Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Urotensin-II Peptidomimetic Incorporating a Non-Reducible 1,5-Triazole Disulfide Bond Reveals a Pseudo-Irreversible Covalent Binding Mechanism to the Urotensin G-Protein Coupled Receptor

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1. Supplementary Figures

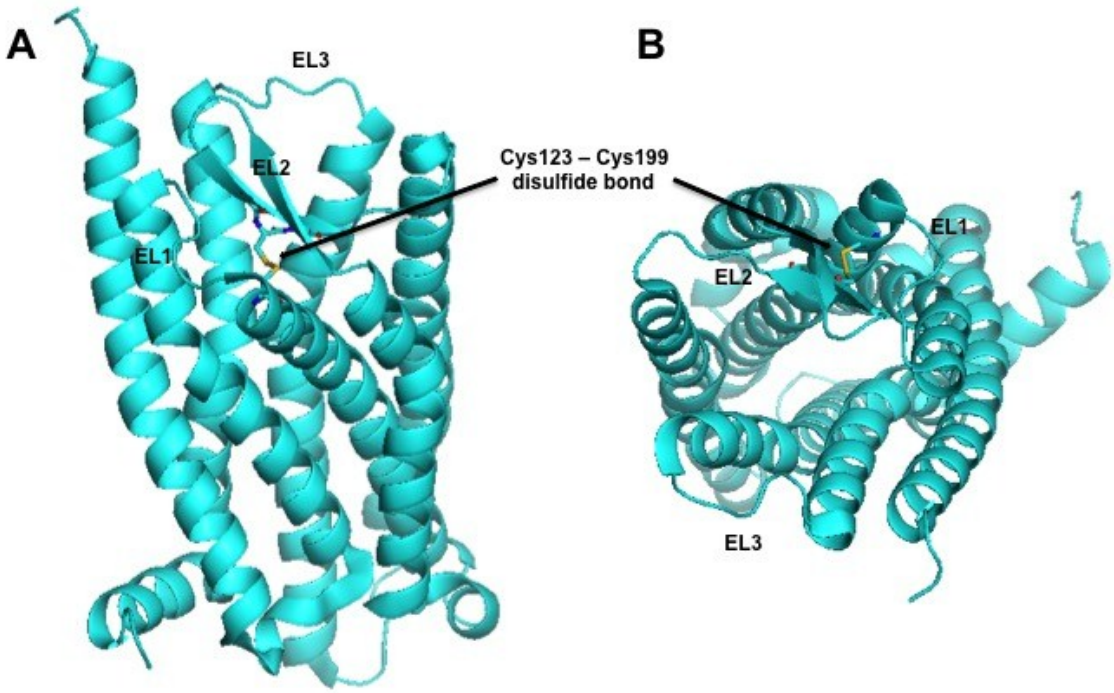


Figure S1. Crystal structure of h-DOR (PDB ID: 4n6h) indicating the disulfide bond between EL1 and EL2. (A) h-DOR side-view; (B) top, extracellular view

