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Synthesis of *N*-Methyl and Azasulfuryl Urotensin-II$_{(4-11)}$ Derivatives

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Introduction

Human urotensin-II (*h*U-II) is a cyclic peptide that is able to regulate cardiovascular homeostasis [1]. The shortest active sequence required for biological activity of *h*U-II contains residues 4-11 in a cycle featuring a disulfide bond between Cys[5]-Cys[10], Asp-*c*[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH (Figure 1), and is suggested to adopt a β-hairpin conformation of prime relevance for interaction with the urotensin-II receptor (UT) [2]. Employing the minimal active sequence, we have developed synthetic strategies to prepare new derivatives in which *N*-methylated (**1**) and azasulfuryl (**2**) residues have been inserted into the cyclic peptide core region (Figure 1). Mono and multiple *N*-methylation of the amide bonds and insertion of *N*-aminosulfamide residues into *h*U-II$_{(4-11)}$ have been performed to survey their influences on intra- and inter-molecular hydrogen bonds, geometry and interactions with UT.

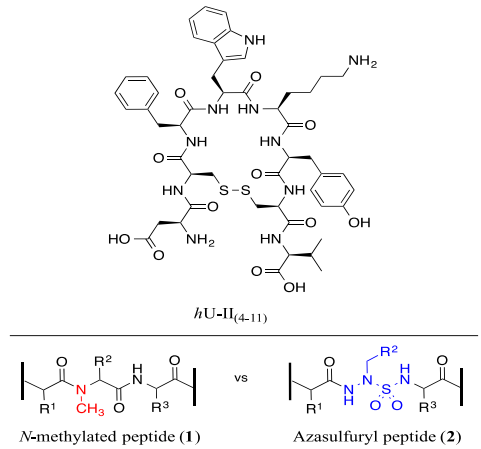
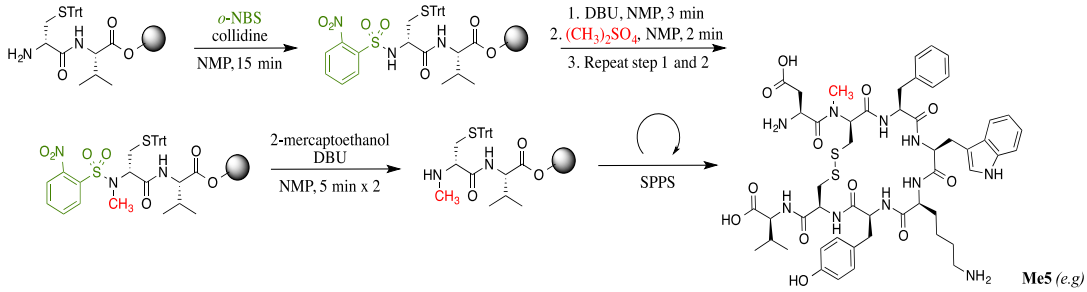


Fig. 1. Structure of hU-II$_{(4-11)}$ and N-methyl (1) and azasulfuryl (2) moieties introduced in its sequence.

