

Solid-Phase Synthesis Utilizing Azido- α -Amino Acids: Reduction of Azido-Protected Proline

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Introduction

During an evaluation of azido- α -amino acids and their utility in solid-phase peptide chemistry, the octapeptide angiotensin II and a tripeptide thyrotropin-releasing hormone (TRH) analog have been prepared using Meldal's procedure [1]. A necessary novel step was the reduction of the azido moiety of L-proline, a secondary amine constrained by a ring, to the corresponding free amine for coupling and peptide elongation. In this step a solution of tin(II)chloride (SnCl_2), thiophenol (PhSH) and triethylamine (TEA) [2] in dimethylformamide (DMF) was added at ambient temperature to the azido-L-proline-peptide-resin to afford the desired free amino group (Figure 1) and make the use of azido- α -amino acids more general for solid-phase peptide synthesis.

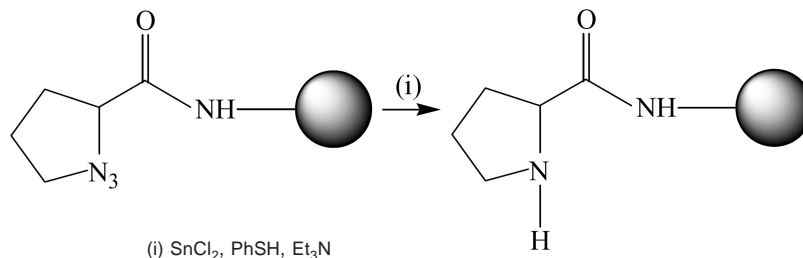


Fig. 1. Reduction of azido-protected proline.

Results and Discussion

Our motivation to explore this chemistry was the use of the azido group in the synthesis of chiral amino acids that could be utilized directly in SPPS, and the reported higher reactivity by use of the acid chloride for coupling combined with the reduced steric bulk of the azido protecting group [1]. The synthesis of the two peptides, a thyrotropin-releasing hormone analog [Phe^2]-TRH and the octapeptide angiotensin II was performed by solid-phase synthesis using the acid chlorides of azido- α -amino acids to evaluate this approach. TRH is responsible for pituitary stimulation of TSH release from the thyroid whereas angiotensin II is a potent vasopressor agent involved in blood pressure homeostasis.

The major problem encountered during the synthesis of angiotensin II was the reduction of the azido-L-prolyl-L-phenylalanyl-resin. During these syntheses, it was discovered that the use of 2 M dithiothreitol (DTT) and 1 M diisopropylethylamine (DIEPA), in DMF at reflux temperature [1], in the reduction of the azido-proline-peptide-resin to the corresponding amino group, was not effective. This azido-reductive method [2] had not been previously applied to SPPS, and hence, it was necessary to optimize its application. The use of SnCl_2 , PhSH and TEA [2] solution in DMF led to the formation of the free amino group of azido-proline. This was sup-

Synthetic Methods

ported by the mass spectroscopy analysis of the cleaved dipeptide (Pro-Phe-OH) resulting in the required molecular ion (MW = 261).

Wong's Cu(II) catalyzed diazo-transfer method [3] was used to generate the azido- α -amino acids that were converted to the acid chlorides with thionyl chloride for coupling. The main byproduct in the preparation of azido- α -amino acids is trifluoromethanesulfonic amide [4], which interferes with purification, analysis and synthesis, but can be completely removed by washing. While it is not necessary to use acid chlorides to activate the carboxylates, they have greater reactivity and increase the rate of reaction, a useful attribute in difficult sequences. The choice of protecting group is crucial; while forming the acid chlorides of the azido-amino acids, acid-labile protecting groups can be cleaved releasing reactive side chains or α -amino groups leading to further side reactions. We chose to use the following side-chain protection during the synthesis of the two peptides: Arg(Tos), Arg(Mtr), Asp(OBut), His(Bom), Pro-OMe·HCl, Pro-OBzl·HCl, Tyr(But), Tyr(2-Br-Z). [Phe²]-TRH was obtained in 65% yield and 58% purity on cleavage from the support. Angiotensin II was obtained in 75% yield and 63% purity on cleavage from the support.

Acknowledgments

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