# The carba-modification of cystine-containing peptides: Synthesis of selectively protected cystathionines and their incorporation into the oxytocin molecule

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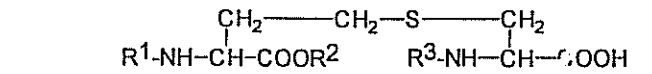
## Introduction

The reduction of disulfide bridge in cystine containing peptidic hormones results in many cases in loss of biologically active conformation. The replacement of the chemically labile disulfide group by an isostere structure is of interest for new stable structures of hormone analogs. The methylene-thio-group (-$CH_2$-S-) was introduced as a suitable replacement of the disulfide bond [1, 2]. Several types of cystine-substituting amino acids were prepared [1-9], but only several examples maintaining all functional groups and the length of the bridge are known [10, 11]. In this study, the selectively protected monocarba-isosteres of cystathionines were prepared as building blocks and applied in a combined stepwise solid phase/solution synthesis for a series of analogs of the neurohypophyseal hormone oxytocin.

## Results and Discussion

Selectively protected cystathionines **1-5** (Figure 1) were synthesized using S-alkylation [12] of cysteine with fully protected derivatives of 2-amino-4-bromobutyric acid [10] and subsequent introduction of Fmoc-group (compound **3-5**). The derivatives **2** and **3** were used for the synthesis of carba-1-oxytocin ($C^1$-OXT) chosen as a model peptide. The procedure using derivative **2**, pMBHA resin and Boc/Bzl strategy gave in 12 synthetic and purification steps 6.8% of $C^1$-OXT. In contrast, our new approach using Fmoc-protected derivative **3**, acid labile handle and Fmoc/Bzl strategy (Figure 2) yielded 63.8% of $C^1$-OXT after optimization. The cyclization was carried out by DPPA/DIEA (10eq) in DMF with 78% preparative yield. For the final deprotection, a mixture TFMSA/TFA/thioanisole/m-cresole/DMS (5/85/5/2.5/2.5) was used with the yield of 81%. As an alternative method, derivative **3** and SCAL-handle [13] with on-the-resin cyclization was tested with the yield 34% of $C^1$-OXT. The synthetic procedure using cystathionine derivative **3** was further applied for a series of analogs of oxytocin modified in position 4 and/or 7 (agonists) and positions 2,4,8 (inhibitors). Biological evaluation of these analogs allowed to differentiate the influence of deamination in position 1 and carba modification of the bridge on their agonistic and/or antagonistic properties.



1. $R^1$=ClZ, $R^2$=Me, $R^3$=Boc
2. $R^1$=Z, $R^2$=Me, $R^3$=Boc
3. $R^1$=ClZ, $R^2$=Bu$^t$, $R^3$=Fmoc
4. $R^1$=Z, $R^2$=Bu$^t$, $R^3$=Fmoc
5. $R^1$=Boc, $R^2$=Bu$^t$, $R^3$=Fmoc

*Fig. 1. Selectively protected cystathionines.*

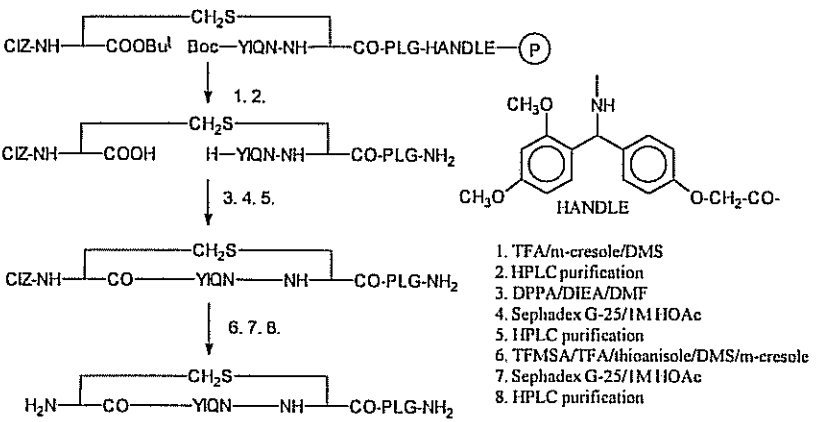



*Fig. 2. Optimized strategy for the synthesis of $C^1$-OXT.*

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