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A new protecting group for tryptophan in solid-phase peptide synthesis which protects against acid-catalyzed side reactions and facilitates purification by HPLC

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The indole nucleus of *Z*-Trp-OBzl is modified by acylation of the indole nitrogen using Boc-*N*-methyl butyric acid followed by catalytic hydrogenation and introduction of the Fmoc group. The resulting derivative, Fmoc-Trp(Boc-Nmbu)-OH, is incorporated into peptide chains via solid-phase peptide synthesis (SPPS). After assembly of the peptide chain, the Boc group is cleaved by treatment with TFA. The peptide is isolated with the tryptophan residue modified with a cationic 4-(*N*-methylamino) butanoyl group, which improves the solubility of the peptide during HPLC purification. On treatment of the purified peptide at pH 9.5, the Nmbu group undergoes an intramolecular cyclization reaction; this results in the fully deprotected peptide and *N*-methylpyrrolidone.

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Highly hydrophobic peptides, especially those prone to β-sheet formation, can cause significant problems in the handling of a peptide after cleavage from the resin.[1] These peptides tend to be difficult to dissolve in the solvents typically used for HPLC purification, hence, only small amounts can be loaded onto the column and the peptides often elute as broad peaks with poor resolution.[2]

Several studies have shown that these problems can be circumvented by adding charged residues to the peptides, especially arginine residues as a 'solubilizing tail'.[3–6] These residues are present during the purification but are cleaved later in a separate step. The efficiency of this approach has been demonstrated in several reports, but this method is dependent on the efficient and mild cleavage of additional cationic residues after purification of the peptide.

In two recent publications, we have suggested an alternative strategy to increase the solubility of synthetic peptides during the purification step.[7,8] In these studies, the phenolic alcohol groups on tyrosine residues or the peptide backbone-protective *N*-(2-hydroxy-4-methoxybenzyl) (Hmb) group were modified with the Boc-*N*-methyl-*N*-[(2-methylamino)ethyl]carbamoyl group (Boc-Nmec) during the synthesis. After cleavage of the peptide from the resin with TFA, the Boc group was removed while the cationic *N*-methyl-*N*-(2-methylamino)ethyl carbamoyl group was still attached to the peptide, thereby increasing the solubility and facilitating purification. By exposing the peptide to slightly alkaline pH, the Nmec group is cleaved via an intramolecular cyclization, giving the fully deprotected peptide. In this study, we propose an analogous strategy for tryptophan.

Tryptophan is a very hydrophobic residue and it can therefore contribute to reducing the solubility of a peptide during purification steps. The indole ring of tryptophan is also susceptible to alkylation by cations formed during acidolytic cleavage of protecting groups, these side reactions can be suppressed by addition of scavengers or by the use of electron-withdrawing protecting groups on the indole nucleus. In the present study, we report the use of a new protecting group, the 4-(*N*-methylamino)butanoyl group (Nmbu) for tryptophan (**1**) that provides protection against modification by reactive cations during the cleavage reaction and at the same time facilitates purification by supplying additional cationic charges during the purification step (Scheme 1).

Esters of 4-aminobutyric acid are known to undergo intramolecular lactamization, giving pyrrolidone and an alcohol.[9] We therefore wanted to investigate whether indolyl derivatives of *N*-methyl-4-amino butyric acid could be cleaved by intramolecular aminolysis at a rate that is sufficiently fast to be of practical use in solid-phase peptide synthesis.

The synthesis of Fmoc-Trp(Boc-Nmbu)-OH is outlined in Scheme 2. The indole nitrogen of *Z*-Trp-OBzl (**4**) was acylated as described by Klausner et al. and Snider and Zeng using the *p*-nitrophenyl ester of Boc-*N*-methyl-4-amino butyric acid (**5**) and KF/18-crown-6, in 67–87% yield.[10,11] The fluoride anion mediated

