to study this question with the model tripeptide **H-Arg(Mtr)-Trp-Gly-OMe**, using HPLC and TLC[4]. Whereas the usual acid-labile protecting groups of the tert.butyl type are cleaved with TFA at r.t., Mtr clearly requires more vigorous conditions. With TFA-water 95:5 (v/v) the model tripeptide was fully deprotected after 80 min. at 50°C[5]. The yield of the correct tripeptide was about 70%, and in addition to several impurities in small amounts approximatly 10% of a lipophilic by-product was formed. This compound was isolated by preparative chromatography on silica gel plates, and its structure 1 confirmed by ^{13}C-NMR.



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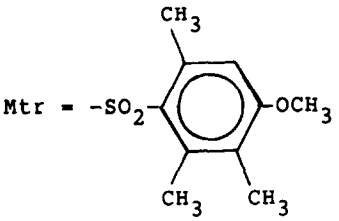
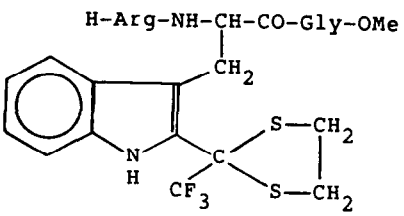


Next we studied the influence of the presence of 10 equivalents of different scavengers. Addition of Ac-Trp-OH, hydroquinone or indole had a negative effect on the yield of the correct tripeptide. On the other hand, thioanisole, dimethyl sulfide, 1-methylindole, m-cresol, 2-methylindole, 4-(methylmercapto)phenol and aniline improved the yield, in that order. Clearly the best scavenger was 1,2-ethanedithiol. Addition of 15 equiv. in 95% TFA at 50°C produced, after 1.5 hours, 85% of the tripeptide and reduced by-product 1 to 3-4%.

With most scavengers, the reaction is slightly slower than with 95% TFA alone. In order to find the optimal conditions, samples were taken after different reaction periods at 50°C. Analysis by TLC and HPLC revealed, in addition to compound 1, the slow appearance of a new lipophilic by-product 2. After 20 hours, the correct tripeptide was completly converted to compound 2, the structure of which was established by FAB-MS and NMR. The analogous derivative of the free amino acid tryptophan could also be prepared by heating under reflux with TFA in the presence of ethanedithiol for 2 days. Beside the main product H-Trp(2'-Mtr)-OH a number of structural related compounds were observed as well[6].



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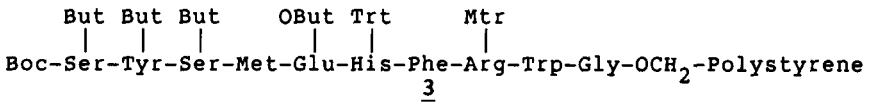
We also examined a combination of the most promising scavengers and ethanedithiol. The best results were obtained with a mixture of 15 equiv. ethanedithiol and 10 equiv. 4-(methylmercapto)phenol or aniline in 95% TFA at 50°C. In both experiments, about 90% of the correct tripeptide was obtained, in the first case after 1.5 hours, in the second after 3 hours.

In order to test the cleavage conditions with a larger peptide, we prepared ACTH-(1-10) 3, which contains the problematic amino acids Tyr, Met, and His in addition to Arg(Mtr) and Trp. The synthesis was performed with standard solid-phase methods using Fmoc-amino acids on a p-alkoxybenzylalcohol polystyrene resin. Cleavage of the peptide from the resin, combined with partial removal of side-chain protecting groups, was performed with TFA-1,2-dichloroethane-ethanedithiol-m-cresol 50:45:3:2 (v/v) in two consecutive steps (30 and 60 min. at r.t.). The rest of the protecting groups, especially the Mtr group, were cleaved with 95% TFA containing 5% ethanedithiol and 5% 4-(methylmercapto)phenol for 1.5 hours at 50°C. According to the HPLC analysis, the decapeptide is obtained in 70% yield. The remaining 30% is the sum of truncated and failure sequences from the solid-phase synthesis and of side products from the acidolytic cleavage of the Mtr group.



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Our experiments confirmed that the Mtr group in tryptophan-containing peptides can be cleaved with a minimal formation of by-products if the acidolysis is performed at 50°C with 95% TFA containing ethanedithiol and 4-(methylmercapto)phenol. It is strongly recommended that the shortest possible reaction time for each individual peptide should be found, in order to keep the formation of the dithioketal by-product 2 at tryptophan residues to a minimum.

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References and Notes.

1. M. Fujino, M. Wakimasu, and C. Kitada, Chem. Pharm. Bull., 29, 2825 (1981).

2. E. Atherton, C.J. Logan, and R.C. Sheppard, J. Chem. Soc., Perkin Trans. I, 1981, 538.

3. A.N. Eberle, E. Atherton, A. Dryland, and R.C. Sheppard, J. Chem. Soc., Perkin Trans. I, 1986, 361.

4. HPLC: Vydac column 5 C18, 250x4.6 mm; eluant A: 0.1% aqueous TFA, B: 0.1% TFA in acetonitrile. Elution with a linear gradient of 0-60% B over 40 min. Detection at 214 nm. TLC on silica gel in 3 different systems.

5. 95% TFA produced fewer by-products than 100% TFA, while addition of more than 5% water prolonged the cleavage time considerably.

6. The product H-Trp(2'-Mtr)-OH was purified by counter-current distribution in tert.amylalcohol-acetic acid-water 4:1:5 and crystallization from acetonitrile-water at pH 5-6. mp 203-204°C; $[\alpha]_D^{20}$:-16.2° (c=1, ethanol-water 1:1). UV: ε=10.000, λmax 283 nm. FAB-MS, NMR and combustion analysis are consistent with the assigned structure.

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