Results and Discussion

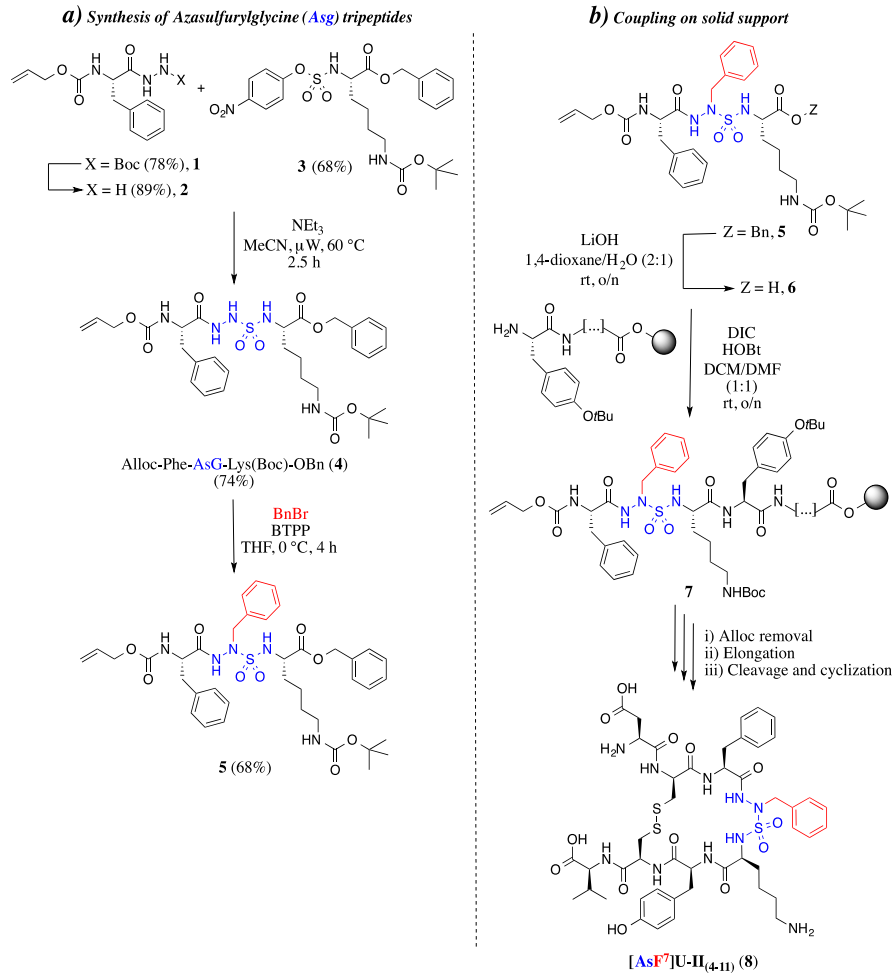
A solid-phase procedure was used to install *N*-methyl groups on amides at residues 5-10 consisting of three fundamental steps: i) amine protection with the *o*-nitrobenzenesulfonyl group (*o*-NBS), ii) amine alkylation with methylsulfate and 1,8-diazabicycloundec-7-ene (DBU) as base, and iii) selective removal of the *o*-NBS group on solid support (Scheme 1) [3].

N-Aminosulfamides are peptidomimetics in which the $C_\alpha H$ and the carbonyl of an amino amide residue are respectively replaced by a nitrogen atom and a sulfuryl group. Azasulfurylphenylalanine (AsF) analogue **8** was synthesized by an approach consisting of two phases: i) the synthesis of the AsF tripeptide building block **5** by solution phase chemistry with protected functional groups compatible



*Scheme 1. Three-step procedure for the synthesis of N-methylated U-II analogues is illustrated for the preparation of [N-(Me)Cys[5]]hU-II$_{(4-11)}$ (**Me5**).*

with solid phase peptide synthesis (Scheme 2a); ii) incorporation of AsF tripeptide **6** into the peptide sequence on solid support using a Fmoc/*t*Bu orthogonal protection strategy (Scheme 2b). In [AsF^7]*h*U-II$_{(4\text{-}11)}$ (**8**), the Trp^7 residue was replaced by azasulfurylphenylalanine with the phenyl group serving as a mimic of the indole moiety. Initially, the AsF tripeptide building block **5** was synthesized using a solution-phase approach featuring chemoselective alkylation of azasulfurylglycine (AsG) **4** with benzyl bromide and *tert*-butylimino-tri(pyrrolidino)phosphorane (BTPP) as base [4].



