Supplementary Information (SI) for RSC Medicinal Chemistry.
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Supplementary data

Structure and activity of conopressins: Insights into *in silico* oxytocin/V2 receptor interactions, anti-inflammatory potential, and behavioural studies.

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List of Figures:

Supplementary Figure 1: The analytical HPLC chromatograms of the purified peptides: a) oxytocin, b) Mo1033, c) ^{D}R4-Mo1033, d) Mo1034, e) Mo1061, f) Tr-Mo976, and g) Tr-Mo977. The chromatograms depict more than 95% purity for all the peptides.

Supplementary Figure 2 Representative ESI-QTOF mass spectra of a) oxytocin, b) Mo1033, c) ^{D}R4-Mo1033, d) Mo1034, e) Mo1061, f) Tr-Mo976, and g) Tr-Mo977.

Supplementary Figure 3: 500 MHz ^{1}H NMR spectra of Mo1033 in water at 278 K. Unidentified impurities are marked with an asterisk.

Supplementary Figure 4: Partial ^{1}H 1D spectra highlighting the variation of chemical shifts with temperature (273 - 313 K) in Mo1033.

Supplementary Figure 5: Variation of intensities of NH resonances of Mo1033 in different ratios of H_2O: D_2O with time after dissolution at 278 K.

Supplementary Figure 6: Partial ^{1}H-^{13}C HSQC spectra indicating the assignment of ^{13}C chemical shifts of Pro7 Cβ and Cγ resonances of Mo1033

Supplementary Figure 7: Measurement of the $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$ using selective TOCSY experiments. 1D selective TOCSY experiment obtained by irradiating the $C^{\alpha}H$ of Arg4 in the Mo1033. The expanded regions are shown, and the vicinal coupling constant $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$ value is indicated on the spectrum.

Supplementary Figure 8: The ball and stick representation of the Mo1033 a) NMR-derived structure and b) AlphaFold predicted structure model_0. The backbone dihedral angles (ϕ and ψ) and the disulfide dihedral angles are highlighted, demonstrating the structural similarities between the predicted and experimentally determined conformations.

Supplementary Figure 9: 500 MHz ^{1}H NMR spectra of ^{D}R4-Mo1033, in water at 278 K. Unidentified impurities are marked with an asterisk.

Supplementary Figure 10: Variation of intensities of NH resonances of ^{D}R4-Mo1033 in different ratios of H_2O: D_2O with time after dissolution at 278 K.

Supplementary Figure 11: Partial ^{1}H 1D spectra highlighting the variation of chemical shifts with temperature (273 - 313 K) in ^{D}R4-Mo1033

Supplementary Figure 12: Partial ^{1}H-^{13}C HSQC spectra indicating the assignment of ^{13}C chemical shifts of Pro7 Cβ and Cγ resonances of ^{D}R4-Mo1033

Supplementary Figure 13: Measurement of the $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$ using selective TOCSY experiments. 1D selective TOCSY experiment obtained by irradiating the $C^{\alpha}H$ of Arg4 in the ^{D}R4-Mo1033. The expanded regions are shown, and the vicinal coupling constant $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$ value is indicated on the spectrum.

Supplementary Figure 14: 500 MHz ^{1}H NMR spectra of Mo1034 in water at 273 K. Unidentified impurities are marked with an asterisk.

Supplementary Figure 15: 500 MHz ^{1}H NMR spectra of Mo1061 in water at 273 K. Unidentified impurities are marked with an asterisk.

Supplementary Figure 16: 500 MHz ^{1}H NMR spectra of Tr-Mo976 in water at 273 K. Unidentified impurities are marked with an asterisk.

Supplementary Figure 17: 500 MHz ^{1}H NMR spectra of Tr-Mo977 in water at 273 K. Unidentified impurities are marked with an asterisk.

Supplementary Figure 18: Variation of intensities of NH resonances of Mo1034 in 100% D_2O with time after dissolution at 273 K.

Supplementary Figure 19: Variation of intensities of NH resonances of Mo1061 in 100% D_2O with time after dissolution at 273 K.

Supplementary Figure 20: Variation of intensities of NH resonances of Tr-Mo976 in 100% D_2O with time after dissolution at 273 K.

Supplementary Figure 21: Variation of intensities of NH resonances of Tr-Mo977 in 100% D_2O with time after dissolution at 273 K.

Supplementary Figure 22: Partial ROESY spectrum of Mo1034 at 500 MHz in water at 278 K highlighting a) NH-C$^{\alpha}$H; b) NH-NH; and c) C$^{\delta}$H-C$^{\alpha}$H and C$^{\delta}$H-C$^{\beta}$H NOEs. d) NMR-derived overlay backbone structure of (average heavy atom RMSD: 0.67 ± 0.23 Å) and e) the representative structure of Mo1034 shows backbone and disulfide dihedral angles.

Supplementary Figure 23: Partial ROESY spectrum of Mo1061 at 500 MHz in water at 278 K highlighting a) NH-NH; b) NH-C$^{\alpha}$H; c) C$^{\delta}$H-C$^{\alpha}$H NOEs. d) NMR-derived overlay backbone structure of (average heavy atom RMSD: 0.71 ± 0.15 Å) and e) the representative structure of Mo1061 shows backbone and disulfide dihedral angles.

Supplementary Figure 24: Partial ROESY spectrum of Tr-Mo976 at 500 MHz in water at 278 K highlighting a) NH-C$^{\alpha}$H; b) NH-NH; c) C$^{\delta}$H-C$^{\alpha}$H NOEs. e) NMR-derived overlay backbone structure of (average heavy atom RMSD: 0.63 ± 0.10 Å) and f) the representative structure of Tr-Mo976 shows backbone and disulfide dihedral angles.

Supplementary Figure 25: Partial ROESY spectrum of Tr-Mo977 at 500 MHz in water at 278 K highlighting a) NH-NH; b) NH-C$^{\alpha}$H; c) NH-side chain d) C$^{\delta}$H-C$^{\alpha}$H and C$^{\delta}$H-C$^{\beta}$H NOEs. e) NMR-derived overlay backbone structure of (average heavy atom RMSD: 0.52 ± 0.04 Å) and f) the representative structure of Tr-Mo977 shows backbone and disulfide dihedral angles.

Supplementary Figure 26: Partial ^{1}H-^{13}C HSQC spectra indicating the assignment of ^{13}C chemical shifts of Pro7 Cβ and Cγ resonances of a) Mo1034, b) Mo1061, c) Tr-Mo976, and d) Tr-Mo977

Supplementary Figure 27: Partial 500 MHz ^{1}H 1D NMR spectra of a) Mo1033, b) ^{D}R4-Mo1033, at 278 K and c) Mo1034, d) Mo1061, d) Tr-Mo976, and e) Tr-Mo977 in H_2O: D_2O in the presence of copper ions at 273 K.

Supplementary Figure 28: Partial 500 MHz ^{1}H 1D NMR spectra of a) Mo1033, b) ^{D}R4-Mo1033, at 278 K and c) Tr-Mo976, d) Mo1034, d) Mo1061, and e) Tr-Mo977 in H_2O: D_2O in the presence of magnesium ions at 273 K.

Supplementary Figure 29: Partial 500 MHz ^{1}H 1D NMR spectra of a) Mo1033, b) ^{D}R4-Mo1033, at 278 K and c) Mo1034, d) Mo1061, d) Tr-Mo976, and e) Tr-Mo977 in H_2O: D_2O in the presence of calcium ions at 273 K.

Supplementary Figure 30: RMSD of the atomic positions for the ligand a) oxytocin and, b) vasopressin (in red, Lig fit Prot) and the receptor oxytocin and vasopressin (Cα positions in blue) of the 100 ns molecular dynamics simulations using Desmond package. Protein-ligand contacts of c) OT receptor- oxytocin d) V2 receptor – vasopressin for a simulation time of 100 ns through hydrogen bond (green), hydrophobic (grey) and ionic interactions (pink), and water bridges (blue).

Supplementary Figure 31: The comparison of MD simulated and the cryo-EM interactions. a) Superposition of OT receptor cryo-EM (blue) and MD simulated (orange) structures; the seven transmembrane helices aligned over C^{α} atoms are shown as a cartoon (PDB ID: 7RYC) with an RMSD of 1.67 Å. b) Cryo-EM oxytocin-OT receptor interactions and c) MD simulation oxytocin-OT receptor interactions, the oxytocin ligand (carbon green), the interacting receptor residues (carbon cyan) are represented as sticks, and the transmembrane helices are represented as cartoon (violet). d) Superposition of V2 receptor cryo-EM (blue) and MD simulated (orange) structures; the seven transmembrane helices aligned over C^{α} atoms are shown as a cartoon (PDB ID: 7DW9). b) Cryo-EM vasopressin-V2 receptor interactions and c) MD simulation vasopressin-V2 receptor interactions, the vasopressin ligand (carbon green), the interacting receptor residues (carbon cyan) are represented as sticks, and the transmembrane helices are represented as cartoon (violet) and the hydrophobic cleft is shown as surface (red).

Supplementary Figure 32: RMSD of the atomic positions for the ligand a) Mo1033, b) ^{D}R4-Mo1033, c) Mo1034, d) Mo1061, e) Tr-Mo976, and f) Tr-Mo977 (in red, Lig fit Prot) and the receptor oxytocin (Cα positions in blue) of the 100 ns molecular dynamics simulations using Desmond package.

Supplementary Figure 33: Protein-ligand contacts of oxytocin receptor a) Mo1033, b) ^{D}R4-Mo1033, c) Mo1034, d) Mo1061, e) Tr-Mo976, and f) Tr-Mo977 for a simulation time of 100 ns through hydrogen bond (green), hydrophobic (grey) and ionic interactions (pink), and water bridges (blue).