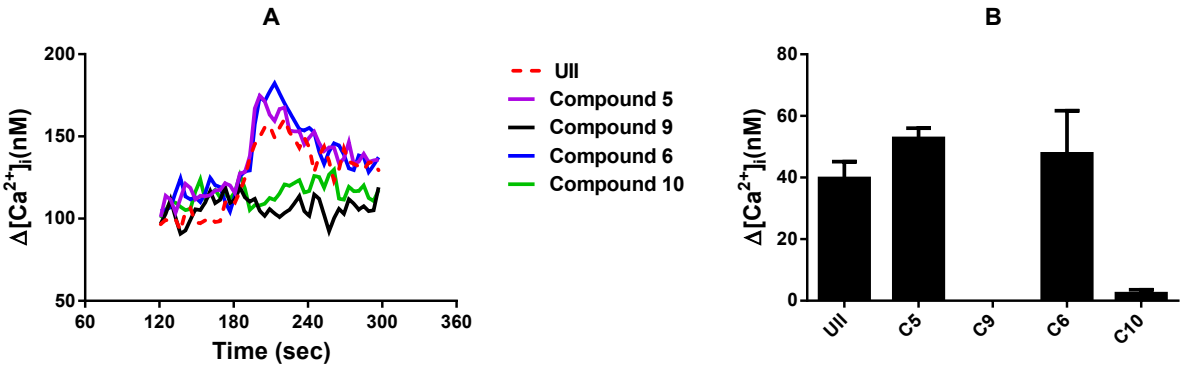


Figure S2. Intracellular Ca^{2+} data. U-II, compounds **5** and **6** increased intracellular Ca^{2+} in SJCRH30 cells. Data are Mean ± SEM (n = 3).

2. 3D Modeling and Calculations

UrotensinII_NMR.pdb was imported into Schrödinger Maestro (*Schrödinger*, LLC). This is used as a reference structure for of U-II $^{4-11}$. The disulfide connection between residues 5 and 10 was removed, retaining the respective Cβ atoms. Then, the corresponding triazole linkages were modelled into the molecule. A conformational search was performed (MMFFs + mixed torsional/low-mode sampling, in water) for the triazole linkages and Cβ atoms in **5-6** and **9-10**, whilst keeping the rest of Urotensin frozen. The lowest energy conformer for each respective case underwent an energy minimization (MMFFs + PRCG, in water) for the full structure. The obtained structures were aligned at the respective Cα and Cβ atoms of residue 5 and 10 using the "Atom pair superposition" task. Distances between Cα atoms of residues 5 and 10 were calculated using the "Measurement" tool. The route mean square deviations (RMSD) were calculated for the respective Cα and Cβ atoms of residues 5 and 10 at the compared structures using the "RMSD" superposition tool.

Entry	d($C_{\alpha 5}$-$C_{\alpha 10}$) [Å]	RMSD
U-II $^{4-11}$	3.95	
9	6.23	1.173
10	5.19	0.783
5	3.97	0.607
6	4.23	0.182

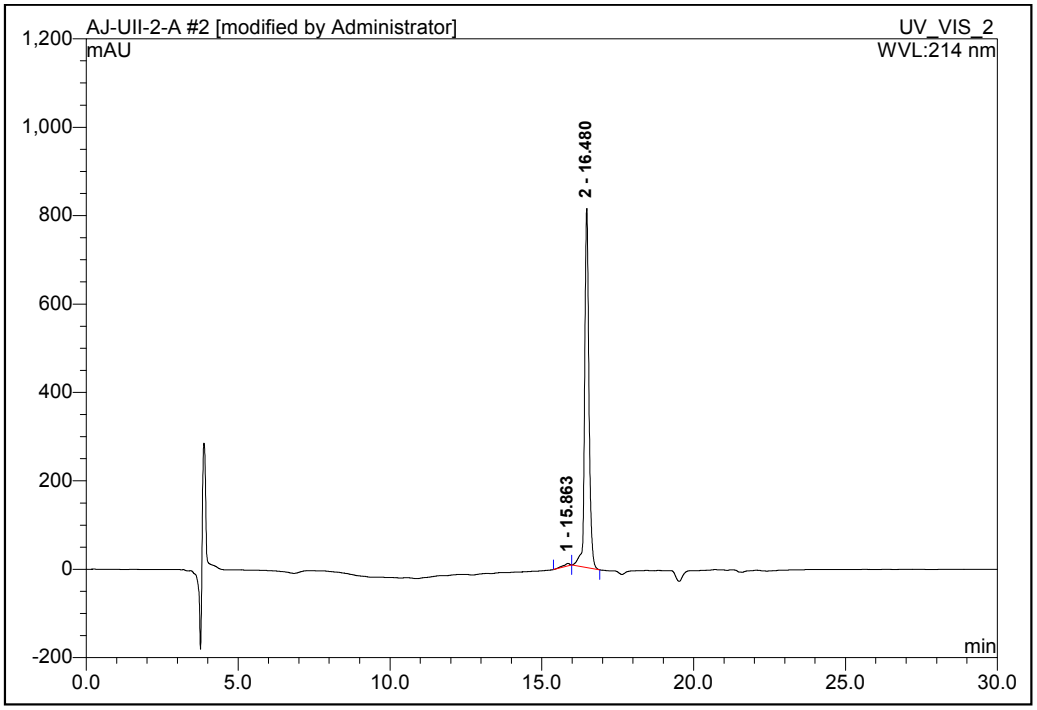
3. Table S1: Yields of purified analogues