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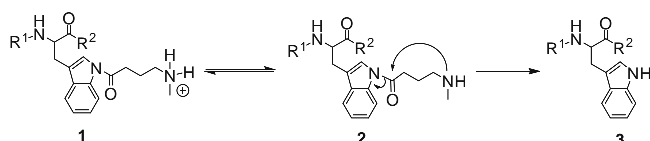
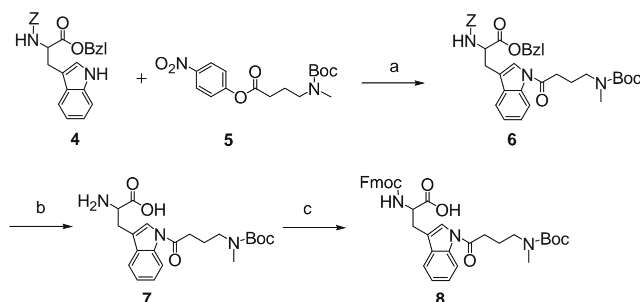


Scheme 1. General scheme for the use of 4-(*N*-methylamino)butanoyl (Nmbu)-protected tryptophan in SPPS. After cleavage from the resin, the peptide is isolated with the cationic Nmbu group attached to the tryptophan residue of **1**; R^1, R^2 = peptide chains. In this form, the peptide has improved solubility and can be purified by reverse-phase HPLC. After purification, the peptide is exposed to alkaline pH during which the Nmbu group is cleaved via an intramolecular cyclization reaction as shown in **2** to give the fully deprotected peptide **3**.



Scheme 2. Synthesis of Fmoc-Trp(Boc-Nmbu)-OH. Reagents (a) KF/18-crown-6/*N,N'*-diisopropylethylamine, THF; (b) H_2/10% Pd on charcoal, EtOH; (c) (i) Trimethylsilyl chloride, (ii) *N,N'*-diisopropylethylamine, Fmoc-Cl, MeCN.

reaction was carried out in dry tetrahydrofuran instead of dry acetonitrile, as was recommended by Klausner and co-workers. In our hands, no product was obtained with acetonitrile as solvent. The purified product **6** was hydrogenated with H_2/Pd, to remove the benzyloxycarbonyl and the benzyl protecting groups. The α-nitrogen of H-Trp(Boc-Nmbu)-OH (**7**) was protected with Fmoc-Cl as described by Bolin and co-workers, and the product was purified by flash chromatography giving Fmoc-Trp(Boc-Nmbu)-OH (**8**) in 97% yield.[12] The ^{1}H NMR and ^{13}C NMR spectra were in accordance with the expected structure. A detailed description of the synthesis and the NMR data are given in Supplementary data.

The Nmbu group is an acyl type protecting group, and in this respect it is similar to the nucleophile-sensitive formyl group used to protect tryptophan in Boc chemistry. The formyl group prevents

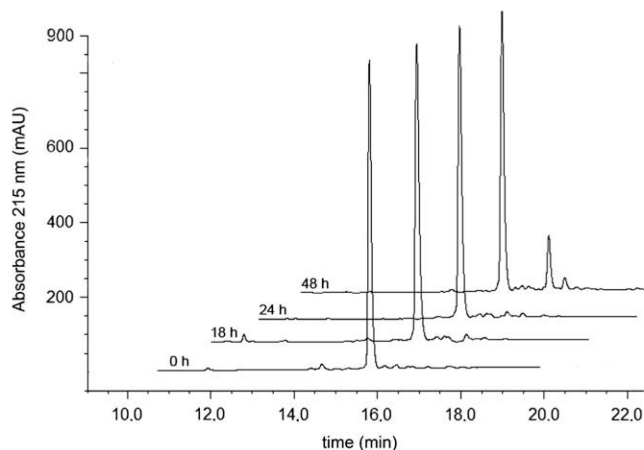


Figure 1. Stability of the Boc-Nmbu group to piperidine treatment. The peptide Fmoc-Lys(Boc)-Trp(Boc-Nmbu)-Leu-Pro-Phe-Leu-Ala attached to a solid phase was exposed to 20% piperidine in DMF for 18, 24 or 48 h. The peptide was then cleaved from the resin with a mixture of TFA (95%), triisopropylsilane (2.5%) and water (2.5%) and was analyzed by reverse-phase HPLC at 215 nm.

alkylation of tryptophan and can be removed by nucleophiles such as piperidine or, under acidic conditions, by ethanedithiol.[13] Although the formyl protecting group is very sensitive to nucleophiles, studies have shown that other acyl derivatives are much more stable. Arai and co-workers used indole as a protecting group for carboxylic acids and cleavage required 3 M NaOH in methanol for 2.5–35 h for complete deprotection, depending on the structure of the carboxylic acid.[14]