Supplementary Figure 34: RMSD of the atomic positions for the ligand a) Mo1033, b) ^{D}R4-Mo1033, c) Mo1034, d) Mo1061, e) Tr-Mo976, and f) Tr-Mo977 (in red, Lig fit Prot) and the vasopressin V2 (Cα positions in blue) of the 100 ns molecular dynamics simulations using Desmond package.

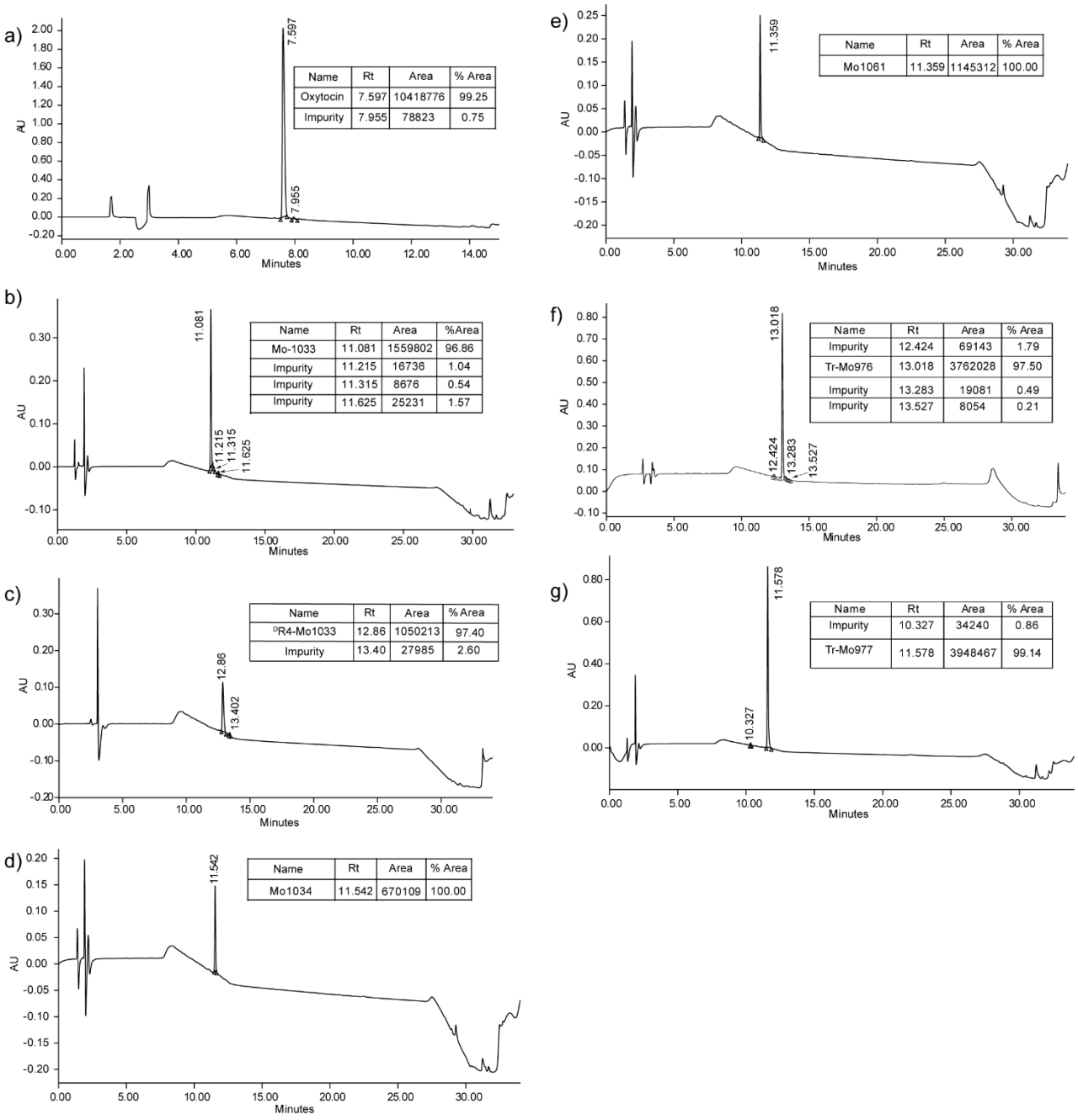
Supplementary Figure 35: Protein-ligand contacts of vasopressin V2 receptor a) Mo1033, b) ^{D}R4-Mo1033, c) Mo1034, d) Mo1061, e) Tr-Mo976, and f) Tr-Mo977 for a simulation time of 100 ns through hydrogen bond (green), hydrophobic (grey) and ionic interactions (pink), and water bridges (blue).

Supplementary Figure 36: The cytotoxicity of the conopressins was determined with the MTT assay after 24 H of treatment with different doses of conopressins. All the conopressins showed safety up to a concentration of 3 μM, while Mo1034, Tr-Mo976, and Tr-Mo977 showed ~20-40% toxicity at 10 μM concentration.

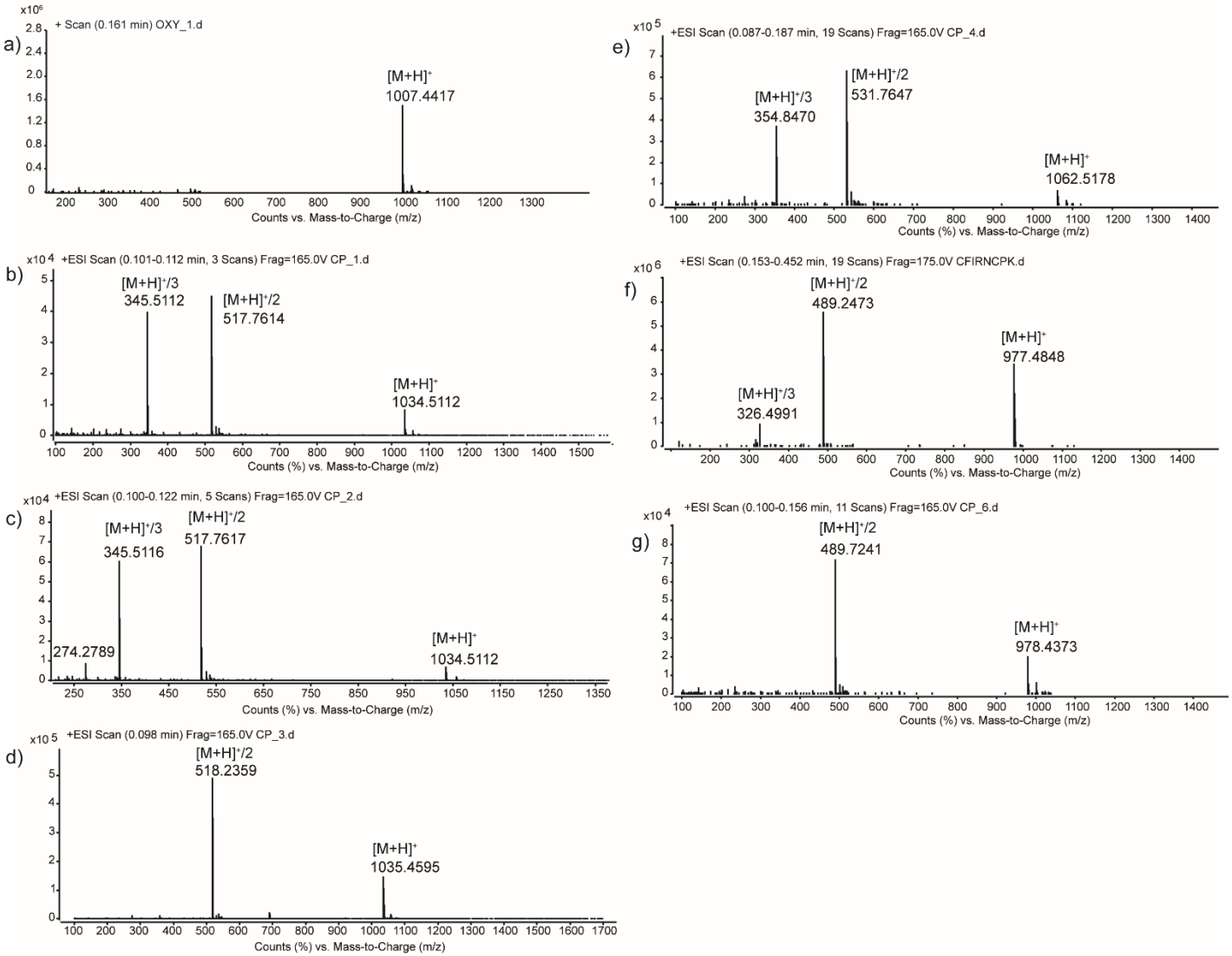
Supplementary Figure 37: Effects of conopressins on the production of TNF-α, and IL-6 in LPS-activated RAW 264.7 cells. Pro-inflammatory cytokine (TNF-α and IL-6) production was measured in the culture medium to determine the anti-inflammatory activity of the conopressins. LPS-activated RAW 264.7 cells were treated with the conopressins at the concentrations 1 and 3 μM. The proinflammatory cytokine production was significantly reduced as compared to the LPS treated group. The values are expressed as the means ± SD. ### $p < 0.001$, compared with the control group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with the LPS group, $n = 3$.