Analogue	Yield (mg)	Yield	Purity	MS $[M+H]^+$	
				Calcd.	**Found**
1,5-Triazole Bridged Urotensin II(4-11) **5**	15 mg	14 %	98%	1106.5060	1106.5077
1,5-Triazole Bridged Urotensin II(4-11) **6**	10 mg	8 %	97%	1120.5216	1120.5220
1,4-Triazole Bridged Urotensin II(4-11) **9**	20 mg	18 %	99%	1106.5060	1106.5054
1,4-Triazole Bridged Urotensin II(4-11) **10**	17 mg	15 %	97%	1120.5216	1120.5195

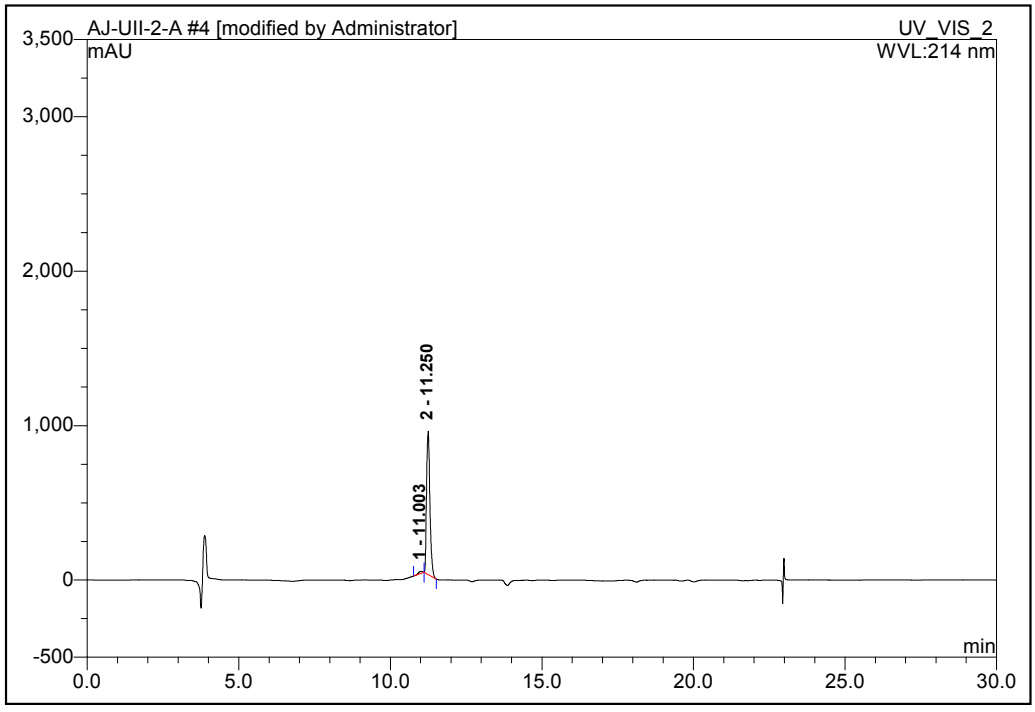
4. RP-HPLC and MS data

1,5-Triazole Bridged Urotensin II$^{(4\text{-}11)}$ **5**

Analytical RP-HPLC: Gradient: 5-100% B, over 15 min.