To investigate the stability of Boc-Nmbu to piperidine treatment, Fmoc-Trp(Boc-Nmbu)-OH **8** was incorporated into the model peptide Fmoc-Lys(Boc)-Trp(Boc-Nmbu)-Leu-Pro-Phe-Leu-Ala attached to a Wang resin, and was treated with 20% piperidine in DMF for 18, 24 or 48 h, followed by treatment with TFA. As can be seen in Figure 1, only small amounts of the Nmbu group were cleaved after 48 h, showing that the Boc-Nmbu group is sufficiently stable to be used even for large peptides in Fmoc SPPS.

Cleavage of the Nmbu group under aqueous alkaline conditions was studied by exposing the peptide H-Lys-Trp(Nmbu)-Leu-Pro-Phe-Leu-Ala-OH to Tris buffer (Fig. 2). It was found that the rate of cleavage was slow below pH 9.0 but proceeded with a half-life of about 5–7 min at pH 9.5, as measured by absorption at 240 nm. The reaction mechanism was (at least partly) an intramo-

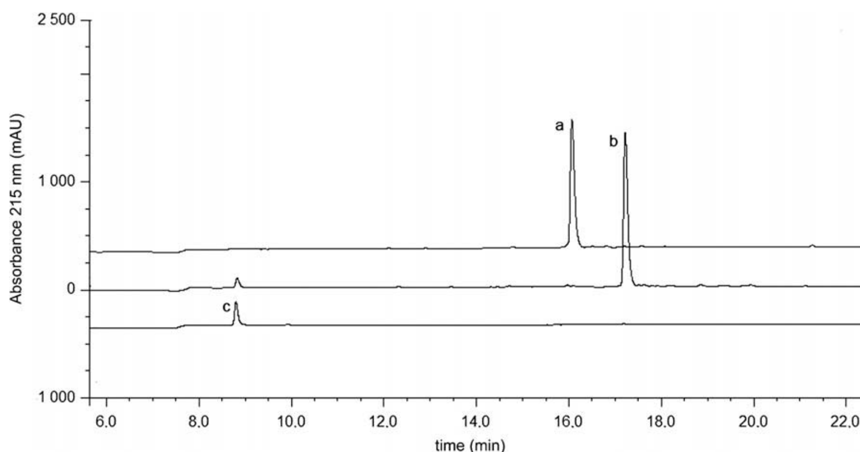


Figure 2. HPLC profiles, monitoring the cleavage of Nmbu-protected tryptophan in the peptide H-Lys-Trp(Nmbu)-Leu-Pro-Phe-Leu-Ala-OH exposed to Tris buffer, pH 9 (a) H-Lys-Trp(Nmbu)-Leu-Pro-Phe-Leu-Ala-OH; (b) H-Lys-Trp-Leu-Pro-Phe-Leu-Ala-OH after 90 min showing complete deprotection of tryptophan; (c) *N*-methylpyrrolidone.

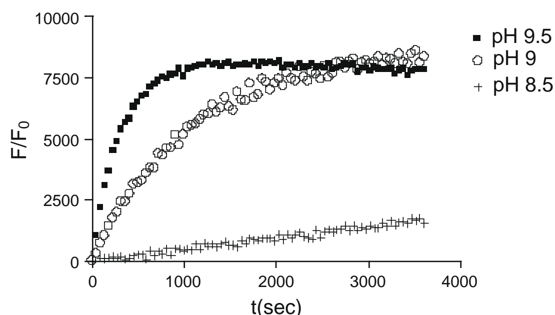


Figure 3. Kinetics of the cleavage of the Nmbu group in the peptide H-Lys-Trp(Nmbu)-Leu-Pro-Phe-Leu-Ala-OH as measured by the increase in fluorescence (F/F_0) at 360 nm and at different pH values.

lecular cyclization reaction, as HPLC analysis at 215 nm showed that a product was formed which had the same elution time as *N*-methylpyrrolidone.