Supplementary Figure 38: Effects of conopressins on NO production in LPS-activated RAW 264.7 cells. NO production was measured in the culture medium to determine the anti-inflammatory activity of the above compounds. LPS-activated RAW 264.7 cells were treated with the above conopressins at concentrations 1 and 3 μM. NO production was significantly reduced compared to that of the LPS-treated group. The values are expressed as the means ± SD. ### $p < 0.001$, compared with the control group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with the LPS group, $n = 3$.

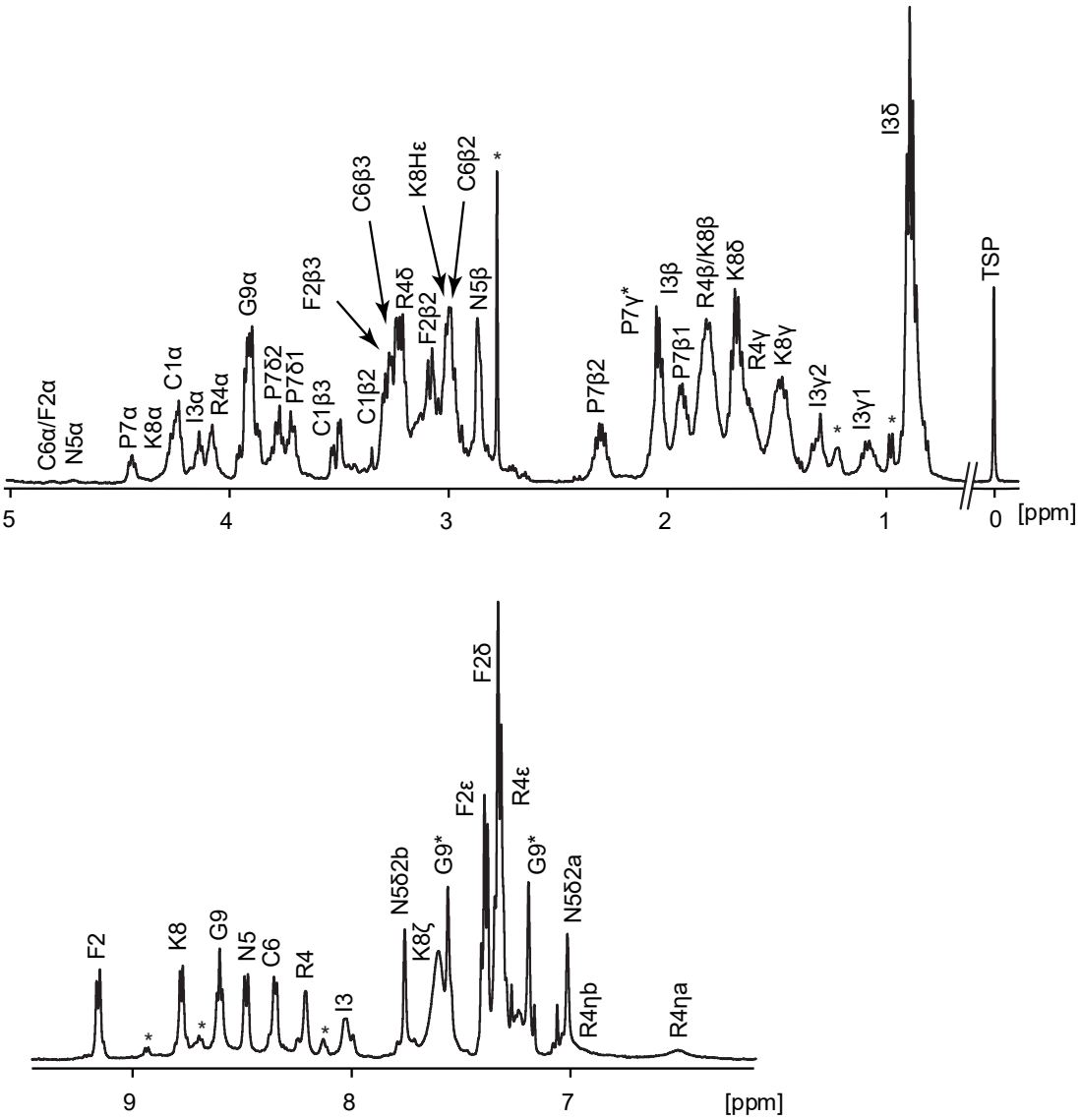
List of Tables:



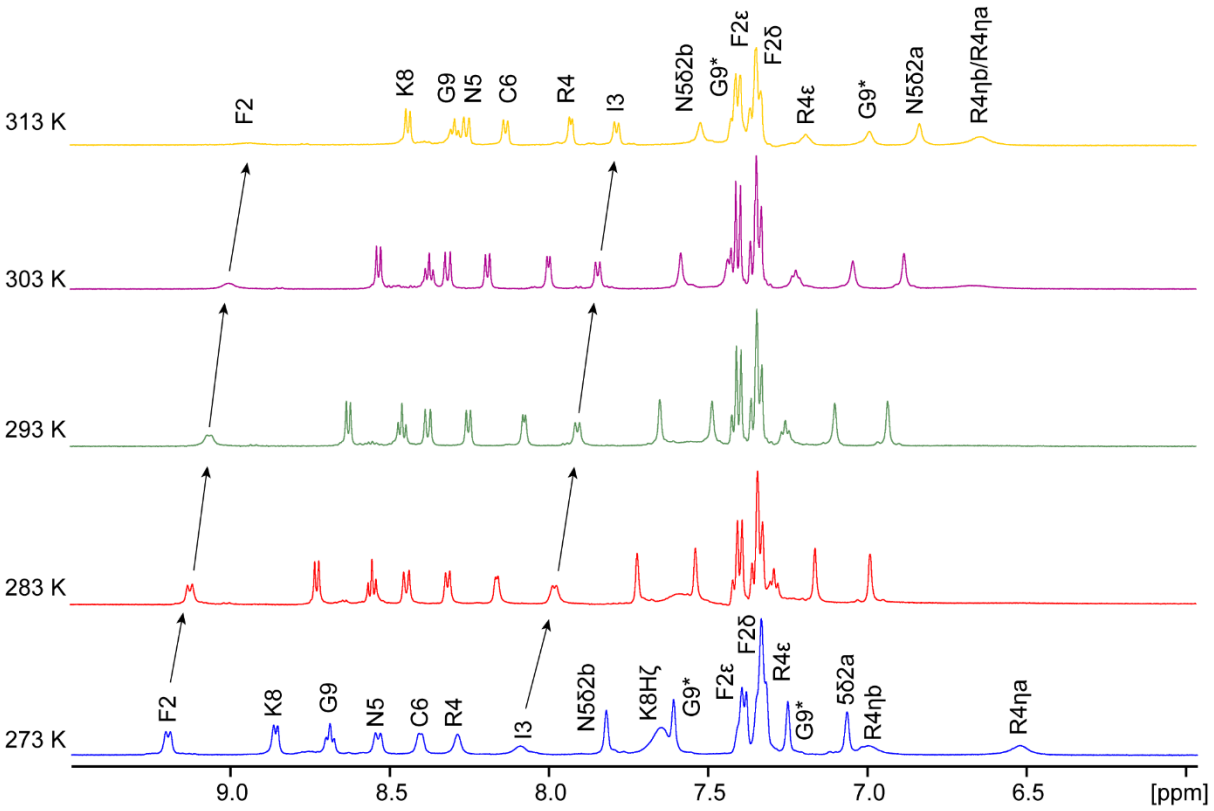
Supplementary Figure 1: The analytical HPLC chromatograms of the purified peptides: a) oxytocin, b) Mo1033, c) ^{D}R4-Mo1033, d) Mo1034, e) Mo1061, f) Tr-Mo976, and g) Tr-Mo977. The chromatograms depict more than 95% purity for all the peptides.



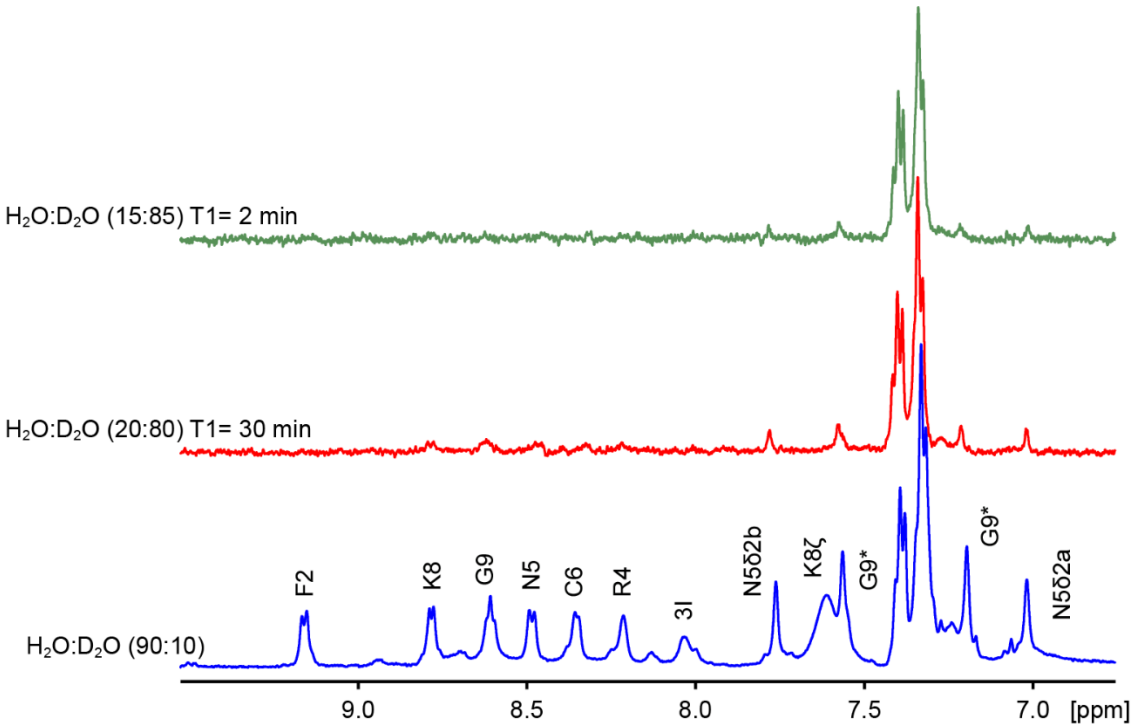
Supplementary Figure 2 Representative ESI-QTOF mass spectra of a) oxytocin, b) Mo1033, c) ^{D}R4-Mo1033, d) Mo1034, e) Mo1061, f) Tr-Mo976, and g) Tr-Mo977.