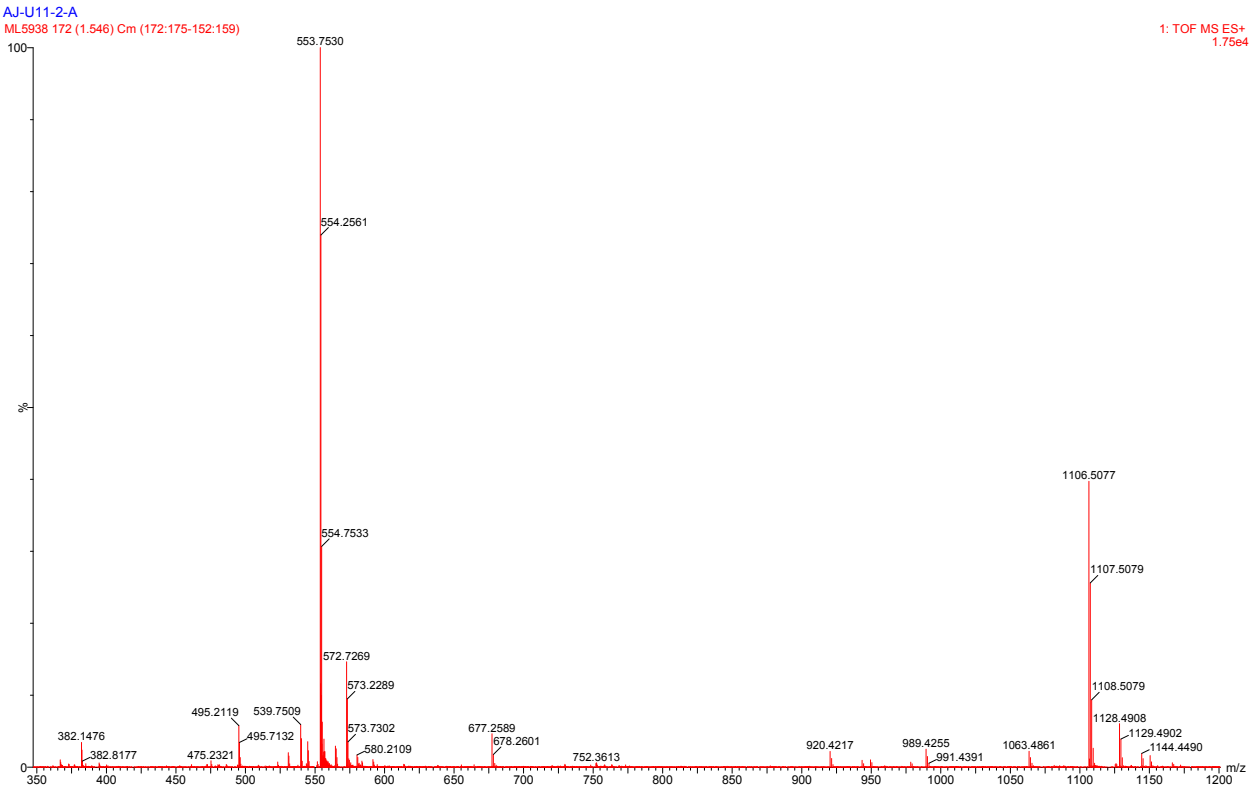


Analytical RP-HPLC: Gradient: 5-50% B, over 15 min.



