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UTEROGLOBIN-LIKE PEPTIDE CAVITIES I. SYNTHESIS OF ANTIPARALLEL AND PARALLEL DIMERS OF BIS-CYSTEINE PEPTIDES

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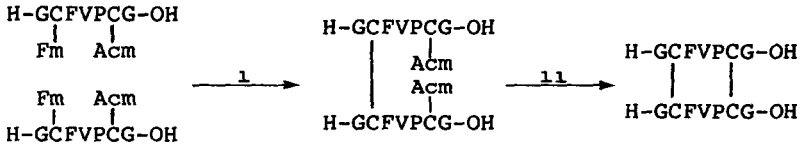
Abstract: Syntheses of parallel and antiparallel dimers of bis-cysteine peptides using four different protecting groups for the cysteine side chain are described.

Uteroglobin, a small globular protein, is formed by two identical chains of 70 amino acid residues linked by two disulfide bridges in antiparallel sense[1]. It is found in uterine secretions of the pregnant and pseudopregnant rabbits[2]. Uteroglobin presents the property of binding the progesterone that is the same steroid which induces its biosynthesis[3]. Likewise, the recently isolated β -hANP has been found to be the antiparallel dimer of α -human atrial natriuretic peptide[4]. Thus, the development of a methodology to prepare dimers of peptides containing cysteines constitutes an obvious need. Finally, these molecules are the basis for the design of a new class of molecular cavities that can recognize and bind substrates.

We have followed several strategies to obtain parallel and antiparallel dimers of bis-cysteine peptides based on the use of four different protecting groups for the thiol function of cysteine: the HF stable, Fluorenylmethyl (Fm)[5,6], acetamidomethyl (Acm)[7], and 3-nitro-2-pyridinesulfenyl (Npys)[8]; and HF labile 4-methylbenzyl (MeBzl)[9]. These protecting groups present an excellent level of orthogonality[10]: it is possible to remove the Acm or Npys in presence of anyone of the rest, Fm in presence of Acm, and the MeBzl in presence of Fm and Acm.

For this study, we have chosen the peptide sequence -Gly-Cys-Phe-Val-Pro--Cys-Gly-, the antiparallel dimer of this peptide is similar to [Phe^4, Val^6] antamanide, an active analogue with C_2 symmetry[11,12], with the substitution of a proline and a phenylalanine residues by cysteine.

The parallel dimer was prepared from a single peptide with the two cysteine residues protected by a Fm and an Acm groups. This protected peptide was synthesized on a Pab-resin[13]. The first glycine was anchored onto the resin according to the cesium salt method[14]. The rest of amino acids were incorporated following a standard procedure[13]. At the end of the synthesis, the peptide-resin was subjected to the HF reaction in presence of 10% of p-cresol. The crude product was washed with CH_2Cl_2 and used without further purification to obtain the parallel dimer (17% of total yield from Boc-Gly-OCH_2-Pab-resin) by treatment with piperidine, followed by oxidation with I_2, and purification by Sephadex G-10 column and MPLC (scheme 1)[15].

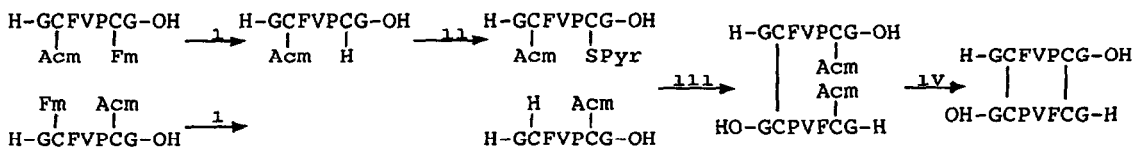


i) a: piperidine-DMF (1:1), 150 min. 25°C. b: washings with CH_2Cl_2; ii) a: I_2 in 80% AcOH, 60-90 min. 25°C. b: Zn. c: Sephadex G-10 in 0.1M AcOH. d: MPLC on Vydac C_{18} column with a convex gradient (AcCN/H_2O/TFA mixtures).

Scheme 1

The synthesis of the antiparallel isomer requires the concourse of two peptides with the same amino acid sequence but with different protecting groups for the cysteine residues. Nevertheless, the preparation of both peptides can be carried out simultaneously in solid phase using two polymers: the conventional, polystyrene-1 %-divinylbenzene, and Kel-F-g-styrene[16] which have different densities and can, therefore, be separated by flotation[17] before the incorporation of the first protected cysteine. Then, the two peptide-resins are mixed again and the syntheses jointly continued until the second cysteine residue where the process is repeated. Peptides were synthesized using Pab-handles following the same protocol above described. After the incorporation of the last residue of glycine, the two peptide-resins were separately treated with HF in presence of 10% p-cresol.

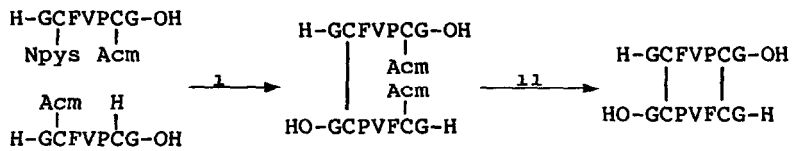
The antiparallel dimer has been obtained by two alternative strategies using the Kel-F/polystyrene methodology. The first (scheme 2) involves the use of only two protecting groups: Fm and Acm. The Fm group of both peptides was removed with piperidine in presence of β-mercaptoethanol in order to obtain both peptides with the thiol function free. One of these was allowed to react with dithiodipyridine and, after purification by a Sephadex G-10 chromatography, mixed with the other peptide to form the first disulfide bridge. The final oxidation with I_2 and purification lead to the antiparallel dimer (15 % yield from Boc-Gly-OCH_2-Pab-resin).



i) piperidine-DMF-β-mercaptoethanol (45:45:10), 2 h, 25°C; ii) a: dithiopyridine in Tris pH 8-isopropanol (1:1), overnight, 25°C, under Ar atmosphere. b: Sephadex G-10 in O.1M AcOH; iii) a: acetate buffer pH 5, 1 min, 25°C. b: MPLC; iv) a: I_2 in 80% AcOH, 60-90 min, 25°C; b: Zn; c: Sephadex G-10 in 0.1M AcOH; d: MPLC.

Scheme 2

The second strategy (scheme 3), similar to the one described by Sakakibara et al.[18] for the synthesis of β -hANP, involves the synthesis of a peptide with a Npys and an Acm as protecting groups of cysteines and a second one with a free thiol and an Acm in cysteine two. During the solid-phase assembling of this peptide, cysteine six was protected with the MeBzl group which is removed during the HF reaction. Peptides were purified by MPLC. Equivalent amounts of both peptides were mixed and, after lyophilization, the crude was purified by MPLC. The antiparallel dimer was obtained (11% from Boc-Gly-OCH_2-Pab-resin) after treatment with I_2 and subsequent purification by Sephadex G-10 and MPLC.



i) a: acetic acid solution at pH 3.0. b: MPLC; ii) a: I_2 in 80% AcOH, 60-90 min, 25°C; b: Zn; c: Sephadex G-10 in 0.1M AcOH; d: MPLC.

Scheme 3

In conclusion, this work describes different alternatives for the unequivocal synthesis of antiparallel and parallel dimers of peptides containing two cysteine residues. Current experiments in our laboratory show that spontaneous dimerization of monomers with one or two free thiols affords mixtures of the three possible dimers.

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17. The peptide-resins were suspended in CH_2Cl_2-trifluoroethanol (8:2) for 30 min. After separation, both peptide-resins were independently subjected to the same process.

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