Supporting Information

Peptidic Macrocycles - Conformational Sampling and Thermodynamic Characterization

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Figure S1 Conformational space of cyclo-(Pro-Ser-leu-Asp-Val) captured in 1µs cMD simulation. (A) The cMD ensemble is color-coded according to the free energies and depicted as projection onto the first two eigenvectors of the aMD dihedral PCA. (B) Occupied $\omega_{\text{Val-5}}$ torsional angles, the simulation is started in the cis state and does not exhibit any snapshots in the trans state.

|                  | cyclo-(Pro-Ser-leu-Asp-Val) | cilengitide | cyclo-(Arg-Arg-Trp-Trp-Arg-Phe) |
|------------------|-----------------------------|-------------|---------------------------------|
| $E_{\text{dihed}}$ | 100.0                       | 74.0        | 110.0                           |
| $\alpha_{\text{dihed}}$ | 1.0                          | 4.0         | 2.4                             |
| $E_{\text{tot}}$  | -12400.0                    | -16208.0    | -18182.0                        |
| $\alpha_{\text{tot}}$ | 600.0                       | 857.0       | 914.0                           |
**Figure S2** Cartesian PCA of cyclo-(Pro-Ser-leu-Asp-Val). The aMD ensemble is color-coded according to the reweighted free energies and depicted as projection onto the first two PCA eigenvectors.

**Figure S3** Eccentricity of cyclo-(Pro-Ser-leu-Asp-Val). A value of $\varepsilon$ near 1 describes an aspherical compound and 0 indicates perfect globularity. The aMD snapshots are color-coded according to the conformational state indicated by $\omega_{Val-5}$. Structures with $|\omega_{Val-5}| \leq 90^\circ$ were considered as cis- (blue) and the remaining structures with $|\omega_{Val-5}| > 90^\circ$ as trans-state (red).
Figure S4 Reweighted distribution of $\omega_{Val-5}$ in cyclo-(Pro-Ser-leu-Asp-Val). Reweighting all snapshots of the aMD ensemble results in a trans (red) to cis (blue) state ratio of 25/75.

Figure S5 Comparison to bioactive conformation of cilengitide. The starting structure (red) and cluster representative c16 (orange) (see Figure 5) are superposed with the target-bound conformation of cilengitide. The RMSD to the bioactive conformation are 1.0 Å for the starting structure and 0.6 Å for the cluster representative from the aMD simulation.
Table S2  Dihedral angles of cyclo-(Pro-Ser-leu-Asp-Val) cluster representatives depicted in Figure 4

| Cluster-ID | $\phi_{\text{Pro-1}}$ | $\psi_{\text{Pro-1}}$ | $\omega_{\text{Pro-1}}$ | $\phi_{\text{Ser-2}}$ | $\psi_{\text{Ser-2}}$ | $\omega_{\text{Ser-2}}$ |
|------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| c5         | -53                 | 144                  | -36                  | -52                  | 131                  | -167                 |
| c19        | -53                 | -52                  | 170                  | -75                  | 147                  | -166                 |
| c23        | -85                 | -68                  | 174                  | -44                  | 73                   | 175                  |

| Cluster-ID | $\phi_{\text{leu-3}}$ | $\psi_{\text{leu-3}}$ | $\omega_{\text{leu-3}}$ | $\phi_{\text{Asp-4}}$ | $\psi_{\text{Asp-4}}$ | $\omega_{\text{Asp-4}}$ |
|------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| c5         | 92                   | -133                 | -172                 | -88                  | -30                  | 123                  |
| c19        | 55                   | -92                  | 171                  | -78                  | -69                  | -167                 |
| c23        | 102                  | -96                  | 148                  | -62                  | 124                  | -177                 |

| Cluster-ID | $\phi_{\text{Val-5}}$ | $\psi_{\text{Val-5}}$ | $\omega_{\text{Val-5}}$ |
|------------|----------------------|----------------------|----------------------|
| c5         | -87                  | 142                  | 174                  |
| c19        | -139                 | 131                  | -47                  |
| c23        | -25                  | 123                  | -5                   |

All values given in °

Table S3  Dihedral angles of cilengitide selected cluster representatives and the bioactive conformation of cilengitide, as depicted in Figure 8.