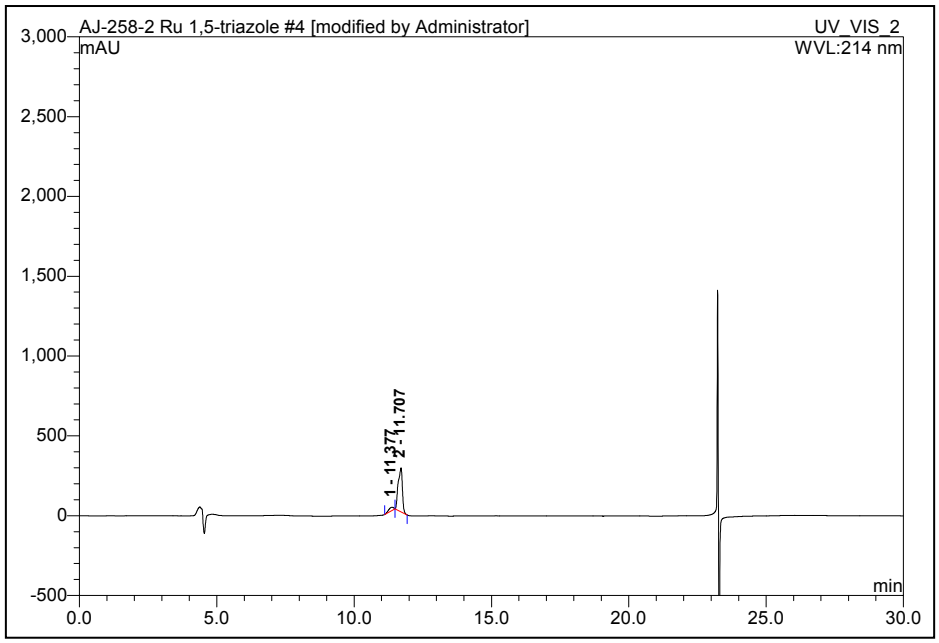
ESI Mass Spectrometry

1,5-triazole bridged Urotensin II(4-11) **5** - Chemical Formula: $C_{54}H_{67}N_{13}O_{13}$; MW 1106

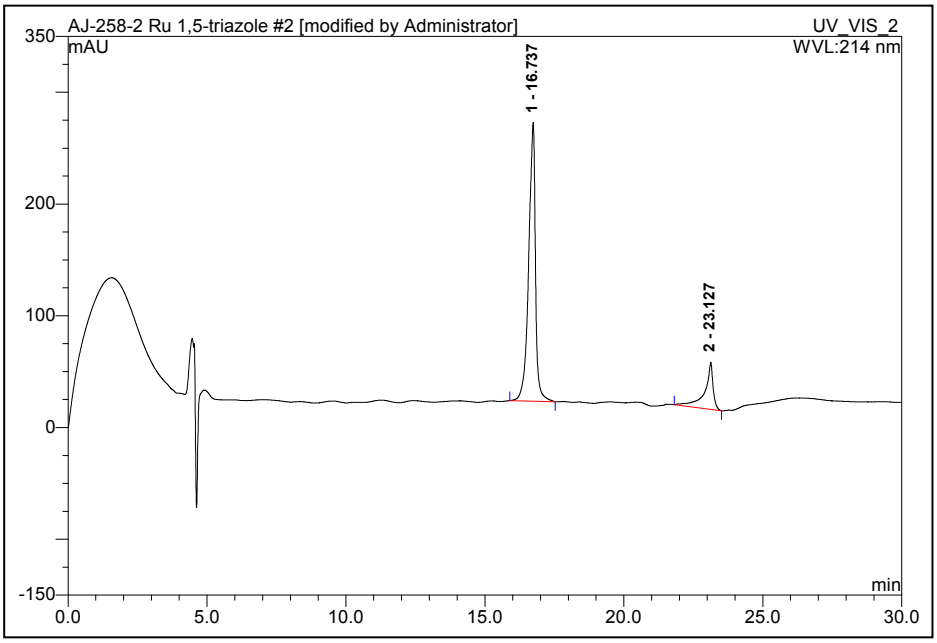


1,5-Triazole Bridged Urotensin II(4-11) **6**

Analytical RP-HPLC: Gradient: 5-100% B, over 15 min.



Analytical RP-HPLC: Gradient: 5-50% B, over 15 min.

