Novel Selectively Cleavable Dmb-Based Protecting Groups for Side Chain Amino Groups of Amino Acids

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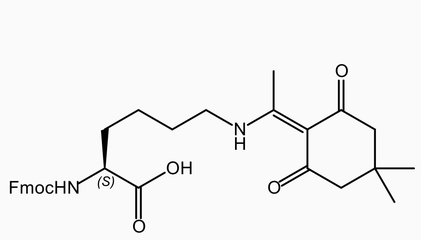
Abstract

We are presenting a **novel selectively cleavable protecting group** for the side-chain amino groups of lysine or other diamino acids, which play an important role during peptide synthesis, e.g., for modification of a particular side chain on the synthesis resin.

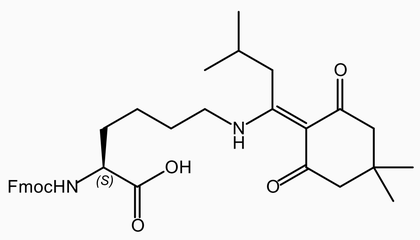
Trityl-based, mildly acidic cleavable protecting groups such as Mtt or Mmt are incompatible with strongly acid-labile resin linkers like trityl. Therefore, amino acid derivatives carrying the **hydrazinolysis-susceptible protecting group Dde or ivDde**[A] are frequently used for the synthesis of branched, cyclic, or side chain-modified peptides. However, **Dde might migrate to free lysine epsilon-amino groups ("scramble")**[B] and for the more robust **ivDde, total removal is hardly possible**.

A newer group for the orthogonal protection of amino groups is **MeDmb (methyl dimethylbarbituric acid)**.[C]

In our study we present the possibilities and limitations of the novel lysine Dmb derivatives **(MeDmb, EtDmb, ivDmb)** and compare their properties with the existing protecting groups Dde and ivDde.



FAA1390
Fmoc-L-Lys(Dde)-OH



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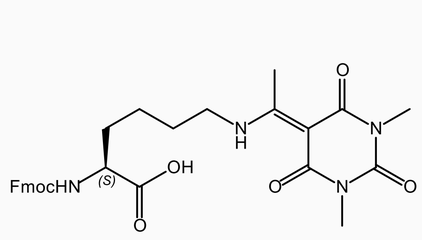
Fmoc-L-Lys(ivDde)-OH



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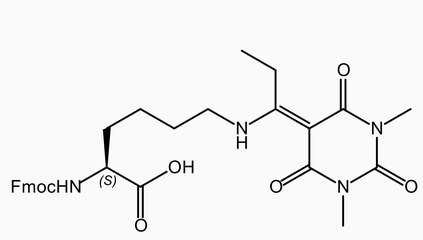
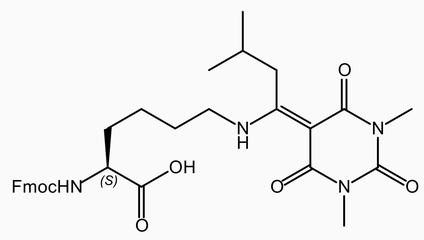
Fmoc-L-Lys(MeDmb)-OH



