Fmoc Solid-Phase Peptide Synthesis of Human α-Calcitonin Gene-Related Peptide and Two Fluorescent Analogs

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Introduction

Human α-Calcitonin Gene-Related Peptide (h-α-CGRP) is a naturally occurring 37 amino acid vasodilatory neuropeptide amide, ACDTATCVTHRLAGLLSRSGGKVKNFVPTNVGSKAF, with a disulfide bond between residues 2 and 7. The peptide is found in primary afferent sensory nerves and is widely distributed throughout the central and peripheral nervous systems in the body [1]. Structure activity studies of h-α-CGRP have shown that the middle and C-terminal part of the peptide allow the formation of the appropriate conformation required for the interaction with the receptor, while the N-terminus is essential for biological activity and onset of signal [2]. Fluorescent h-α-CGRP analogs are useful for investigating the mechanism behind (re)uptake of h-α-CGRP into the sensory nerve terminals and monitoring trafficking of CGRP receptors. As part of an ongoing study on the mechanism of action behind h-α-CGRP-induced vasodilation, we here present an Fmoc strategy for the synthesis of [Cys2,7(Acm)] h-α-CGRP (1), h-α-CGRP (2), and two fluorescent h-α-CGRP analogs labelled with 5-carboxyfluorescein [3] (5CF) at the side-chain of K24. The first analog, [Cys2,7(Acm), 5CFK24] h-α-CGRP (3) is linear, while the second [5CFK24] h-α-CGRP (4), contains the native disulfide bond.

Results and Discussion

The peptides (1) and (2) were synthesized using standard Fmoc chemistry on a TentaGel RAM resin (50 mg, loading 0.24 mmol/g) (Figure 1). Activation of the Fmoc amino acids was carried out using HATU/HOAt/DIEA (4:4:8) [4].

Fig. 1. Strategy for the synthesis of compound (4).
Fmoc-Cys(Acm)-OH was used for residue 2 and 7. Fmoc deprotection was accomplished by treatment with 20% piperidine in DMF (3x4 min) and final wash with DMF/DCM/DMF (3x/3x/5x). The peptides were cleaved from the solid support along with the permanent side chain protection groups using TFA/H₂O/TIS (90:2.5:2.5 v/v) for 2 h. The crude peptides were purified by preparative HPLC and characterized by MALDI-TOF-MS (Figure 2). The peptides (3) and (4) were synthesized as above with the following modifications: Fmoc-Lys(ivDde)-OH was used at residue 24. Following SPPS, the ivDde was cleaved by treatment with 2% hydrazine hydrate in DMF (12x5 min). This is significantly longer than reported in the literature but a cleavage study using the model peptide Boc-A-F-S-K(ivDde)-S-F-NH-Resin showed that it was necessary. After DMF wash, 5-carboxyfluorescein was coupled overnight to the side-chain of K24 using HATU/HOAt/DIEA (5:5:10 eq). Following resin cleavage, disulfide bond formation for compound 2 and 4 was achieved by dissolving the HPLC-purified and Acm-protected peptides in I₂/acetic acid (20mM) and 60 mM HCl [5]. MALDI-TOF-MS indicated that the reaction was completed after 30 min. Next, 9 vol. eqv. of ice-cold ether was added and cooled on dry ice for 10-15 min. The suspension was then centrifuged, decanted and purified by RP-HPLC.

In conclusion, we present an Fmoc strategy for the syntheses of [Cys2,7(Acm)] h-α-CGRP (1), h-α-CGRP (2), and two fluorescent h-α-CGRP analogs labeled with 5-carboxyfluorescein at the side-chain of K24. The first analog, [Cys2,7(Acm), 5CFK24] h-α-CGRP (3) is linear, while the second [5CFK24] h-α-CGRP (4), contained the native disulfide bond. However, the compounds were obtained in low yields. Additional future work will include protocol optimization and performing binding and functional studies.

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References