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New Synthesis of the Cyclic Tetrapeptide Tentoxin Employing an Azlactone as Key Intermediate

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Abstract : An improved preparation of the cyclic tetrapeptide Tentoxin is reported employing an azlactone as key intermediate. This new synthetic route offers the advantage over existing methodologies that the dehydro amino acid would easily be varied, thus allowing the simple preparation of analogues.

Tentoxin is a cyclic tetrapeptide having the sequence cyclo(MeAla-Leu-MeΔPhe-Gly) and possessing phytotoxic properties[1-3]. As for all small peptides of four residues, the twelve membered ring system is strained and is therefore a challenging synthetic target. Cyclization is the limiting step, since intramolecular reaction occurs only in poor to moderate yields[4]. Furthermore, the presence of an α,β-unsaturated α-amino acid unit in its structure adds significant difficulty to the preparation of the linear precursor. The presence of the double bond in a dehydro amino acid dramatically decreases both the nucleophilicity of the amino group and the reactivity of the carboxylic group, making incorporation of dehydro amino acid into a peptide chain difficult[5]. As a result of the weak nucleophilicity of the enamine group, the acylation of a dehydro amino acid is not a satisfactory method for the formation of dehydropeptide[6,7]. This suggests that the double bond should be introduced into the molecule after peptide bond formation. Previous reports of synthesis of Tentoxin have employed saturated precursors for Z-dehydrophenylalanine (Δ^{Z}Phe) which require both additional preparative steps prior to peptide elaboration and separation of configurational isomers.

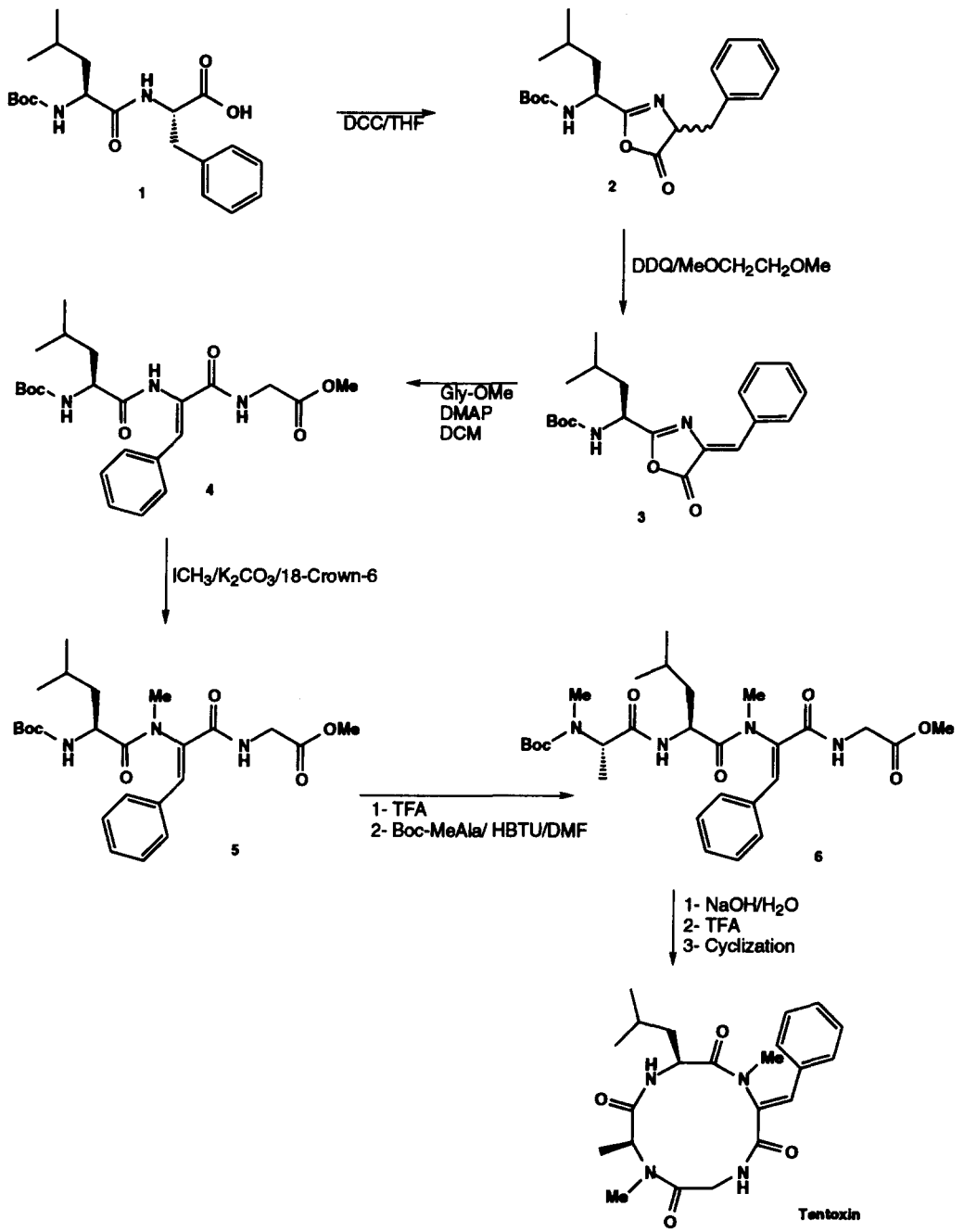
In the first synthesis of Tentoxin, Rich and al.[8,9] used the threo isomer of N-*tert*butyloxycarbonyl-N-methyl-3-benzylthiophenylalanine. After incorporation of this saturated derivative into a peptide chain, the double bond was introduced by oxidation and β-elimination. This synthesis involved many steps and required separation of the starting isomers .

