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A method for the one-pot regioselective formation of the two disulfide bonds of α-conotoxin SI

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Abstract

A one-pot method for the regioselective formation of the disulfide bridges of α-conotoxin SI employing temperature-controlled orthogonal protecting groups is reported. The pairing of *t*-butyl and 4-methylbenzyl side-chain protecting groups was chosen for the four cysteine residues. Cleavage of the peptide from a solid support afforded a fully *S*-protected crude product. The first disulfide bridge was formed directly from the crude material by simultaneous cleavage and oxidation of the *t*-butyl groups in TFA:DMSO:anisole (97.9:2:0.1) at room temperature. The subsequent heating of this solution resulted in the cleavage of the 4-methylbenzyl groups with simultaneous oxidation yielding the desired bicyclic product.

Keywords: disulfides; peptide; regioselection; protecting groups.

The regioselective formation of disulfide bridges in peptides and proteins still remains a significant challenge to peptide chemists. Although several cysteine protecting groups are orthogonal and have been successfully employed in the stepwise formation of multiple disulfides,[1] there still exists a need for alternative strategies. A common feature of most of the protocols currently in use today is the discrete steps of *S*-deprotection/oxidation followed by the separation of folded or partially folded products. The complex nature of this process often means that syntheses are both time-consuming and low yielding. The present study simplifies the folding conditions by utilising the dimethylsulphoxide (DMSO)/trifluoroacetic acid (TFA) oxidation[2] to regioselectively form two disulphides in a one-pot reaction eliminating the need for the isolation of intermediates.

During the course of our work we noted that the *S*-protecting groups 4-methylbenzyl[3] (4-MeBn) and acetamidomethyl[4] (Acm) became progressively more labile in TFA/DMSO mixtures at elevated temperatures. Furthermore, cleavage was accompanied by the simultaneous formation of disulfide bonds. In contrast cysteine residues protected with the *t*-butyl[5] (*t*-Bu) groups were rapidly cleaved and converted to cystine at room temperature, conditions where the 4-MeBn and Acm groups (data not shown) remained intact. We postulated that temperature-controlled orthogonality could potentially facilitate a one-pot

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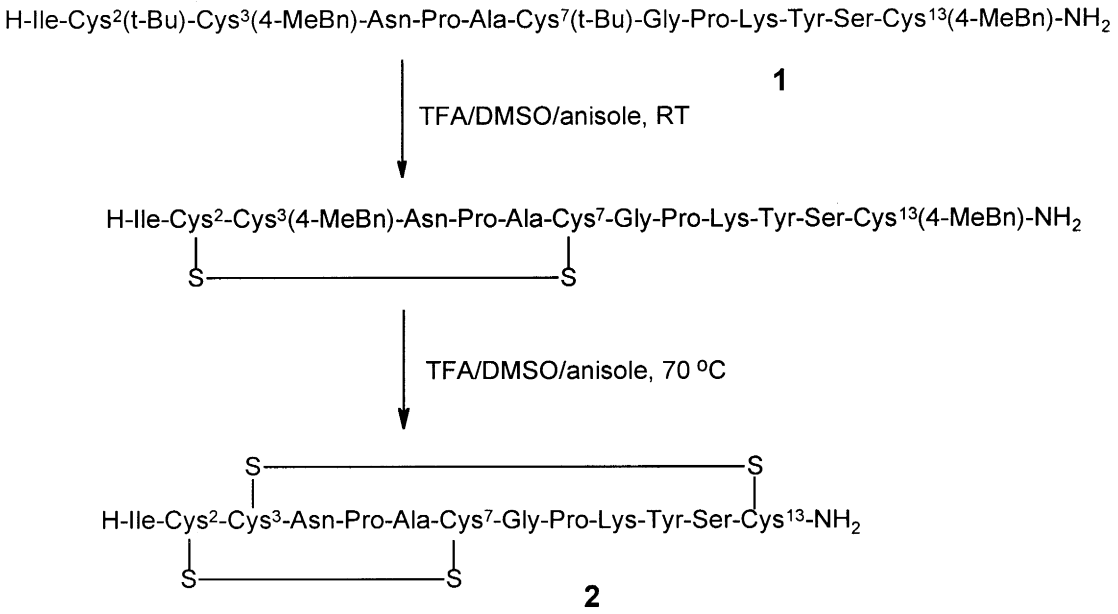
regioselective formation of multiple disulfide bonds. In order to test this hypothesis we selected the peptide α-conotoxin SI, a tridecapeptide amide from the marine cone snail venom, as a challenging target.[6] Several studies have been reported for the synthesis of this peptide using a variety of combinations of protecting groups.[7] The naturally occurring sequence comprises two interlocking disulfide bridges between cysteine residues 2–7 and 3–13. In this paper we wish to report on a method for the one-pot conversion of the cysteine protected peptide to the fully folded product as shown in Scheme 1.



