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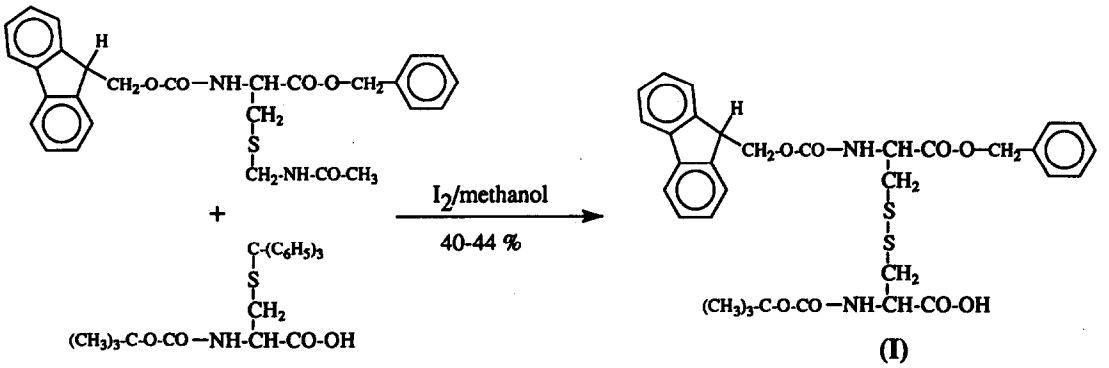
SEQUENTIAL SYNTHESIS OF AN UNSYMMETRICAL TWO-CHAIN DISULFIDE PEPTIDE ON SOLID-PHASE

Erika E. Büllesbach and Christian Schwabe
Medical University of South Carolina
Department of Biochemistry and Molecular Biology
171 Ashley Avenue
Charleston S.C. 29425

The synthesis of an unsymmetrical cystine-derivative (Nα'-9-fluorenyl-methyloxycarbonyl-Nα"-tert.butyloxycarbonyl-cystine-O'-benzyl ester) allowed the sequential synthesis of a disulfide-linked two-chain polypeptide on solid-phase.

Proteohormones of the insulin family such as relaxin (1), bombyxin (2), and molluscan insulin-related peptide (3), are two-chain polypeptides with at least two interchain and one intrachain disulfide link. Typically, the cysteine at or close to the C terminus of the A chain is crosslinked to a cysteine in the B chain. In order to synthesize peptides of such complex disulfide structure it is adventageous to be able to synthesize each disulfide bond specifically. This approach has been used successfully in the synthesis of fully biologically active insulin (4) and relaxin (5). The distribution of the cysteines in these hormones suggested to us an even more efficient strategy involving an unsymmetrical cystine in which the two amino groups are protected by orthogonally removable protecting groups and wherein one of the two carboxyl groups is semipermanently protected. The only free carboxyl group of the cystine derivative is specifically activated and introduced into a growing peptide chain. Further extension of the peptide is possible by the liberation of one amino group at a time. In the present paper we report on the synthesis of Fmoc-Cys(Boc-Cys-OH)-OBzl (I) and its application in the production of a bis-cystinyl peptide on solid phase.

For the synthesis of Fmoc-Cys(Boc-Cys-OH)-OBzl, Boc-Cys(Trt)OH (1mmol) (Bachem, Torrance, CA) and Fmoc-Cys(Acm)-OBzl (6) (1mmol) in 30 ml of methanol were reacted with 3 mmol of iodine (in 10 ml of methanol) for 30 minutes at room temperature. As previously reported (7) under these conditions a nonstatistical distribution of the unsymmetrical cystines is observed. The reaction was quenched with an excess of aqueous thiosulfate. The cystine derivative was extracted into ethyl acetate (30ml, 3 times), the pooled organic layers washed with water, dried over $MgSO_4$, filtered, and dried *in vacuo*. Symmetrical and unsymmetrical disulfides were



separated on silicagel (2.5 cm x 30 cm; Bio-Sil A, 100-200 mesh, Biorad) in benzene/acetic acid (9:1 v/v). The unsymmetrical disulfide was concentrated *in vacuo* and rechromatographed on Bondapak C_{18}/Porasil B, 37-75 microns (Waters, Milford, MA) (column: 2.0 cm x 30 cm) in acetonitrile/water 8:2 v/v. The yield of the product was 40-44%. The cystine derivative was homogeneous by thin layer chromatography and showed the expected ^{1}H-NMR-spectrum(8).

In order to test the applicability of the Fmoc-Cys(Boc-Cys-OH)-OBzl an unsymmetrical disulfide peptide was synthesized on an automatic peptide synthesizer (ABI-430A, Applied Biosystems, Forster City, CA), using standard Boc-chemistry. The unsymmetrical cystine was introduced on a manual shaker, using a 2.5-fold excess of preactivated 1-hydroxybenzotriazole (HOBT) ester. The condensation was completed after 2 h and the automatic synthesis was continued by Boc-chemistry. The N terminus of the B-chain was acetylated, the Fmoc-group removed by treatment with