Fmoc-L-Lys(EtDmb)-OH



FAA7975
Fmoc-L-Lys(ivDmb)-OH

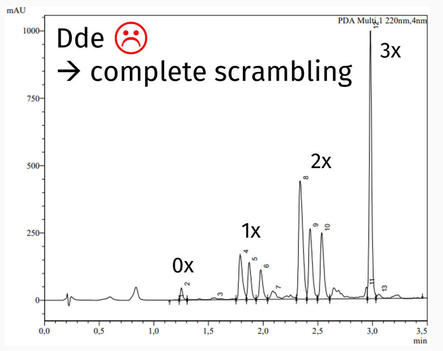
Results & Discussion

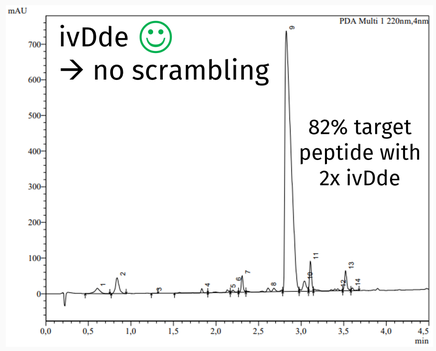
- **Stability**

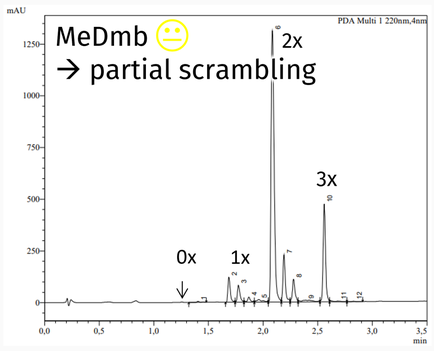
  → Fmoc-Lys(MeDmb/EtDmb/ivDmb)-OH are clearly soluble in DMF (0.5 M), stable for > 3d in closed vials at RT in DMF, and stable in TFA/H_2O for at least 4.5h

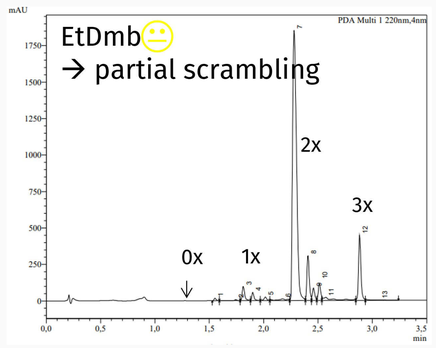
- **Scrambling on side chains**

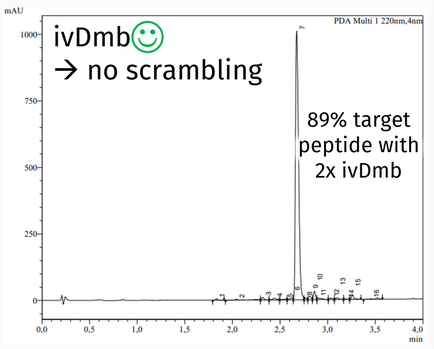
  → Synthesis of Boc-Lys(Fmoc)-Ala-Lys(**X**)-Pro-Lys(**X**)-Ala-(2CT-resin); 50% Pip/DMF, 30 min; cleavage 20% HFIP/DCM; LCMS: Boc-Lys-Ala-Lys(**X**)-Pro-Lys(**X**)-Ala-OH





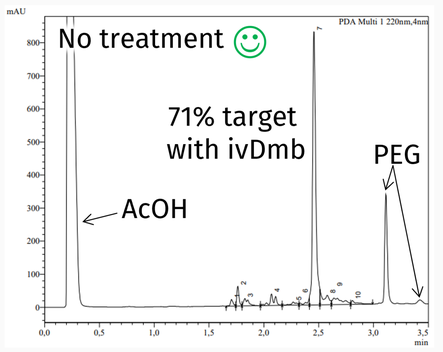


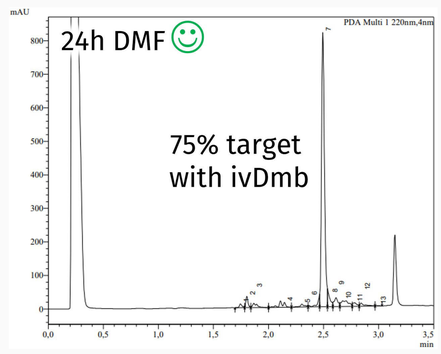


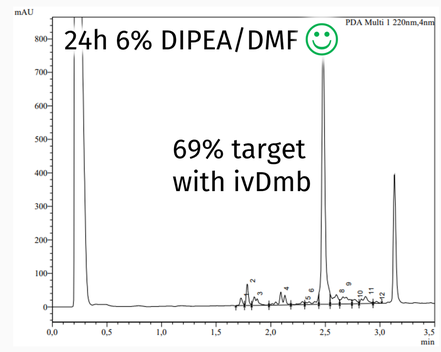


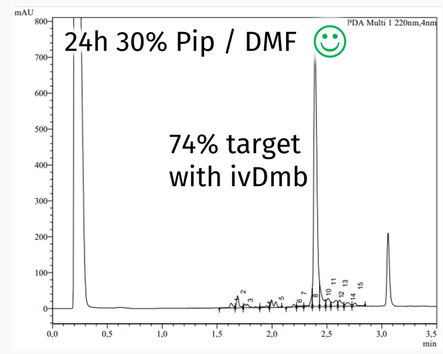
- **Stability during synthesis/cleavage**