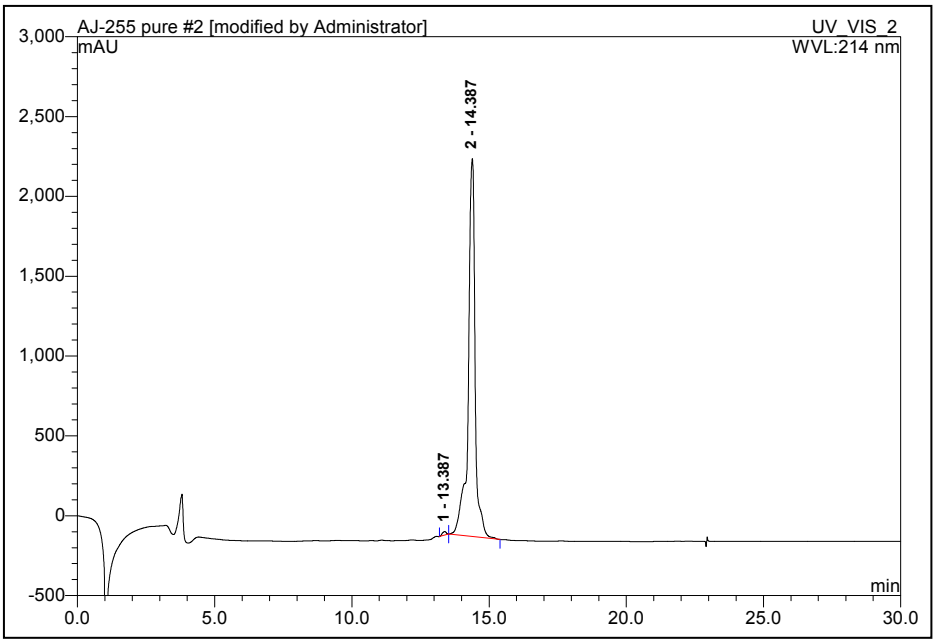


ESI Mass Spectrometry

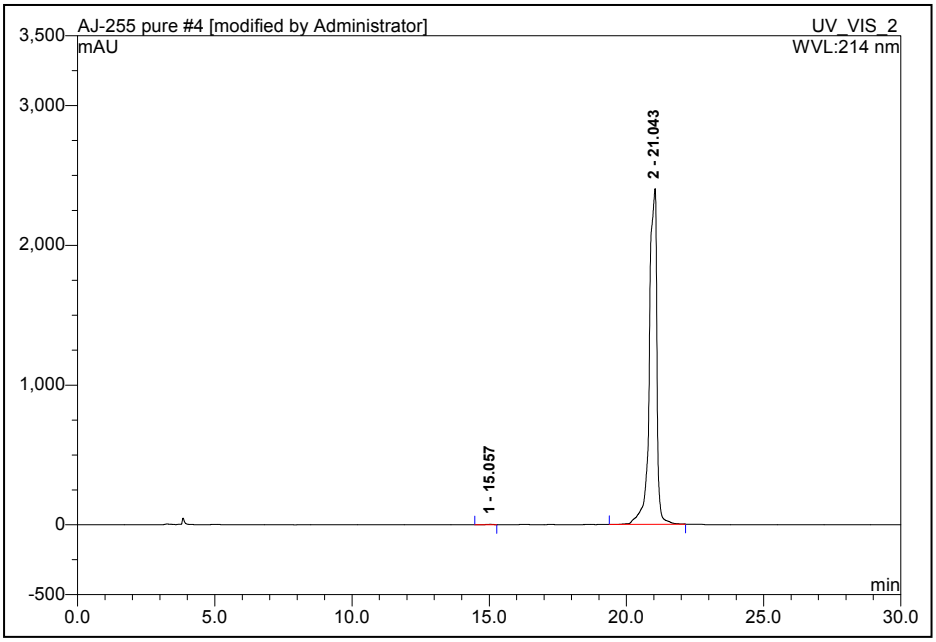
1,5-triazole bridged Urotensin II(4-11) **6** - Chemical Formula: $C_{55}H_{69}N_{13}O_{13}$; MW 1120

1,4-Triazole Bridged Urotensin II(4-11) **9**

Analytical RP-HPLC: Gradient: 5-100% B, over 15 min.

