FMOC DEPROTECTION BY tert-BUTYLAMINE AND ITS COMPARISON IN SOLUTION AND SOLID PHASE SYNTHESIS

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\textit{tert}-Butylamine was employed as the alternative to piperidine for Fmoc deprotection in liquid and solid phase peptide synthesis. Basic kinetic parameters of deprotecting reaction were checked in chosen Fmoc amino acid derivatives. Solid phase synthesis of cyclic part of oxytocin (tocinamide) was worked out and used for CD spectra measurement.

Keywords: \textit{tert}-Butylamine (TBA); Fmoc deprotection; Oxytocin cycle; CD spectra.

Deprotection of the Fmoc group is carried out under basic conditions by 20\% piperidine in DMF\textsuperscript{1}. For peptide difficult sequences, diazabicycloundecene (DBU, 2\% in DMF) can be an alternative. Both agents are unfortunately not very suitable in the liquid phase synthesis. For this reason, a permanent effort and search for the agents easily removable during intermediate isolation is taking place\textsuperscript{2}. \textit{tert}-Butyl amine (TBA, 30\%) in DMF was chosen as the substitute of piperidine in liquid phase. The linear peptide as the precursor corresponding to the cyclic part of oxytocin (tocinamide) was manually synthesized on the solid phase. In this synthesis either 20\% piperidine in DMF or TBA were compared for the Fmoc group removal. TBA 100\% in the mixture with 1-octadecanethiol (C18-SH) was then checked for deprotection of some Fmoc-AA derivatives to study the cleavage condition affordable later for liquid phase. The choice of the model peptide was motivated by its possible exploitation either for CD spectra measurement and/or for the study of its biological effects in CNS during the study of stress.

RESULTS AND DISCUSSION

Syntesis of Fmoc-Cys(Trt)-Tyr(t-Bu)-Ile-Gln(Trt)-Asn(Trt)-Cys(Trt)-Rink-resin was carried out in two batches by the usual scheme. Fmoc derivatives were used in 3 eq. excess, DIC/HOBt was used for coupling, and deprotection
was carried out for 5 and 20 min in 20% piperidine/DMF solution. Similar scheme was applied in the second batch, but the 30% TBA in DMF with 0.1% dithiothreitol (DTT) for 5 and 40 min was used for the Fmoc removal. The deprotecting reaction gave closely similar results in both methods (Table I).

At the assembling end, peptides were detached from the resin in the cocktail of 95% TFA, 3% anisole and 2% water during 3 h, precipitated by diethylether, dissolved in methanol, acidified by AcOH (pH 4.5, 1 mg/ml), and oxidized by 1% iodine in MeOH for the closing of disulfide bridge. After 30 min, the yellowish solution was decolorized by ascorbic acid, and the solution gently evaporated at 40 °C in vacuum. Cyclized peptide was dissolved in water, desalted and purified on HPLC C18 column. After lyophilization the identity of peptides was confirmed by mass spectra (MS). MS analysis surprisingly showed the iodinated Tyr aromatic ring as the main product in both batches.

**Table I**

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<tr>
<td>tert-Butylamine</td>
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<td>0.63</td>
<td>0.64</td>
<td>0.69</td>
<td>0.73</td>
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**Fig. 1**

HPLC of Fmoc deprotection. (Fmoc-Gln(Trt)-OH, TBA cleavage)
Fmoc-Gln(Trt) was chosen for the study of the cleavage reaction in solution (Fig. 1). Fmoc group was fully eliminated by TBA in 30–60 min in tested Fmoc-AA (C,F,Y) derivatives. To scavenge dibenzofulvene (DBF), DTT\textsuperscript{3} or preferably C18-SH was added in 5 molar excess to 30% solution of TBA in ACN or to 100% TBA.

The primary product of the Fmoc elimination is dibenzofulvene (DBF) as reactive intermediate, which in the reversible Michael addition reacts with nucleophiles to form adducts with piperidine, primary or secondary amines or preferably with the soft nucleophiles-thiols. In the solid phase, the large excess of the base guarantees full conversion of DBF to piperidine adduct. In the liquid phase, hydrogenolysis (Pd/C)\textsuperscript{4,5}, various bases\textsuperscript{1,2}, AlCl\textsubscript{3}/toluene\textsuperscript{6}, catalytic amount of DBU/C8SH\textsuperscript{3} were described. DBF at higher concentration polymerizes and if not fully scavenged reacts reversibly with the amino groups on the peptide chain producing secondary peptide amines. tert-Butylamine has the convenient properties for Fmoc removal. It is a strong base (pK\textsubscript{a} 10.7, b.p. 46 °C), and its reactivity towards amide formation is limited due to its bulky chain. TBA (30%) in DCM, DMF or TBA as such, were used for Fmoc deprotection in solution synthesis. Isolation of intermediates with free amino group is easy often by simple precipitation by ether, heptane or petroleum ether. DBF can be scavenged by C18-SH and very lipophilic adduct then extracted into the sitable solvent.

Figure 2 shows comparison of CD spectra (measured on Jasco J-815 spectrometer in neutral – pH 7, 10 mM PBS buffer and 0.1 cm quartz cell) of the diiodinated OT6 with oxytocin. The intense negative band at 190–210 nm and also the smaller positive band at 220–250 nm are nearly identical for both compounds. The red shift of about 10 nm in the spectra of OT-6 J2 vs.

![Fig. 2](image)

CD spectrum of oxytocin and tocinamide (OT6 J2)
OT-9 is due to the effect of diiodinated tyrosine aromatic ring. The band due to iodinated tyrosine nucleus is also discernible in absorption at about 310 nm, (measured in 1 cm cell on Jasco 815). Hence, since CD spectra of the complete OT nonapeptide remain similar to spectra of just the ring part, even the ring with modified tyrosine, suggests that the ring conformations are similar for both compounds and that the C-terminal linear tripeptide possesses only minor if any effect on the spectral properties.

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REFERENCES