Scheme 1. One-pot method for the folding of α-conotoxin SI

Partially protected α-conotoxin SI, H-Ile-Cys(*t*-Bu)-Cys(4-MeBn)-Asn-Pro-Ala-Cys(*t*-Bu)-Gly-Pro-Lys-Tyr-Ser-Cys(4-MeBn)-NH_2 **1**, was assembled by 9-fluorenylmethoxycarbonyl solid-phase chemistry[8] on a Rink Amide polymer then cleaved in TFA containing the scavengers water and triisopropylsilane. In a typical experiment 50 mg of crude **1** was dissolved in 100 mL of TFA:DMSO:anisole (97.9:2:0.1) in a round bottom flask and the mixture stirred for 40 min at room temperature. MALDI-TOF analysis of the peptide solution revealed that the cleavage of the *t*-Bu groups and oxidation of the first disulfide was complete. The flask was then fitted with a condenser and placed in an oil bath pre-heated to 70°C and a further 2 mL of DMSO added. After heating for 3 h MALDI-TOF analysis showed that the 4-MeBn groups had been removed and a new species corresponding to fully folded product **2** had appeared. The excess TFA was then removed in vacuo and the product precipitated by the addition of diethyl ether. Following trituration with diethyl ether and air drying, the crude product was purified by preparative HPLC yielding the desired peptide **2** in 53% yield (HPLC purity >98%). Fig. 1 shows the analytical HPLC data for the partially protected starting material, the crude folded product following work-up and the purified final product. The conversions proceeded almost quantitatively with few noticeable side-reactions. The correct folding pattern was confirmed by co-elution[9] with an authentic sample of α-conotoxin as the other disulphide isomers are known to be separated by analytical HPLC.[10] In addition amino acid analysis and accurate mass analysis were performed to further characterise the product.[11]

In conclusion we have demonstrated a novel and convenient one-pot method for the regioselective synthesis of α-conotoxin SI. The conversion was clean, high yielding and reproducible offering a new

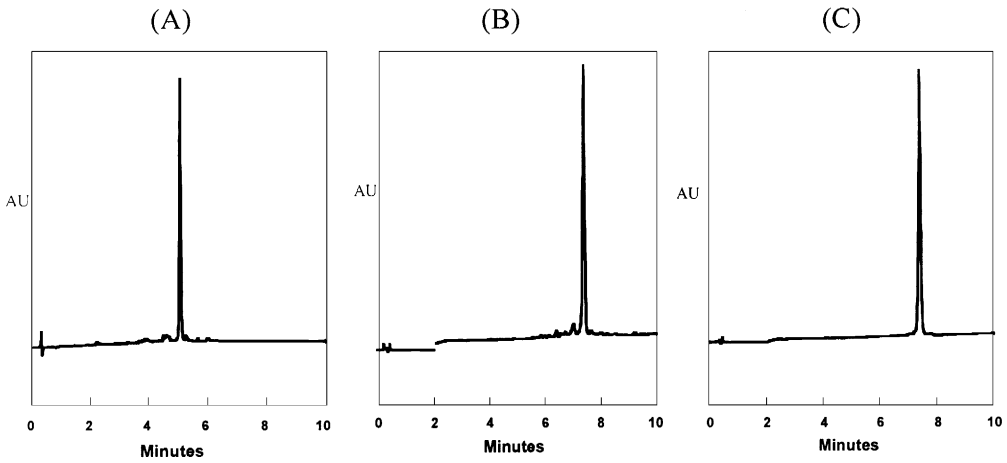


Fig. 1. Analytical HPLC of (A) the crude cysteine protected peptide (B) the crude fully folded peptide and (C) pure peptide following purification. All samples were run on a Luna C18 column (10×4.6 mm) at a flow rate of 2 mL/min where solvent A=0.1% TFA/water and B=0.1% TFA/acetonitrile employing gradients of 20–70% B for (A) and 0–30% B for samples (B) and (C)

approach to the rapid synthesis of this class of peptide toxin. Although it is anticipated that the utility of this methodology may be limited by amino acid composition it should still provide an interesting addition to current protocols. It is hoped that this simple procedure may find application either in combinatorial chemistry or in the large-scale synthesis of peptides containing multiple disulfide bridges.

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9. HPLC co-elution: Product **2** and an authentic sample of α-conotoxin SI (Bachem) were co-injected on a Vydac 218TP54 analytical column. The peptide was eluted using a gradient of 0 to 30% B over 40 min where A=water/0.1% TFA and B=acetonitrile/0.1% TFA at a flow rate of 1 mL/min. The product was a single peak with a retention time of 28.3 min.
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11. Analytical data for product **2**. Amino acid analysis: Asn 1 (1.01); Ile 1 (0.97); Cys 4 (3.95); Pro 2 (1.95); Ala 1 (1.01); Gly 1 (1.00); Lys 1 (1.00); Tyr 1 (0.94); Ser 1 (1.02). Mass spectrometry: accurate mass measurement was performed on a laser-desorption mass spectrometer coupled with delayed extraction. In the MALDI analysis, standard peptide signals (Angiotensin I, 1296.6853 Da. and Glu1-Fibrinopeptide B, 1570.6774 Da.) were used to accurately measure the protonated $[M+H]^+$ monoisotopic sample ion. The ion was analysed eight times in this way and the average value calculated, *m/z* calculated $C_{55}H_{84}N_{16}O_{16}S_4$: 1352.5134, found: 1353.5091 $[M+H]^+$.