Proceedings of the 24th American Peptide Symposium
Ved Srivastava, Andrei Yudin, and Michal Lebl (Editors)
American Peptide Society, 2015 *http://dx.doi.org/10.17952/24APS.2015.264*

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, ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF, 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].

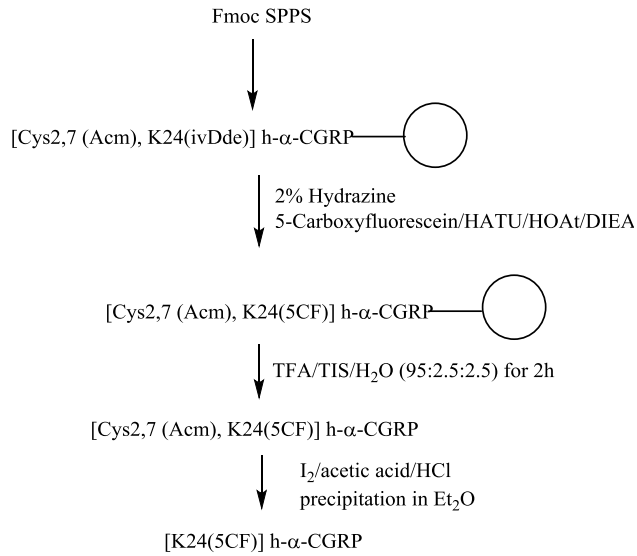

Fig. 1. Strategy for the synthesis of compound (4).

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/H_2O/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 I_2/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.

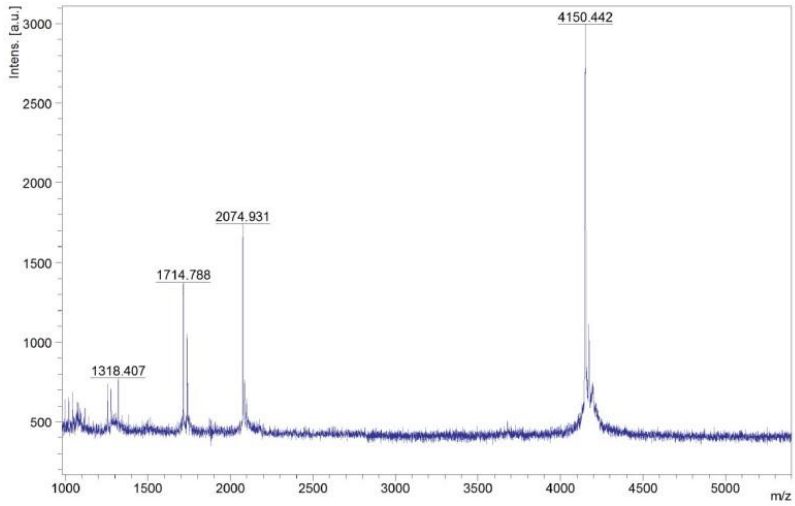

Fig. 2. MALDI-TOF-MS of compound 4.

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.

Acknowledgments

Birgitte Simonsen is thanked for excellent technical assistance. This work was supported by an ERASMUS grant to M. Fuente-Moreno.

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