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Elimination of partial cleavage of acid labile groups during removal of Mtt protection

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Introduction

Lys(Mtt) is an important building block for construction of complex branched or multicyclic peptides[1,2]. It was shown earlier that Mtt cleavage by 1% TFA in presence of various scavengers leads to a loss of Trt protecting groups and also to a loss of peptide from the Wang and Rink resin [3]. Multiple exposure to diluted TFA solution was recommended as an optimal method [4]. During the synthesis of several peptides requiring Lys(Mtt) deprotection, we have found either incomplete cleavage of Mtt or unacceptable loss of peptide chain from the Rink amide resin. We decided to study this reaction and find optimal conditions for the Mtt removal.

Results and Discussion

As a model peptide we prepared Fmoc-Tyr(But)-Gly-Lys(Mtt)-RinkResin. We compared the following conditions (i) 1%TFA/DCM, (ii) 1%TFA/1%MeOH/DCM, and (iii) 1%TFA /1%TIS/DCM. Protected peptide resin (15 mg) was prewashed (60sec) with the TFA solution (0.5 ml) and exposed to the particular solution (5 ml) for a given time, resin was quickly washed with DCM, DMF, 5%DIEA/DMF and acetylated by Ac_2O/DIEA/DMF solution. Samples were cleaved by 95% TFA/5% Water and analyzed by HPLC. Peak 1 is Fmoc-Tyr-Gly-Lys-NH_2 – result of total deprotection and cleavage by 95% TFA without previous removal of Mtt and acetylation, peak 2 is Fmoc-Tyr-Gly-Lys(Ac)-NH_2 – Mtt deprotection, acetylation and total deprotection and cleavage, and peak 3 is Fmoc-Tyr(Ac)-Gly-Lys(Ac)-NH_2 – Mtt and But deprotection, acetylation and total deprotection and cleavage.

We have found that addition of small amount of MeOH into the cleavage solution prevents formation of typical yellow color and we speculated that Mtt cation is very effectively quenched. However, the fact that cleavage of Mtt is dramatically slower may be the reason of no coloration. We have also found that even after 1h, tert-butyl protection from tyrosine is cleaved in 1%TFA without any additives as well as in the presence of triisopropylsilane. After 16 hour exposure the tBu cleavage was substantial (see Figure 1). Presence of MeOH completely prevented this undesirable side reaction. Preliminary experiments let us believe that the premature cleavage from the Rink amide resin was prevented as well.

Surprisingly, we have found that cleavage of Mtt by 10% TFE / 20% HFIP / 70% DCM, complete in 16 h, leads also to partial cleavage of tBu type groups. Eventhough the extent of this cleavage is substantially lower than in the case of 1% TFA solution (2% vs. 15%), it can also be prevented by addition of 1% MeOH.

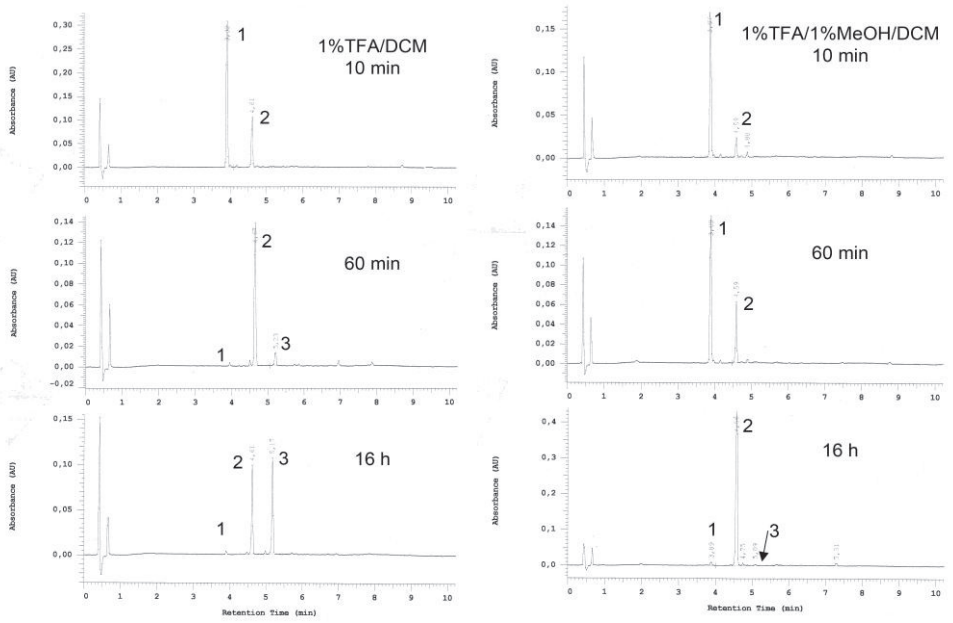

Figure 1. HPLC traces of model peptide Fmoc-Tyr-Gly-Lys.

Our tentative conclusion is that methanol may be an additive of choice for selective removal of Mtt group.

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