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Synthesis of Phytochelatins by the Continuous Flow Solid Phase Procedure

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Dedicated to Professor Ernst Bayer on the occasion of his 65th birthday

Summary: A nona- and an undecapeptide corresponding to phytochelatins with the general structure H-[γ-Glu-Cys]$_n$-Gly-OH were each synthesized by the continuous flow solid phase method using two different methodologies. Fmoc-amino acid derivatives were used as precursors, and two different H_2N-POE-PS supports were employed. Different procedures were used to remove Acm protecting groups from Cys residues. In a second synthesis, Acm groups were removed before cleavage of the peptides from the polymer supports. The partially protected peptides of the first synthesis were purified by preparative HPLC. The purity and identity of all the synthesized peptides were verified by analytical HPLC and IS-MS and in some cases by amino acid analysis.

Key terms: Continuous flow synthesis, phytochelatins, heavy metal toxicity, POE-PS.

Phytochelatins (PCs) are small peptides with the general structure H-[γ-Glu-Cys-]$_n$-Gly-OH ($n = 2–11$)[1,2] which play a major role in detoxification of heavy metals in plants, fungi and algae. Upon exposure to heavy metal ions, such as $Cd^{2\oplus}$, $Zn^{2\oplus}$, $Cu^{2\oplus}$, $Hg^{2\oplus}$, $Pb^{2\oplus}$, etc., plants synthesize PCs, which then form complexes with the ions.

PCs have generally been isolated from cell suspension cultures, although a very small number of PCs has also been chemically synthesized[3–5]. Since only small quantities of phytochelatins can be obtained by isolation, we considered it of interest to synthesize some of these compounds, in order to make them available for further physicochemical investigations such as metal binding studies using NMR and ESR methods.

Recently we succeeded in synthesizing a few PCs by the classical solution synthesis using *St*Bu protecting groups for masking the thiol function of Cys[6].

We now describe the continuous flow solid phase synthesis of two of such compounds ($n = 4$ and 5) on two different H_2N-POE-PS supports[7–9], using an acid-labile linker[10] and the Acm group for protection of the SH groups of Cys residues. Several procedures were tested for removal of the protecting group. A novel procedure in the present investigation, which yielded excellent results, was the deprotection of Acm groups by iodine before cleavage of the peptides from the polymer support. It is more usual in SPPS to perform the deprotection of side-chain protecting groups and cleavage of the peptide-resin linkage simultaneously. Another common practice is the de-

Abbreviations:
The abbreviations used are in agreement with the rules of the IUPAC-IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.* **138**, 9–37 (1984); *Biochem. J.* **219**, 345–373 (1984); *Int. J. Pept. Protein Res.* **24**, 9–37 (1984); *Pure Appl. Chem.* **56**, 595–624 (1984).
Other abbreviations: Acm, acetamidomethyl; CF-SPPS, continuous flow solid phase peptide synthesis; DCM, dichloromethane; DIC, diisopropylcarbodiimide; DMAP, *N,N*-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; DTT, 1,4-dithiothreitol; EDT, 1,2-ethanedithiol; Fmoc, fluoren-9-ylmethoxycarbonyl; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HMPA, 4-hydroxymethyl-3-methoxyphenoxyacetic acid; HOBt, 1-hydroxybenzotriazole; IS-MS, ion-spray mass spectrometry; ME, mercaptoethanol; NMM, *N*-methylmorpholine; POE = PEG, polyoxyethylene; PS, polystyrenedivinylbenzene (1%); SPPS, solid phase peptide synthesis; *t*Bu, *tert*-butyl; TEA, triethylamine; TFA, trifluoroacetic acid.