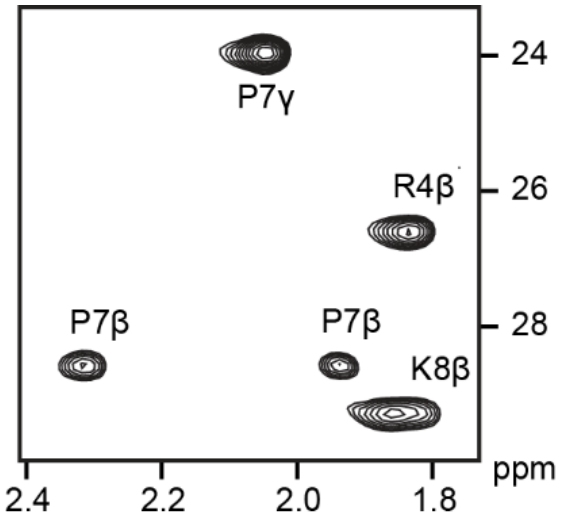
Supplementary Figure 3: 500 MHz ^{1}H NMR spectra of Mo1033 in water at 278 K. Unidentified impurities are marked with an asterisk.



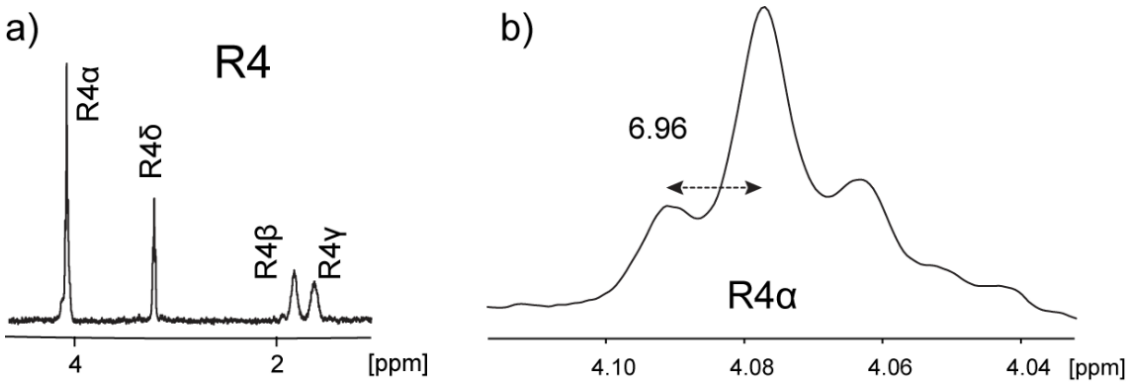
Supplementary Figure 4: Partial ^{1}H 1D spectra highlighting the variation of chemical shifts with temperature (273 - 313 K) in Mo1033.



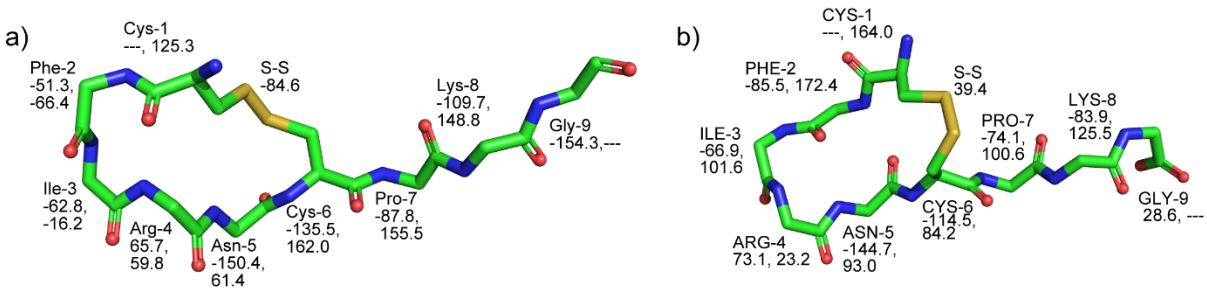
Supplementary Figure 5: Variation of intensities of NH resonances of Mo1033 in different ratios of H_2O: D_2O with time after dissolution at 278 K.



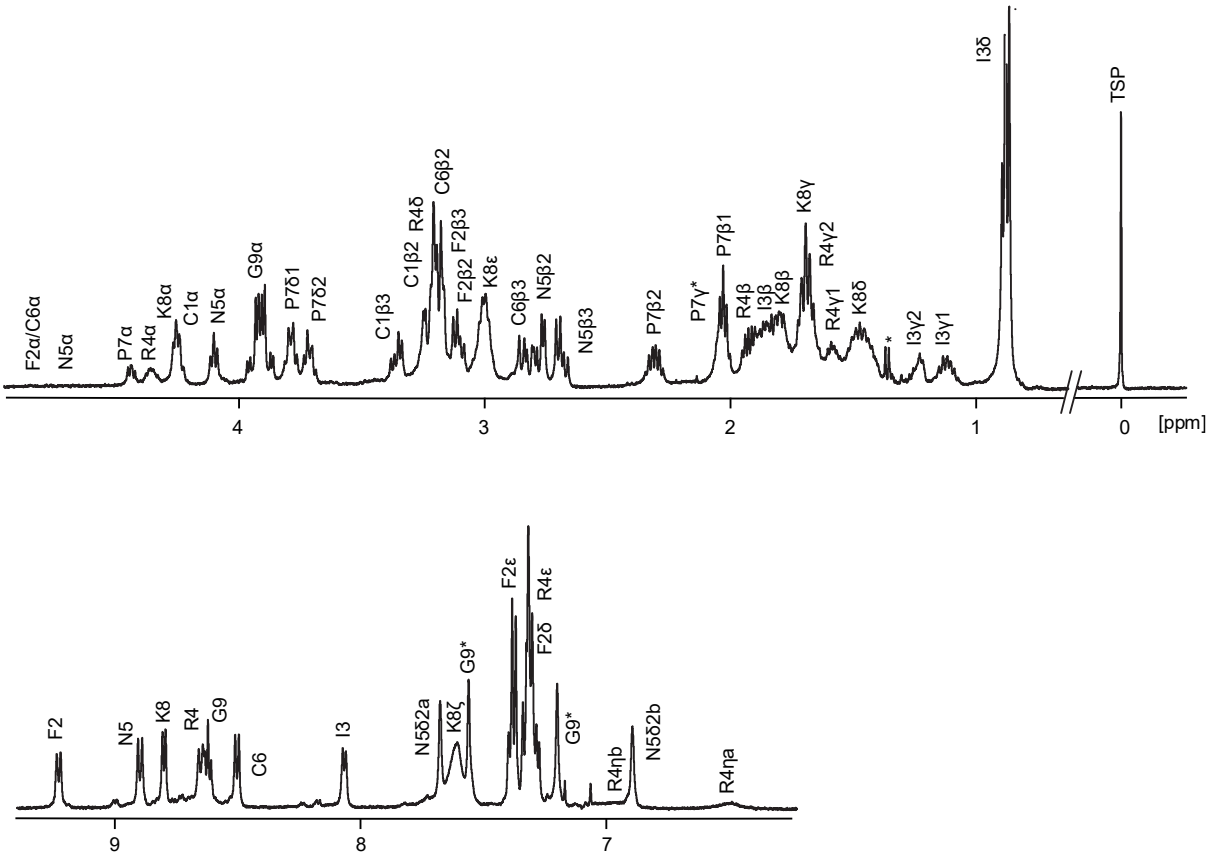
Supplementary Figure 6: Partial ^{1}H-^{13}C HSQC spectra indicating the assignment of ^{13}C chemical shifts of Pro7 Cβ and Cγ resonances of Mo1033