*Scheme 2. Synthesis of [AsF^7]hU-II$_{(4\text{-}11)}$ (**8**): a) preparation of azasulfurylphenylalainine (AsF) tripeptide **5**; b) introduction of AsF tripeptide into [AsF^7]U-II$_{(4\text{-}11)}$ (**8**).*

With AsF tripeptide building block **6** in hand, solid phase chemistry was performed on Rink amide resin. Azasulfuryl tripeptide **6** was coupled to the Tyr^9 residue using di-*iso*-propylcarbodiimide and hydroxybenzotriazole. Subsequently, the *N*-terminal Alloc group was removed using palladium catalysis and the sequence was elongated by conventional solid phase peptide synthesis (SPPS) [5]. Cleavage and disulfide bond formation were accomplished in a one-pot reaction using a cocktail of 10% DMSO in TFA in the presence of anisole as scavenger to afford azasulfuryl peptide **8**, which was purified by HPLC on a C18 bonded silica column and characterized by LCMS and HRMS analysis [t_R = 14.18 min, 20 to 80% methanol (0.1% formic acid) in water (0.1% formic acid) over 15 min;

Table 1. Biological data for mono N-methylated hU-II$_{(4-11)}$ analogues.

Peptide	*Sequence*	*Biological data*	
		pEC$_{50}$[a]	*pK$_D$/pK$_i$*[b]
***h*U-II$_{(4-11)}$**	Asp-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH	8.20±0.01	8.34±0.04
Me5	Asp-c[(NMe)Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH	8.26±0.03	8.53±0.08
Me6	Asp-c[Cys-(NMe)Phe-Trp-Lys-Tyr-Cys]-Val-OH	6.36±0.04	6.64±0.10
Me7	**Asp-c[Cys-Phe-(NMe)Trp-Lys-Tyr-Cys]-Val-OH**	**8.50±0.05**	**8.76±0.07**
Me8	Asp-c[Cys-Phe-Trp-(NMe)Lys-Tyr-Cys]-Val-OH	4.91±0.18	=5
Me9	Asp-c[Cys-Phe-Trp-Lys-(NMe)Tyr-Cys]-Val-OH	5.25±0.07	=5
Me10	Asp-c[Cys-Phe-Trp-Lys-Tyr-(NMe)Cys]-Val-OH	8.31±0.08	8.45±0.05

[a]*functional activity was tested on the rat thoracic aorta;* [b]*binding affinity was evaluated on UT receptors expressed on CHO cell line.*

calculated mass: 1059.3733, found: 1059.3706]. Similar procedures are being pursued to broaden the library of azasulfuryl peptide mimics of the *h*U-II$_{(4-11)}$ sequence.

Biological data for the *N*-methyl U-II$_{(4-11)}$ analogs was acquired through experiments performed on CHO cell lines expressing the human UT receptor for binding affinity, and on rat thoracic aorta for functional activity, according to experimental procedures previously described [6]. In agreement with the hairpin conformation, *N*-methylation of amide bonds of U-II$_{(4-11)}$ at Phe^6 and Tyr^9 decreased binding affinity and consequently reduced vasoconstriction in the rat thoracic aorta assay (Table 1).

In contrast, *N*-methylation of Trp^7 (e.g., **Me7**) improved affinity relative to the parent peptide. On the other hand, *N*-methylation of the amide bonds at position 8, which is normally occupied by Lys, decreased affinity, and reduced vasoconstriction in the rat thoracic aorta assay. *N*-Methylation of the Cys residues at positions 5 and 10 gave more active analogs (e.g., **Me5** and **Me10**) without significant change in binding affinity. Preliminary data inspired investigation of a set of di-methylated analogs, which were synthesized by a similar process and are presently being investigated for biological activity. The influence of *N*-methylation on conformation is also being analyzed by NMR spectroscopy.

Other azasulfuryl amino acids are being inserted into the cyclic portion of the *h*U-II$_{(4-11)}$ sequence by replacement of the Trp^7 and Lys^8 amino acid residues. Their synthesis and activity will be reported in due time.

Information from *N*-methylation and *N*-aminosulfamide studies is designed to provide a detailed view of the influences of hydrogen-bonding in the *h*U-II$_{(4-11)}$ sequence. In preliminary results, removal of the N-H group of Trp^7 by methylation enhanced interaction with the UT receptor. Further investigation of the urotensin analogues is ongoing and their impact on structure-activity relationships of the urotensinergic system will be reported in due time.

Acknowledgments

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