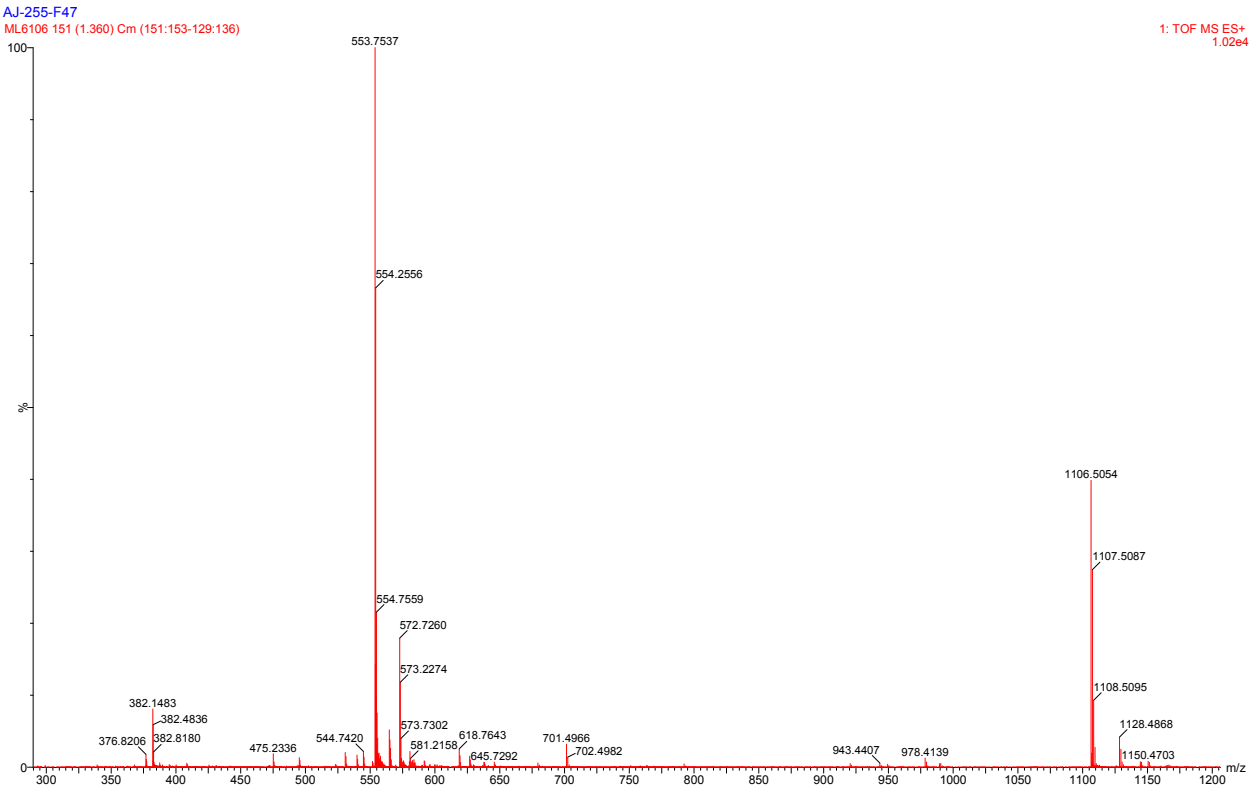


Analytical RP-HPLC: Gradient: 5-50% B, over 15 min.