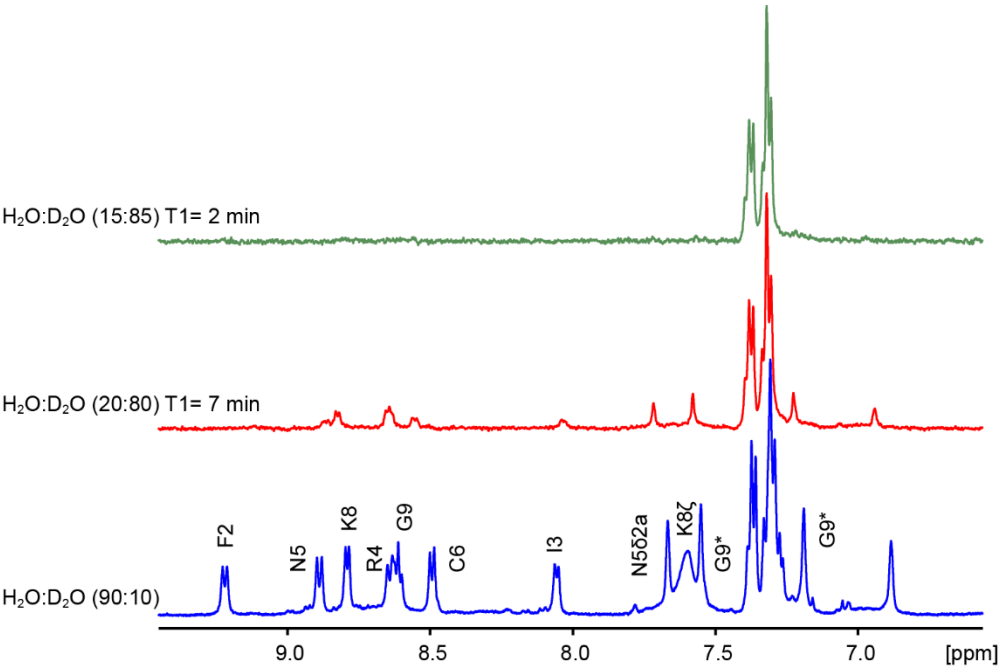
Supplementary Figure 7: Measurement of the $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$ using selective TOCSY experiments. 1D selective TOCSY experiment obtained by irradiating the $C^{\alpha}H$ of Arg4 in the Mo1033. The expanded regions are shown, and the vicinal coupling constant $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$ value is indicated on the spectrum.



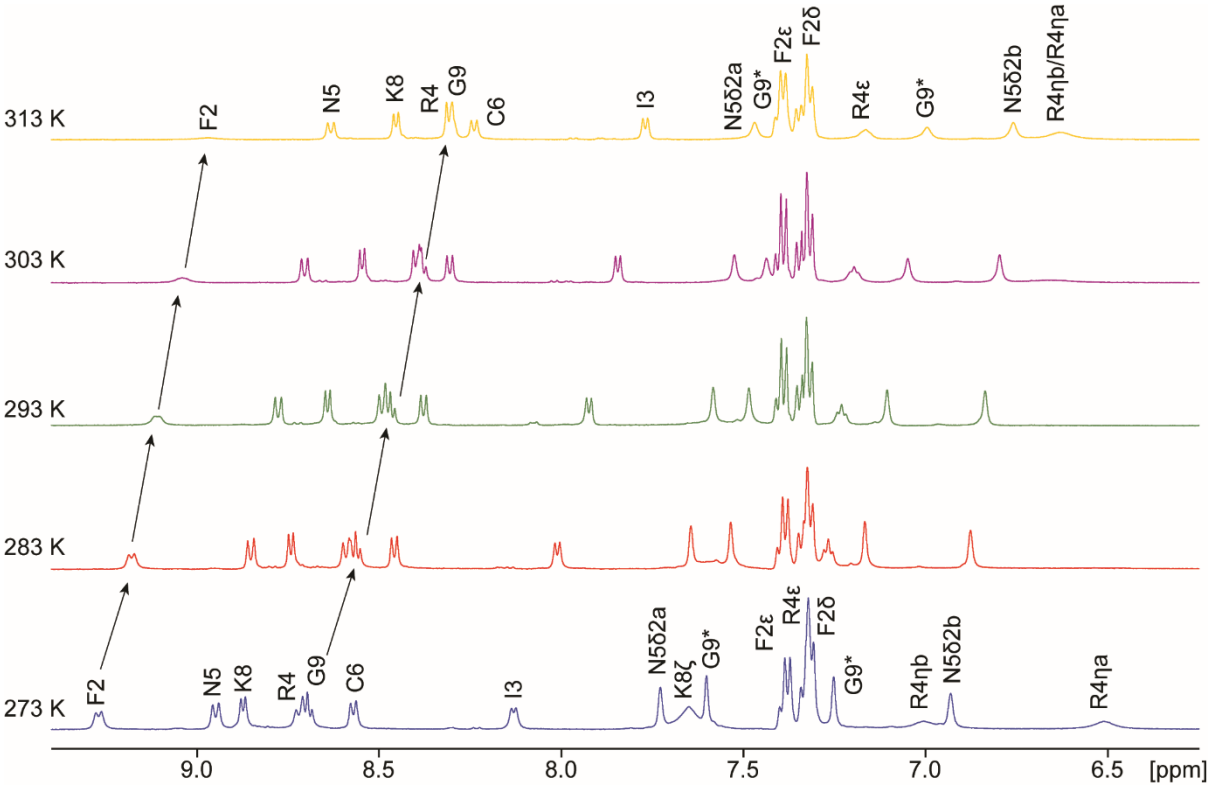
Supplementary Figure 8: The ball and stick representation of the Mo1033 a) NMR-derived structure and b) AlphaFold predicted structure model_0. The backbone dihedral angles (ϕ and ψ) and the disulfide dihedral angles are highlighted, demonstrating the structural similarities between the predicted and experimentally determined conformations.



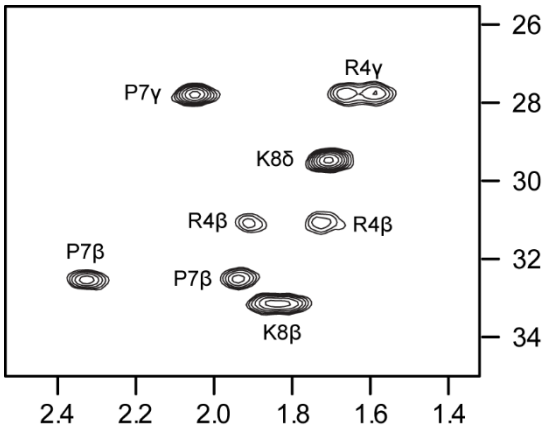
Supplementary Figure 9: 500 MHz ^{1}H NMR spectra of ^{D}R4-Mo1033, in water at 278 K. Unidentified impurities are marked with an asterisk.



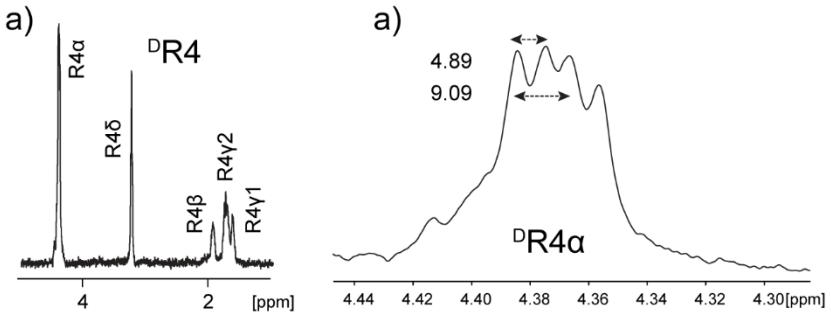
Supplementary Figure 10: Variation of intensities of NH resonances of ^{D}R4-Mo1033 in different ratios of H_2O: D_2O with time after dissolution at 278 K.



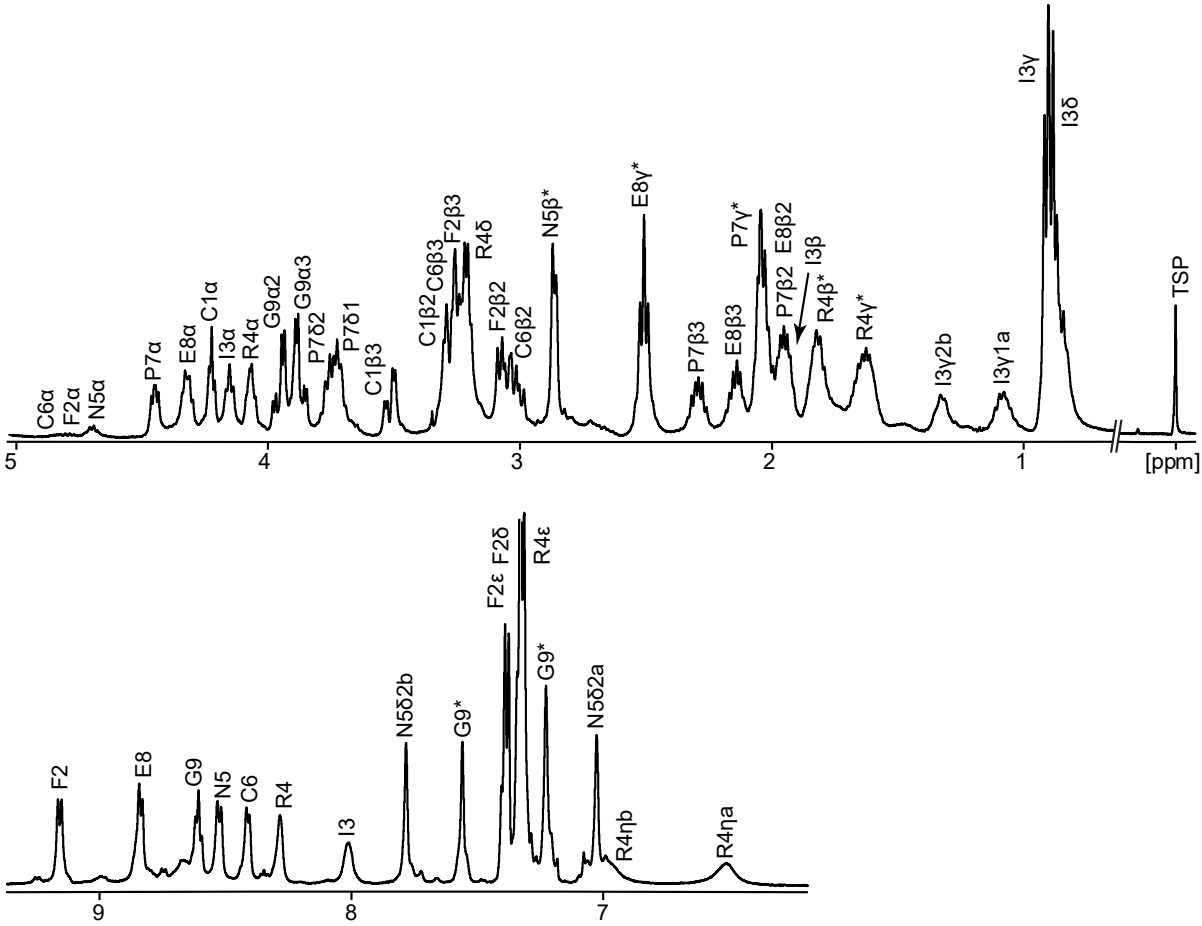
Supplementary Figure 11: Partial ^{1}H 1D spectra highlighting the variation of chemical shifts with temperature (273 - 313 K) in ^{D}R4-Mo1033