In the most recent synthesis described by Edwards and al.[10], the first coupling step was performed on phenylserine as saturated starting material. The double bond was subsequently introduced by concomitant dehydration of Boc-leucyl phenylserine upon azlactonization.

We already described the shortest synthesis of Tentoxin[11] using dehydrophenylalanine itself. We succeeded in coupling dehydrophenylalanine through its N-carboxyanhydride (ΔNCA)[12] derivative.

However, none of these methods are easily applicable to the synthesis of Tentoxin analogues containing dehydro amino acids other than dehydrophenylalanine, which are required for biochemical and conformational

studies currently in progress[13]. For this purpose, we have investigated a new approach starting from proteinogenic aminoacids which would allow easy variation of the dehydo amino acid unit.



SCHEME 1

We report here an improved synthetic route to Tentoxin that affords intermediately a dipeptide derivative of Z-dehydrophenylalanine (Δ^ZPhe) from commercially available phenylalanine (SCHEME 1). We have successfully developed a methodology utilizing dichlorodicyanobenzoquinone (DDQ) oxidation of an azlactone which was used first by Stammer[14]. A single compound was obtained, and this was assigned as the Z-stereoisomer of the azlactone **3**, on the basis of the analogy with previous work on the Erlenmeyer azlactone synthesis[15]. Furthermore, the resulting Tentoxin is identical to the natural compound.

It is known that the ring-closure site has a very significant influence on the yield of the cyclization[4]. In the case of Tentoxin, the choice of linear peptide precursor is limited. The low reactivity of dehydro amino acids means that N-MeΔ^ZPhe cannot be at a terminus, and the racemization risk associated with N-Me amino acids implies that N-MeAla is not suitable for the C-terminal position. Consequently, a sequence with Gly at the C-terminus seems the most rational possibility. The achirality of this residue also means that more forcing conditions can be used in the ring-closure reaction, without any risk of racemization.

Overnight treatment of Boc-Leu-Phe-OH **1** with one equivalent of dicyclohexylcarbodiimide (DCC) at 0 °C in THF afforded the corresponding azlactone **2** as an oil, in quantitative yield. Oxidation by DDQ produced the Z-azlactone **3** [Yield after chromatography with dichloromethane = 57%, R_f 0.53, mp = 117 °C, ^{1}H NMR 250 MHz δ (ppm) ($CDCl_3$) : 1.02 (6H, m, 2xCH_3), 1.28 (2H, m, CH_2), 1.49 (9H, s, C(CH_3)$_3$), 2.06 (1H, m, CH), 4.72 (1H, m, α-CH), 5.11 (1H, d, *J* 8 Hz, NH), 7.19 (1H, s, CH), 7.45 (3H, br s, arom.), 8.10 (2H, br s, arom.), MS Fab (pos) MH^+ 359].

The next step involved ring-opening of **3** by Gly-OMe. A similar coupling was reported[16] to be effected by condensation of the diisopropylethylamine salt of Gly-Pro with an azlactone in dry DMF at room temperature for 24 hours. The expected compound was obtained in 65% yield. More recently[10], the same kind of reaction has been performed at reflux in ethylacetate for 8 hours in 84% yield. We have modified these procedures by using 4-dimethylaminopyridine (DMAP) as catalyst to avoid heating, and the yield of tripeptide **4** has been improved to 97%.

Methylation of **4** using MeI, 18-Crown-6 and K_2CO_3 proved to be highly selective[8]. The last coupling step was essentially uneventful and afforded the fully protected linear precursor **6** in 85% yield. Saponification must be carefully monitored since large excess of sodium hydroxide coupled with long reaction time caused partial degradation of the peptide. Cyclization was performed using diphenylphosphoryl azide (DPPA) under high dilution conditions[8,11] in a yield of 17% after purification by semi-preparative HPLC, or by a mixture of DPPA, hydroxybenzotriazole (HOBt) and DMAP, according to Edwards and al.[10]. In our hands, reproduction of this procedure including work up of the reaction resulted in a 65% yield as quoted, but in a mixture of compounds, though this decreased to 19% in Tentoxin after purification by semi-preparative HPLC. Attempts to improve the yield of the cyclisation step using bromo tris(dimethylamino) phosphonium hexafluorophosphate (BroP reagent)[17] were unsuccessful.

In summary, this newly established method provides a convenient and efficient preparation of a dehydrophenylalanine containing dipeptide. Extension of this method to other dehydro amino acid containing peptides is under investigation in our laboratory.

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