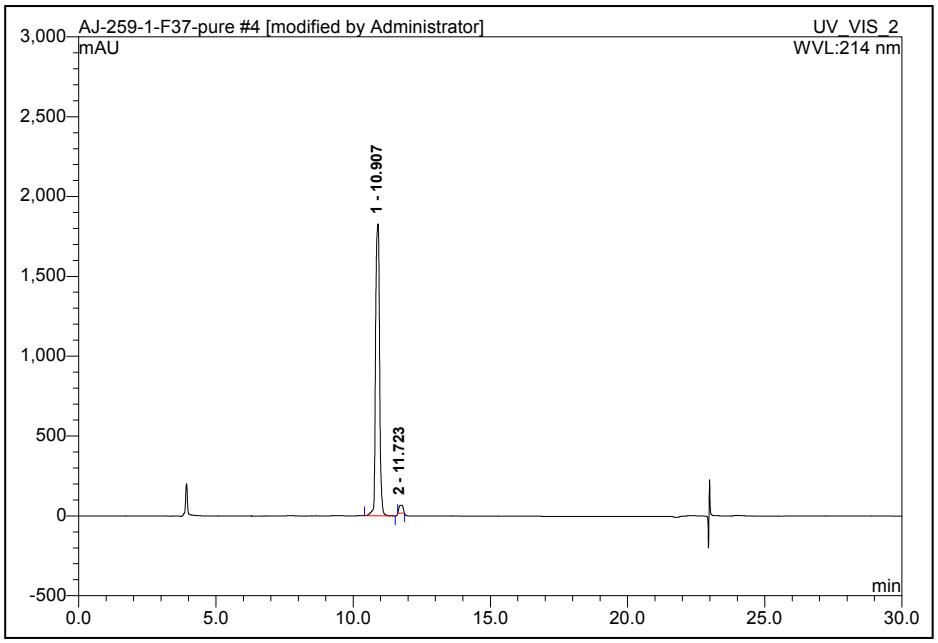
ESI Mass Spectrometry

1,4-Triazole Bridged Urotensin II(4-11) **9** - Chemical Formula: $C_{54}H_{67}N_{13}O_{13}$; MW 1106

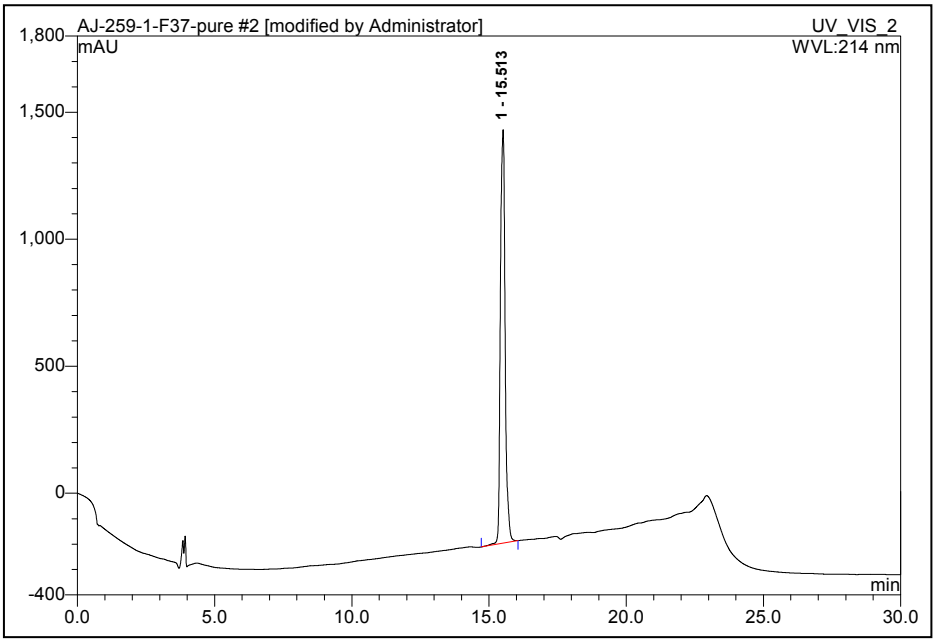


1,4-Triazole Bridged Urotensin II(4-11) **10**

Analytical RP-HPLC: Gradient: 5-100% B, over 15 min.



Analytical RP-HPLC: Gradient: 5-50% B, over 15 min.



ESI Mass Spectrometry

1,4-Triazole Bridged Urotensin II(4-11) **10** - Chemical Formula: $C_{55}H_{69}N_{13}O_{13}$; MW 1120