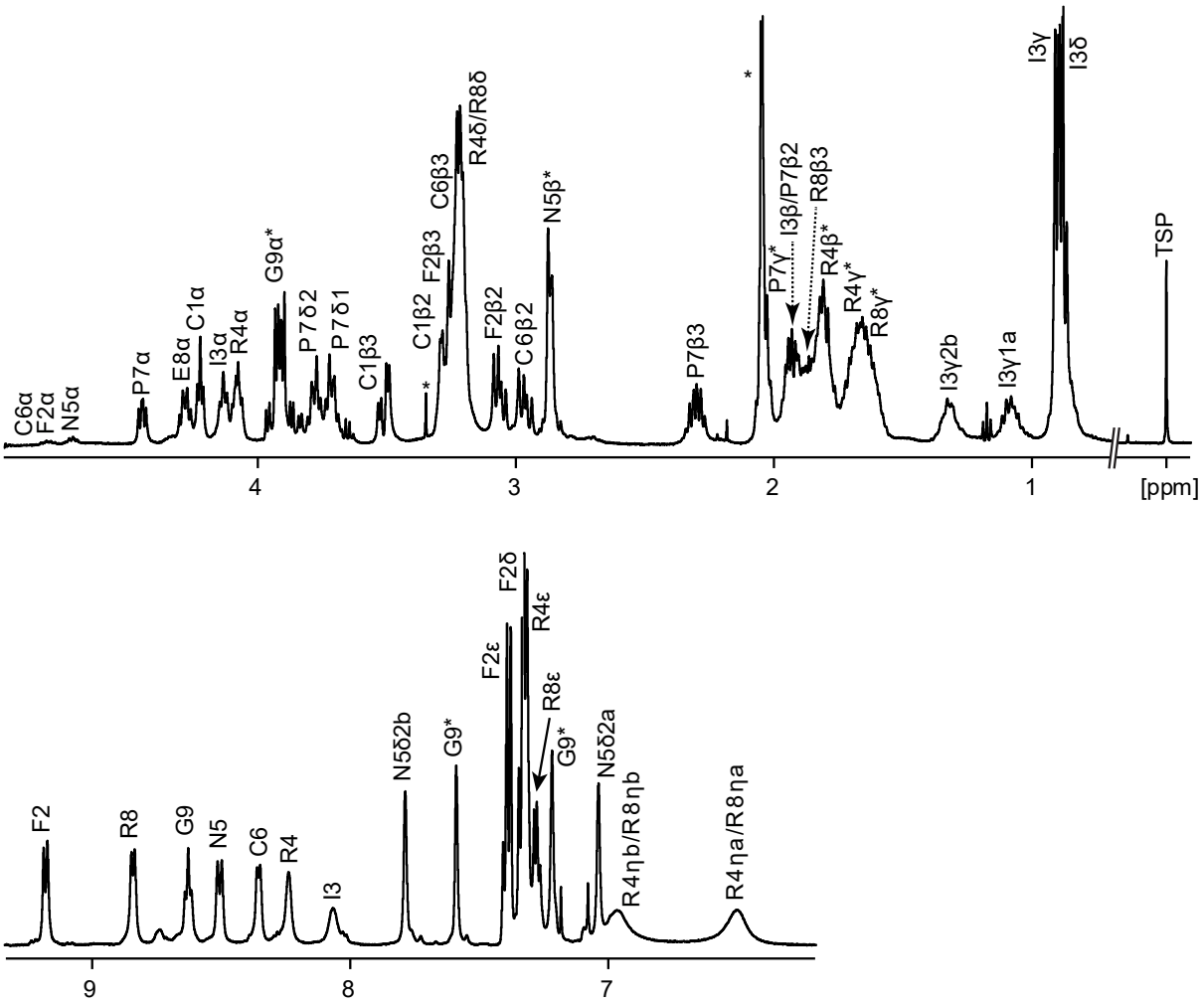
Supplementary Figure 12: Partial ^{1}H-^{13}C HSQC spectra indicating the assignment of ^{13}C chemical shifts of Pro7 Cβ and Cγ resonances of ^{D}R4-Mo1033



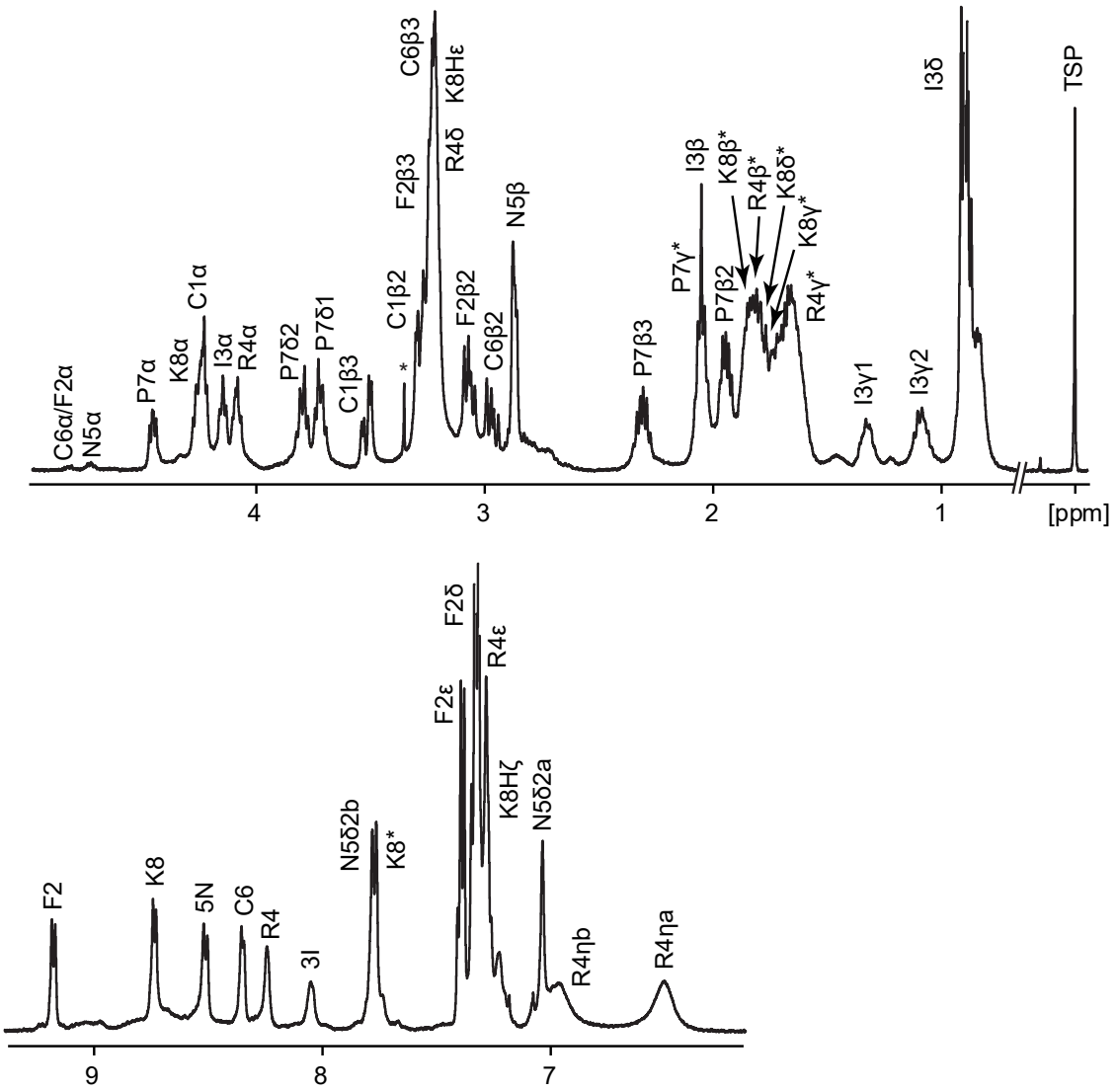
Supplementary Figure 13: Measurement of the $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$ using selective TOCSY experiments. 1D selective TOCSY experiment obtained by irradiating the $C^{\alpha}H$ of Arg4 in the ^{D}R4-Mo1033. The expanded regions are shown, and the vicinal coupling constant $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$ value is indicated on the spectrum.



