**ATTEMPTING TO SYNTHESIZE LASSO PEPTIDES USING HIGH PRESSURE**

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**Table. S1** NMR data for H-Gly-Phe-Gly-Ser-Lys-Pro-Ile-Asp-Ser-Phe-Gly-Leu-Ser-Trp-Leu-NH2 [ppm]

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Res.** | **Cα** | **Cβ** | **Cγ** | **Cδ** | **Cε** | **Cz** | **Hα** | **Hβ** | **Hγ** | **Hδ** | **Hε** | **HN** | **Hz** |
| **Gly1** | 48.891 | - | - | - | - | - | 3.859, 3.767 | - | - | - | - | 8.430 | - |
| **Phe2** | 59.114 | 40.254 | - | 129.101 | 128.904 | 127.320 | 4.213 | 3.169, 2.851 | - | 7.208 | 7.287 | 8.444 | - |
| **Gly3** | 45.517 | - | - | - | - | - | 3.871 | - | - | - | - | 8.485 | - |
| **Ser4** | 58.473 | 64.877 | - | - | - | - | 4.480 | 3.752 | - | - | - | 8 | - |
| **Lys5** | 58.107 | 32.441 | 27.148 | 28.918 | 46.446 | - | 4.310 | 2.174 | 1.224 | 1.450 | 3.331 | 7.898 | - |
| **Pro6** | 63.348 | 33.051 | 27.313 | 50.075 | - | - | 4.349 | 1.721 | 1.510 | 3.281 | - | - | - |
| **Ile7** | 61.451 | 39.358 | 32.503, 17.915 | 13.416 | - | - | 4.061 | 1.719 | 1.779, 0.739 | 0.777 | - | 8.123 | - |
| **Asp8** | 55.561 | 39.958 | - | - | - | - | 4.221 | 3.038 | - | - | - | 7.931 | - |
| **Ser9** | 58.214 | 63.813 | - | - | - | - | 4.617 | 3.738 | - | - | - | 8.134 | - |
| **Phe10** | 59.026 | 42.112 | - | 129.047 | 128.751 | 127.220 | 4.281 | 2.793, 2.610 | - | 7.105 | 7.237 | 8.726 | 7.198 |
| **Gly11** | 51.054 | - | - | - | - | - | 3.729, 3.520 | - | - | - | - | 8.035 | - |
| **Leu12** | 54.638 | 42.575 | 27.785 | 23.649, 23.556 | - | - | 4.521 | 1.507 | 1.902 | 0.786, 0.665 | - | 8.528 | - |
| **Ser13** | 58.449 | 64.245 | - | - | - | - | 4.385 | 3.757 | - | - | - | - | - |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Trp14ᶿ** | 55.183 | 29.641 | | - | 124.558 | 119.554 | | 112.115, 118.257 | | 4.075 | | | 3.209 | - | 7.123 | 7.054 | | 8.230 | | 7.388, 7.523 | |
| **Leu15** | 53.111 | 42.766 | 29.456 | | 25.340, 25.394 | | - | | - | | 4.648 | 1.371 | | 1.586 | 0.831, 0.744 | | - | | 7.804 | | - |

**ᶿ**In Trp14 residue: the additionally signals CH2122.136 ppm, HH2 7.138 ppm are present.

# The accuracy of chemical shift determination is 0.01 ppm for 1H and 0.1 ppm for 13C resonances.

\* Stereospecifically assigned Hβ protons first and second values correspond to Hβ1 and Hβ2, respectively.

$ ring carbons of Mim C3, C4  and C5 (up to down) are connected to the Hδ, Hf and Hε protons, respectively.

**^**For Val Cγcarbons first and second values correspond to Cγ1 and Cγ2, respectively, analogically with the Hγ protons.



**Fig S 1.** LC-MS chromatogram (XIC m/z 796.6) obtained for chaxapeptin LCP analogue (C-terminal amide group) cyclized in ambient and high pressure using mixture of ACN/THF.



**Fig S 2.** ESI-MS spectrum obtained for sungsanpin LCP analogue (C-terminal amide group).



**Fig S 3**. ESI-MS spectrum obtained for linear and isotopically labeled sungsanpin -13C6,15N2 LCP analogue



**Fig S 4**. LC-MS chromatogram (XIC m/z 816.410) obtained for chaxapeptin LCP analogue (C-terminal amide group).



**Fig S 5.** HPLC chromatogram of purified chaxapeptin-amide LCP analogue containing protected (benzyloxycarbonyl group) ε-amino group of lysine.



**Fig S 6.** HPLC chromatogram of purified sungsanpin-amide LCP analogue containing protected (benzyloxycarbonyl group) ε-amino group of lysine.



**Fig S 7.** ESI-MS spectrum obtained for S-Trt-cysteamine (calc. [M+H]+ 320.146). The abundant signal at m/z 243 was formed in the gas phase.



**Fig S 8.** NMR spectrum of S-Trt-cysteamine.



**Fig S 9**. ESI-MS spectrum obtained S-Trityl-2-(ethylamino)ethanethiol (TEE) (calc. [M+H]+ 348.178). The abundant signal at m/z 243 was formed in the gas phase.



**Fig S 10.** LC chromatogram of purified S-trityl-2-(ethylamino)ethanethiol (TEE) at 210 nm.



**Fig S 11.** LC chromatogram obtained for chaxapeptin LCP Cys analogue (C-terminal amide group) containing TEE on the Asp side chain at 210 nm (PDA detector).



**Fig S 12.** LC chromatogram obtained for sungsanpin LCP Cys-analogue (C-terminal amide group) containing TEE on the Asp side chain at 210 nm (PDA detector).



**Fig S 13**. LC-MS chromatogram (XIC m/z 816.410) obtained for sungsanpin branched-cyclic peptide (C-terminal amide group) containing protected ε-amino group of lysine (benzyloxycarbonyl group) cyclized in solution under ambient and high pressure in the mixture of (THF/ACN/DMF; 40:40:20).



**Fig S 14.** ESI-MS spectrum obtained for sungsanpin branched-cyclic peptide Cys-analogue (C-terminal amide group) via tandem acyl shift **A.** cyclization over 24h **B**. Cyclization over 48h



**Fig S 15.** LC-MS chromatogram (XIC) obtained for chaxapeptin branched-cyclic peptide Cys-analogue (C-terminal amide group) via tandem acyl shift**.** Cyclization over 24h



**Fig S 16.** ESI-MS/MS spectrum obtained for chaxapeptin branched-cyclic peptide Cys-analogue (C-terminal amide group) via tandem acyl shift**.** Cyclization over 24h. **A.** Fragmentation of signal at 8.2 min **B.** Fragmentation of signal at 10.1 min