A more accurate determination of the kinetics of intramolecular cyclization can be obtained by fluorescence spectroscopy (Fig. 3). The indole ring of tryptophan emits fluorescence at 353 nm whilst the Nmbu-protected indole ring does not.[15] Thus, the formation of deprotected tryptophan can be measured as an increase in the intensity of fluorescence. As expected, the rate of cyclization was pH-dependent and the half-life of Nmbu-protected tryptophan was 4–5 min at pH 9.5. However, care must be taken to extrapolate these values to reaction conditions in which the peptide is prone to aggregation and is present in much higher concentration during the cyclization reaction. Under these conditions, the reaction rate can be expected to be significantly slower.

During treatment with TFA, tryptophan can undergo oxidation, dimerization and alkylation through reactive cations formed during cleavage of the peptide from the resin.[13] In Fmoc chemistry using N^{in}-Boc protected tryptophan, these side reactions can be suppressed to low levels.[16–18] In order to compare the protection provided with the Nmbu group, we synthesized peptides where tryptophan was left unprotected, or protected by the Boc or the Boc-Nmbu group. This was followed by cleavage with TFA/triisopropylsilane/H_2O in a 95:2.5:2.5 ratio and the products were analyzed by HPLC and MALDI-TOF mass spectroscopy (data not shown). Unprotected tryptophan resulted in the formation of high levels of by-products while both Boc and Boc-Nmbu protection suppressed these side reactions with similar efficiency.

We conclude that the Nmbu protecting group reported in this study, together with the previously reported Nmec protecting group for tyrosine and the Hmb group, can be useful tools in the synthesis of very hydrophobic peptides such as transmembrane segments of membrane proteins. By using these protecting groups, peptides with tryptophan, tyrosine and glycine residues can be purified as cationic peptides after cleavage, even if the sequence contains no cationic residues.

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Supplementary data

Supplementary data (experimental details for the synthesis of Fmoc-Trp(Boc-Nmbu)-OH and ^{13}C NMR and ^{1}H NMR for Fmoc-Trp(Boc-Nmbu)-OH) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.014.

References and notes

1. Fisher, L. E.; Engelman, D. M. *Anal. Biochem.* **2001**, *293*, 102.
2. Bollhagen, R.; Schmiedberger, M.; Grell, E. *J. Chromatogr., A* **1995**, *711*, 181.
3. Choma, C. T.; Robillard, G. T.; Englebretsen, D. R. *Tetrahedron Lett.* **1998**, *39*, 2417.
4. Englebretsen, D. R. *Tetrahedron Lett.* **1996**, *37*, 8431.
5. Englebretsen, D. R.; Robillard, G. T. *Tetrahedron* **1999**, *55*, 6623.
6. Johnson, E. C. B.; Kent, S. B. H. *Tetrahedron Lett.* **2007**, *48*, 1795.
7. Wahlström, K.; Planstedt, O.; Undén, A. *Tetrahedron Lett.* **2008**, *49*, 3921.
8. Wahlström, K.; Planstedt, O.; Undén, A. *Tetrahedron Lett.* **2008**, *49*, 3779.
9. Martin, R. B.; Hedrick, R. I.; Parcell, A. *J. Am. Chem. Soc.* **1964**, *86*, 2406.
10. Klausner, Y. S.; Chorev, M. *J. Chem. Soc., Perkin Trans. 1* **1976**, 627.
11. Snider, B. B.; Zeng, H. B. *J. Org. Chem.* **2003**, *68*, 545.
12. Bolin, D. R.; Sytwu, I. I.; Humiec, F.; Meienhofer, J. *Int. J. Pept. Protein Res.* **1989**, *33*, 353.
13. Jones, J. H. In *Synthesis of Peptides and Peptidomimetics*; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Georg Thieme Verlag: Stuttgart, 2004; pp 342–346.
14. Arai, E.; Tokuyama, H.; Linsell, M. S.; Fukuyama, T. *Tetrahedron Lett.* **1998**, *39*, 71.
15. Lakomicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic/Plenum: New York, 1999.
16. Fields, C. G.; Fields, G. B. *Tetrahedron Lett.* **1993**, *34*, 6661.
17. White, P. In *Peptides Chemistry Structure Biology*; Smith, J. A., Rivier, J. E., Eds.; Proceedings of the 12th American Peptide Symposium; ESCOM: Leiden, 1992; p 537.
18. Franzen, H.; Grehn, L.; Ragnarsson, U. *J. Chem. Soc., Chem. Commun.* **1984**, 1699.