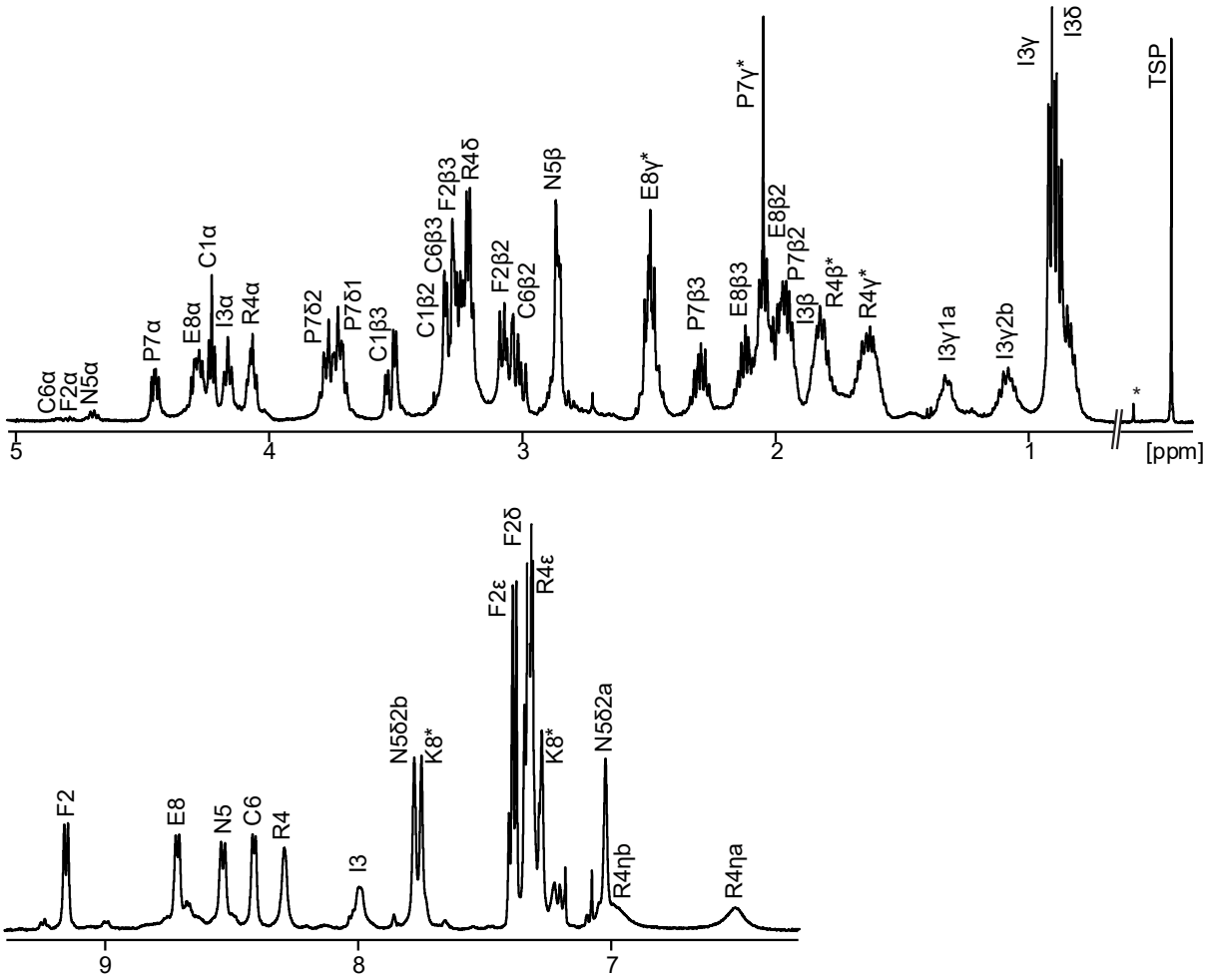
Supplementary Figure 14: 500 MHz ^{1}H NMR spectra of Mo1034 in water at 273 K. Unidentified impurities are marked with an asterisk.



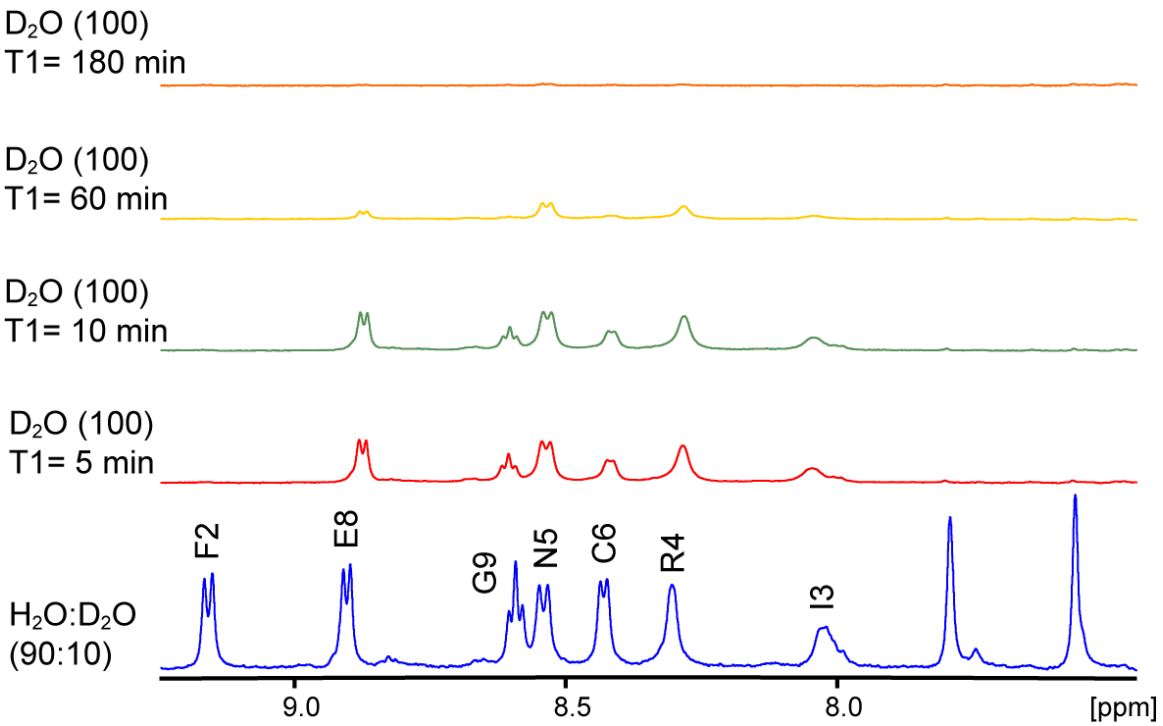
Supplementary Figure 15: 500 MHz ^{1}H NMR spectra of Mo1061 in water at 273 K. Unidentified impurities are marked with an asterisk.



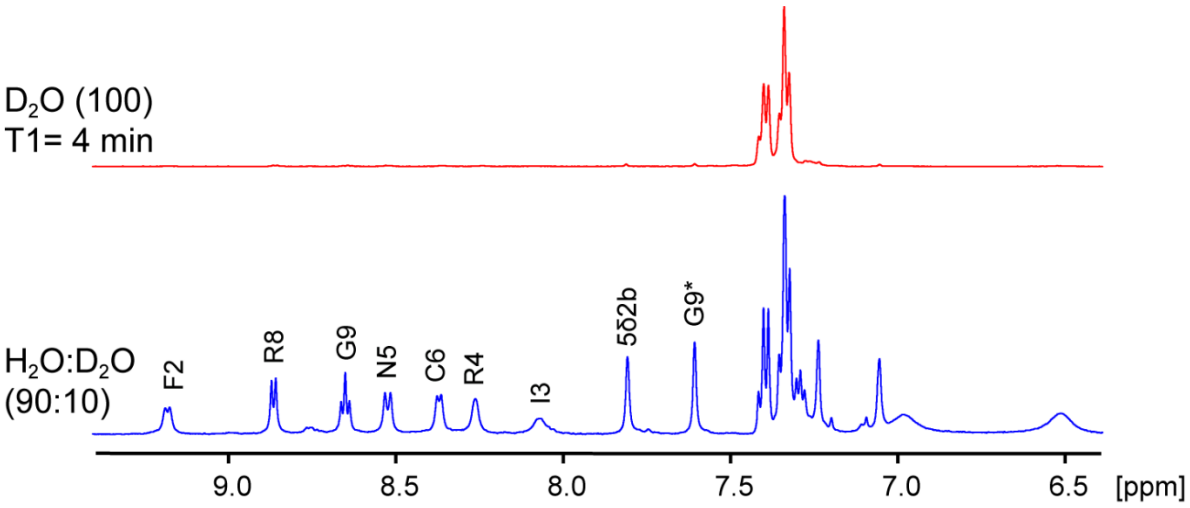
Supplementary Figure 16: 500 MHz ^{1}H NMR spectra of Tr-Mo976 in water at 273 K. Unidentified impurities are marked with an asterisk.