| Cluster-ID | $\phi_{\text{Arg-1}}$ | $\psi_{\text{Arg-1}}$ | $\omega_{\text{Arg-1}}$ | $\phi_{\text{Gly-2}}$ | $\psi_{\text{Gly-2}}$ | $\omega_{\text{Gly-2}}$ |
|------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| c2         | 73                   | -36                  | 161                  | -102                 | -143                 | 174                  |
| c7         | -155                 | -30                  | 172                  | -172                 | -61                  | 134                  |
| c16        | -63                  | 120                  | 173                  | 52                   | -122                 | 170                  |
| X-ray      | -114                 | 130                  | -178                 | 84                   | -136                 | 179                  |

| Cluster-ID | $\phi_{\text{Asp-3}}$ | $\psi_{\text{Asp-3}}$ | $\omega_{\text{Asp-3}}$ | $\phi_{\text{phe-4}}$ | $\psi_{\text{phe-4}}$ | $\omega_{\text{phe-4}}$ |
|------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| c2         | -77                  | 94                   | -156                 | 112                  | -124                 | 169                  |
| c7         | -89                  | 176                  | -172                 | 53                   | -123                 | 169                  |
| c16        | -66                  | 117                  | -151                 | 89                   | -126                 | -177                 |
| X-ray      | -87                  | 61                   | -178                 | 172                  | -123                 | -180                 |

| Cluster-ID | $\phi_{\text{Val-5}}$ | $\psi_{\text{Val-5}}$ | $\omega_{\text{Val-5}}$ |
|------------|----------------------|----------------------|----------------------|
| c2         | -87                  | 127                  | 168                  |
| c7         | -125                 | 62                   | -180                 |
| c16        | -97                  | -64                  | 147                  |
| X-ray      | -77                  | -54                  | 175                  |

All values given in °
Figure S6 Conformational space of cyclo-(Arg-Arg-Trp-Trp-Arg-Phe). The aMD ensemble is color-coded according to the reweighted free energies and depicted as projection onto the first two PCA eigenvectors.
**Table S4** Sum of dihedral entropies. The entropy is calculated for each backbone dihedral and summed up to quantify and compare the global flexibility of the studied peptidic macrocycles. Error estimations derive from block averaging using a block size of 50,000 frames.

| Peptide Structure                  | $S_{\text{sum}} \text{/J/(mol\cdot K)}$ |
|-----------------------------------|-----------------------------------------|
| cyclo-(Pro-Ser-leu-Asp-Val)       | 504.8 ± 41.5                            |
| cyclo-(Arg-Gly-Asp-phe-(N-Me)Val))| 523.2 ± 43.1                            |
| cyclo-(Arg-Arg-Trp-Trp-Arg-Phe)   | 637.1 ± 9.0                             |

**Table S5** Dihedral angles of the cyclo-(Arg-Arg-Trp-Trp-Arg-Phe) cluster representatives as depicted in Figure 11.

| Cluster-ID | $\phi_{\text{Arg-1}}$ | $\psi_{\text{Arg-1}}$ | $\phi_{\text{Pro-1}}$ | $\psi_{\text{Arg-2}}$ | $\phi_{\text{Arg-2}}$ | $\psi_{\text{Arg-2}}$ |
|------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| c1         | -111                   | 16                     | -167                   | 70                     | -78                    | 178                    |
| c11        | -162                   | 175                    | 170                    | -52                    | 112                    | 177                    |
| c13        | -98                    | 170                    | -171                   | -50                    | -37                    | -171                   |

**Figure S7** Interproton distances of selected cluster representatives. The white area indicates distances that fulfill the NMR derived restraints. Interproton distances calculated from the selected cluster representatives are depicted in color. Individual structures of the ensemble show varying violation patterns, only the representative structure of cluster c11 fulfills all experimental distance boundaries.
Figure S8 Θ dihedral distributions of the cis and trans states of cyclo-(ProSer-leu-Asp-Val). (Θ = |ϕ-60°| for L- and Θ = |ϕ+60°| for D-amino acids). The aMD ensemble captures the overall trends as implicated by $^3J_{\alpha NH}$ coupling constants. Θ(Val-5) and Θ(Leu-3) of the cis component is shifting towards smaller angles compared to the trans state. While the sampled Θ(Asp-4) are larger in the cis state than in the trans state. The distribution of Θ(Ser-2) is broader in the cis state and slightly shifted towards higher angles. The difference in $^3J_{\alpha NH}$ is small (0.5 Hz) but implies a tendency for larger angles in the trans state, which we do not observe.