  → Synthesis of Boc-Gly-Ser(tBu)-Ala-Leu-Gly-Lys(**ivDmb**)-Ala-Phe-Gly-Phe-Ser(tBu)-Glu(OtBu)-TG S Trt resin; treatment with various solvents; TFA cleavage



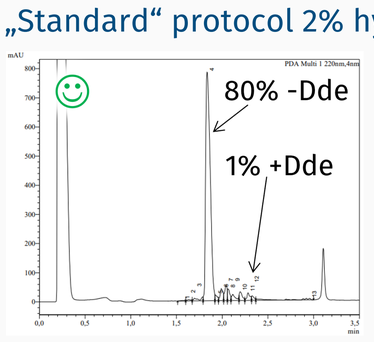


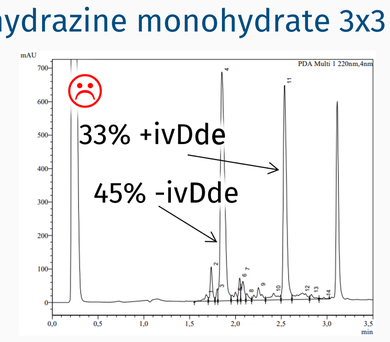


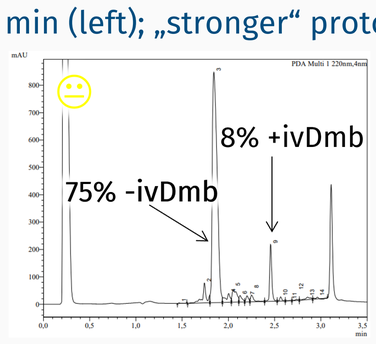


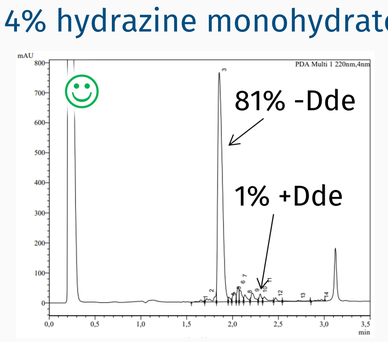
- **Cleavage of the side chain protecting group**

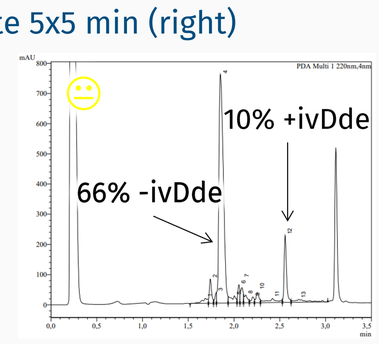
  → „Standard" protocol 2% hydrazine monohydrate 3x3 min (left); „stronger" protocol 4% hydrazine monohydrate 5x5 min (right)

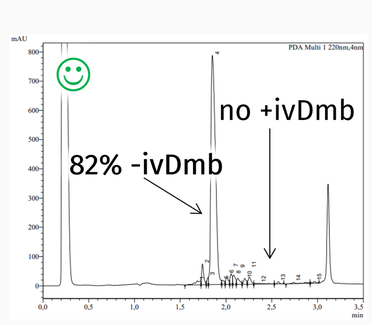












- **„Real" example**

  → Synthesis of Boc-Gly-Ser(tBu)-Ala-Leu-Gly-Lys(**X**)-Ala-Phe-Gly-Phe-Ser(tBu)-Glu(OtBu)-TG S Trt resin (HATU/NMM; 30% piperidine/DMF; 200 mg Glu-TG; 0.19 mmol/g); cleavage of ivDmb/ivDde („stronger" protocol); coupling of 5-carboxyfluorescein; TFA cleavage; HPLC purification (RP18, ACN/H_2O/0.05% TFA)

	ivDde 😐	ivDmb ☺
Crude yield	31 mg	37 mg
Crude purity	82% 5-Fluo + 4% ivDde	86% 5-Fluo + no ivDmb detectable
Isolated yield	10.9 mg (>95%) + 7.8 mg (82%) (repurification)	23.6 mg (>95%)

Summary

- Fmoc-Lys(ivDmb)-OH performs better than previously known orthogonally protected Lysines with regards to scrambling avoidance, deprotection completion, and peptide yield.

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