



## Evaluation of Monomethoxytrityl and Dimethoxytrityl as Orthogonal Amino Protecting Groups in Fmoc Solid Phase Peptide Synthesis

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**Abstract:** Amino acid derivatives protected at the  $\alpha$ - or side chain amino function with mono- as well as dimethoxytrityl were successfully prepared by a one-step procedure. These derivatives were evaluated for their utilization in solid phase peptide synthesis (SPPS), particularly with respect to the selective detritylation in the presence of other acid labile protecting groups to allow for the preparation of multiple, branched or cyclized peptide structures as well as for site specific post-assembly manipulations.

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The selective and site-directed liberation of a functional group besides the temporarily protected  $\alpha$ -amino function required for chain elongation during peptide synthesis on a solid support is a prerequisite to the construction of cyclic or branched peptides, the assembly of e.g. two different sequences on the same support or the attachment of special moieties. An amino group is a convenient target function for this purpose. The methyltrityl group (Mtt) is reported to be cleavable with very weak acid in dichloromethane<sup>1)</sup> which leaves other acid labile protecting groups used in Fmoc synthesis unaffected. However, we observed that Mtt is not as readily cleaved as expected if syntheses are performed on hydrophilic resins such as Tentagel or cellulose. Similar quenching effects of Tentagel resins have been observed for the cleavage of the dimethoxytrityl group (Dmt) during oligonucleotide synthesis.<sup>2)</sup> Therefore, we have evaluated the more acid labile monomethoxytrityl (Mmt) and Dmt derivatives of amino acids. We found that N-Mmt and N-Dmt derivatives are easily accessible by a one-step procedure.<sup>3)</sup> Whereas N-Dmt derivatives slowly decompose in protic solvents, the N-Mmt derivatives are stable and can be handled safely as their diisopropylethylammonium (DIPEAH<sup>+</sup>) salts. Several derivatives

**Table 1.** Data of Some Mmt- and Dmt-Derivatives of Amino Acids Prepared.

Compound	Method <sup>3)</sup>	Yield [%]	Ret. Time <sup>4)</sup> [min]	MW	ESI-MS <sup>4)</sup> [M-H] <sup>-</sup>
Mmt- $\beta$ Ala-OH · DIPEA	A	79	11.0	361.4	361.3
Dmt- $\beta$ Ala-OH · DIPEA	A	16	11.4	391.4	391.3
Boc-Lys(Mmt)OH · DIPEA	A	50	10.2	619.8	618.4
Fmoc-Lys(Mmt)OH · DIPEA <sup>5)</sup>	B	61	18.2	640.8	640.4
Fmoc-Lys(Dmt)OH · DIPEA	B	27	18.0	670.8	670.5

were successfully prepared and relevant data are given in Table 1.

To prove the efficiency of these derivatives as building blocks in solid phase peptide synthesis, the two fully protected model peptides Ac-Ala-His(Trt)-Ala-Lys(Boc)-Ala-Lys(xTrt)-NHCH<sub>3</sub>, with xTrt being either Mtt (peptide I) or Mmt (peptide II), were prepared on Tentagel S OH resin.<sup>6)</sup> These model peptides have incorporated the most acid-sensitive side chain protections used in Fmoc/tBu-peptide synthesis. Aliquots of the resins with the fully protected peptides were transferred into small plastic reaction columns (ABIMED) and some of these were treated repeatedly with a mixture of acetic acid/trifluoroethanol/dichloroethane (1:2:7, v/v)<sup>1)</sup> for 15 min each until no yellow or red trityl cations were detected in the eluent (ca. 4 times, ca. 1h). All products were then cleaved from the resins under non-acidic reaction conditions with 30% methylamine in ethanol to release the peptide C-terminal methylamides. The eluates from each column were subjected to HPLC.<sup>4)</sup> Peak fractions were analyzed by electrospray ionization mass spectrometry.<sup>4)</sup> Both syntheses yielded the correct products in good purity: peptide I with a mass of 1276.8 (calc. 1277.7) and peptide II with a mass of 1293.7 (calc. 1293.7). The Mmt group was efficiently cleaved resulting in the des-Mmt product as a major peak with a mass of 1022.7 (calc. 1021.6), whereas the Mtt group remained completely stable.

## References and Notes

1. Aletras, A.; Barlos, K.; Gatos, D.; Koutsogianni, S.; Mamos, P. *Int. J. Peptide Protein Res.* **1995**, *45*, 488.
2. Weiler, J. *Development of New Methods for the Preparative Automated Oligonucleotide Synthesis*, University of Konstanz, Germany 1995, p 93.
3. 10.0 mmol of amino acid (side chain protected if applicable) was suspended in 20 mL of either abs. dimethylformamide/pyridine (1/1, v/v), Procedure A, or abs. dimethylformamide/dichloroethane (1/1, v/v) with 4.0 mL (24.45 mmol) of DIPEA added, Procedure B. To the stirred solution, 15 mmol of the corresponding tritylchloride derivative (Lancaster, UK) were added and the mixture was heated under reflux for 60-90 min. After cooling, 3 mL of methanol was added and the mixture was evaporated to an oil. This was diluted with 300 mL ethyl acetate and poured into 100 mL of saturated sodium bicarbonate solution. The organic layer was washed twice with 100 mL each of saturated sodium chloride. The combined aqueous layers were extracted twice more with ethyl acetate. The combined organic layers were dried with anhydrous sodium sulfate and evaporated. The product was purified by flash chromatography on 30 g silica gel with toluene/ethyl acetate/methanol (10/10/3 v/v containing 1% DIPEA) as solvent. Coevaporation of the product containing fractions with methanol and dichloromethane resulted in a colourless foam. Alternatively, the crude reaction product was dissolved in 50 ml of toluene and precipitated into 300 mL of petroleum ether under stirring. The suspension was cooled and the product collected as a colourless amorphous powder. The filtrate was evaporated and purified by chromatography as mentioned above to yield another fraction of the product. The products were dried *in vacuo* and stored at -20°C.
4. High Performance Liquid Chromatography (HPLC): MERCK-HITACHI LaChrom L7000 system with an ET 250/8/4 NUCLEOSIL 300-7 C<sub>18</sub> column from Macherey-Nagel (Düren, Germany); gradient of acetonitrile in 0.05 N triethylammonium acetate (pH 7); 0% (3min), 0-100% (20 min), 100% (7 min). - Electrospray Ionization Mass Spectroscopy (ESI-MS): Finnigan MAT TSQ 700-Triple-Quadrupol-Mass Spectrometer; samples of ca. 10 pmol/μL were applied in CH<sub>3</sub>OH or CH<sub>3</sub>CN at a flow rate of 1 μL/min.
5. This derivative has been reported recently for the synthesis of a dipeptide linker within a complex prodrug of an anticancer compound: Dubowchik, G.M.; Radia, S. *Tetrahedron Lett.* **1997**, *38*, 5257-5260.
6. Loading of the resin with Fmoc-Lys(Mtt)-OH or Fmoc-Lys(Mmt)-OH · DIPEA was accomplished by condensation with diisopropylcarbodiimide/N-methylimidazole.<sup>7)</sup> Peptide chains were assembled by coupling the respective Fmoc-amino acid derivatives with TBTU/N-methylmorpholine in dimethylformamide utilizing an AMS222 multiple synthesizer (ABIMED Analysen-Technik GmbH, Langenfeld, Germany) following the instructions of the manufacturer.
7. Eichler, J.; Bienert, M.; Stierandová, A.; Lebl, M. *Peptide Res.* **1991**, *4*, 296-307.