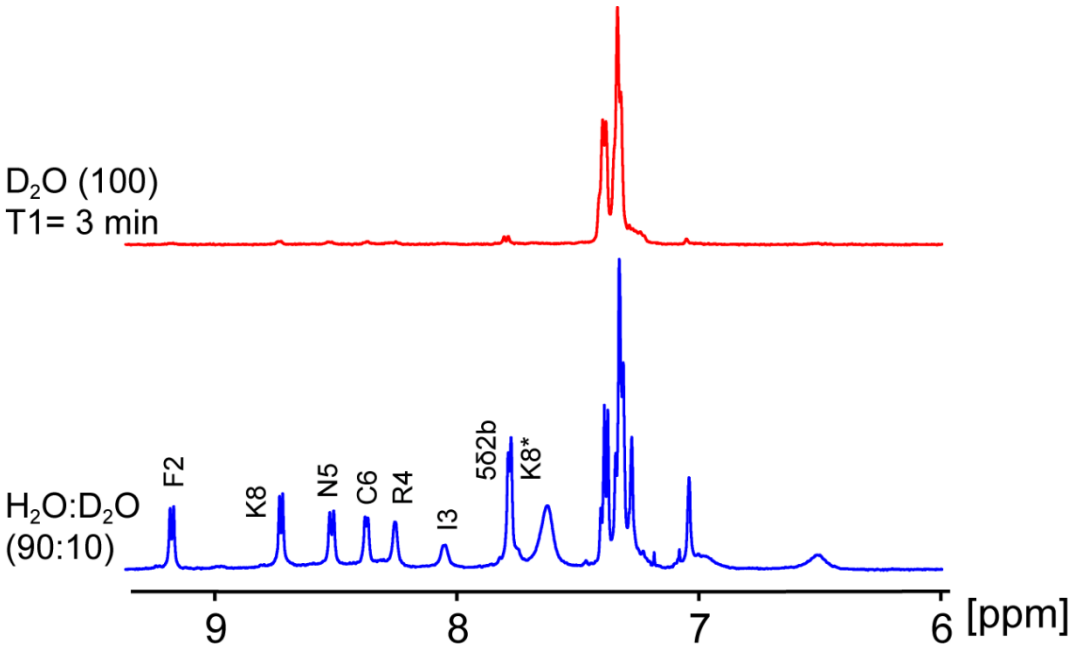
Supplementary Figure 17: 500 MHz ^{1}H NMR spectra of Tr-Mo977 in water at 273 K. Unidentified impurities are marked with an asterisk.



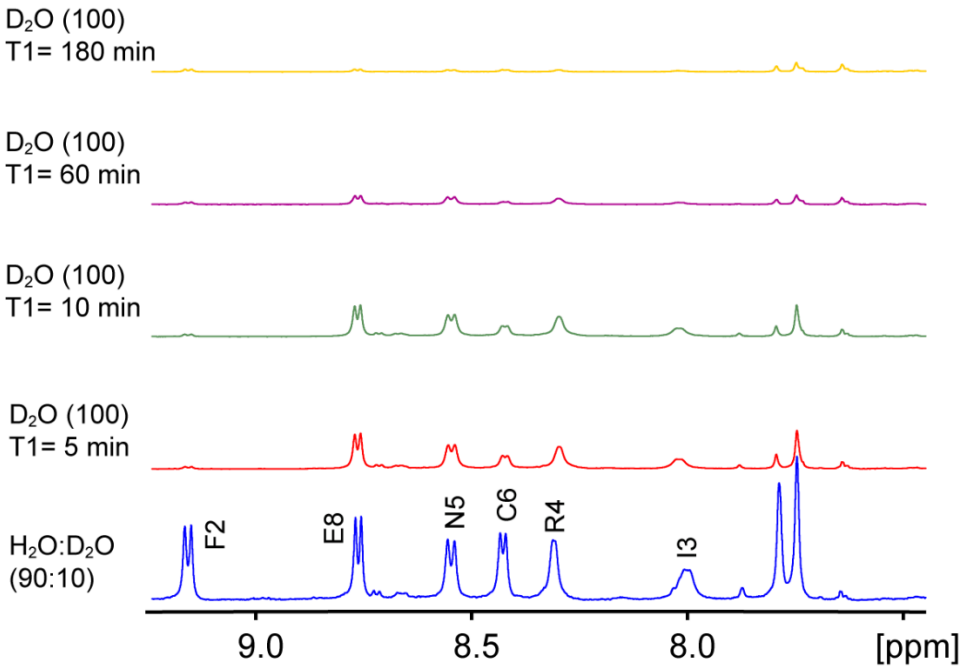
Supplementary Figure 18: Variation of intensities of NH resonances of Mo1034 in 100% D_2O with time after dissolution at 273 K.



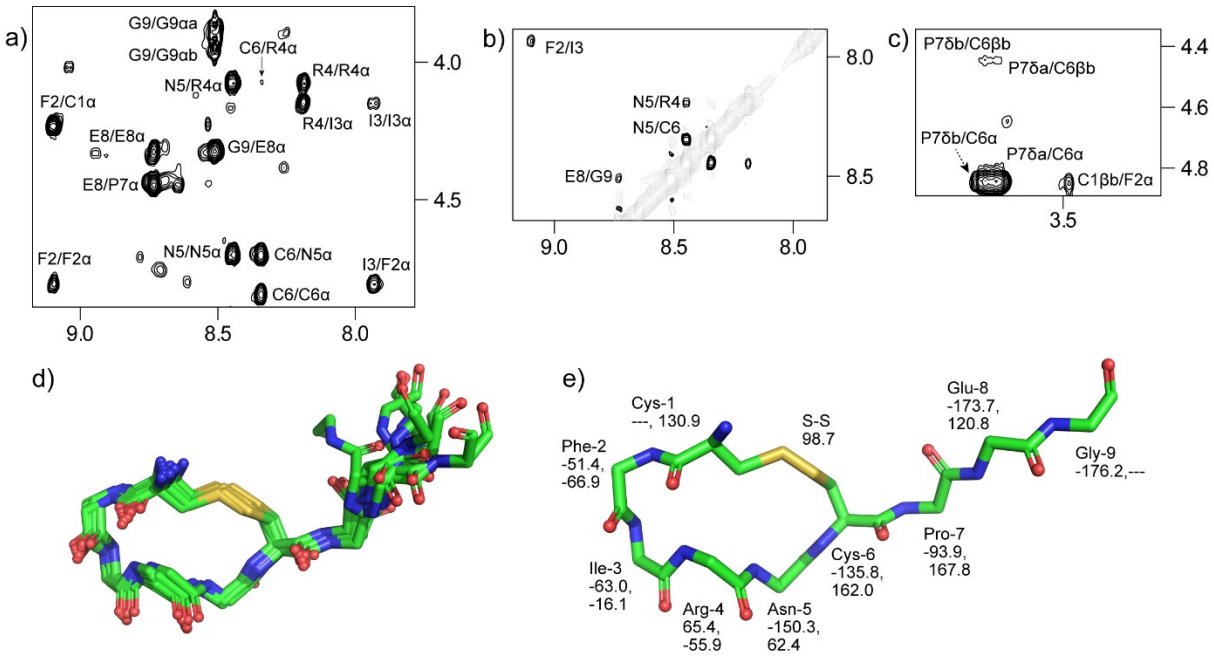
Supplementary Figure 19: Variation of intensities of NH resonances of Mo1061 in 100% D_2O with time after dissolution at 273 K.



Supplementary Figure 20: Variation of intensities of NH resonances of Tr-Mo976 in 100% D_2O with time after dissolution at 273 K.



Supplementary Figure 21: Variation of intensities of NH resonances of Tr-Mo977 in 100% D_2O with time after dissolution at 273 K.



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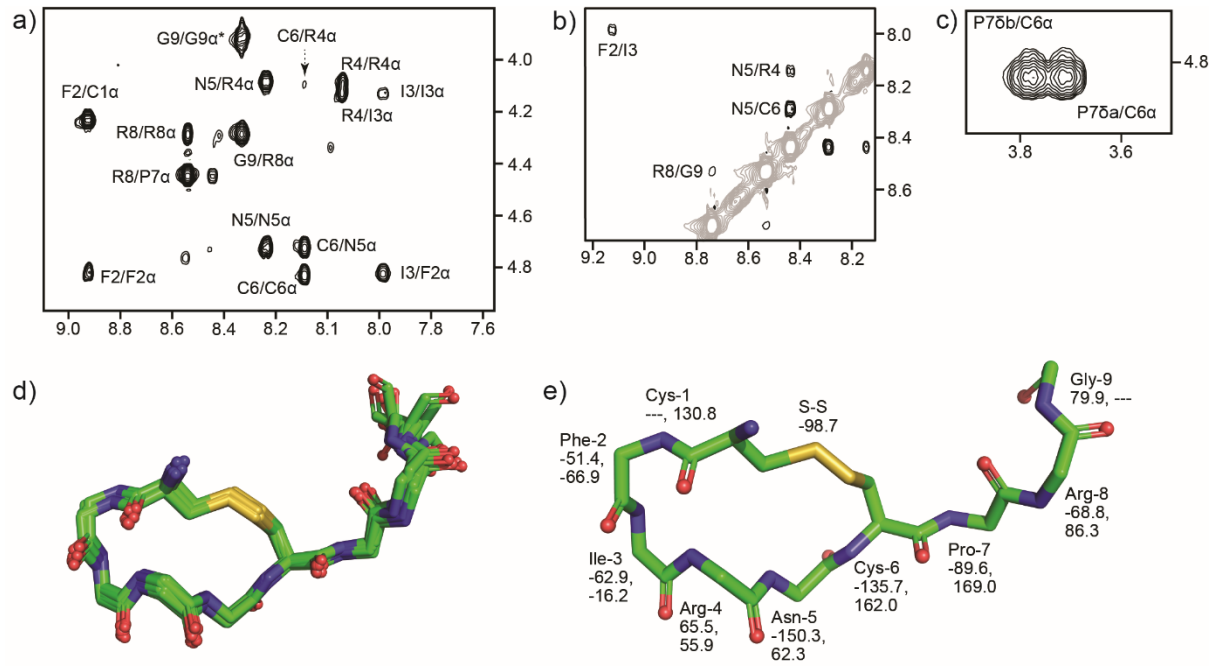

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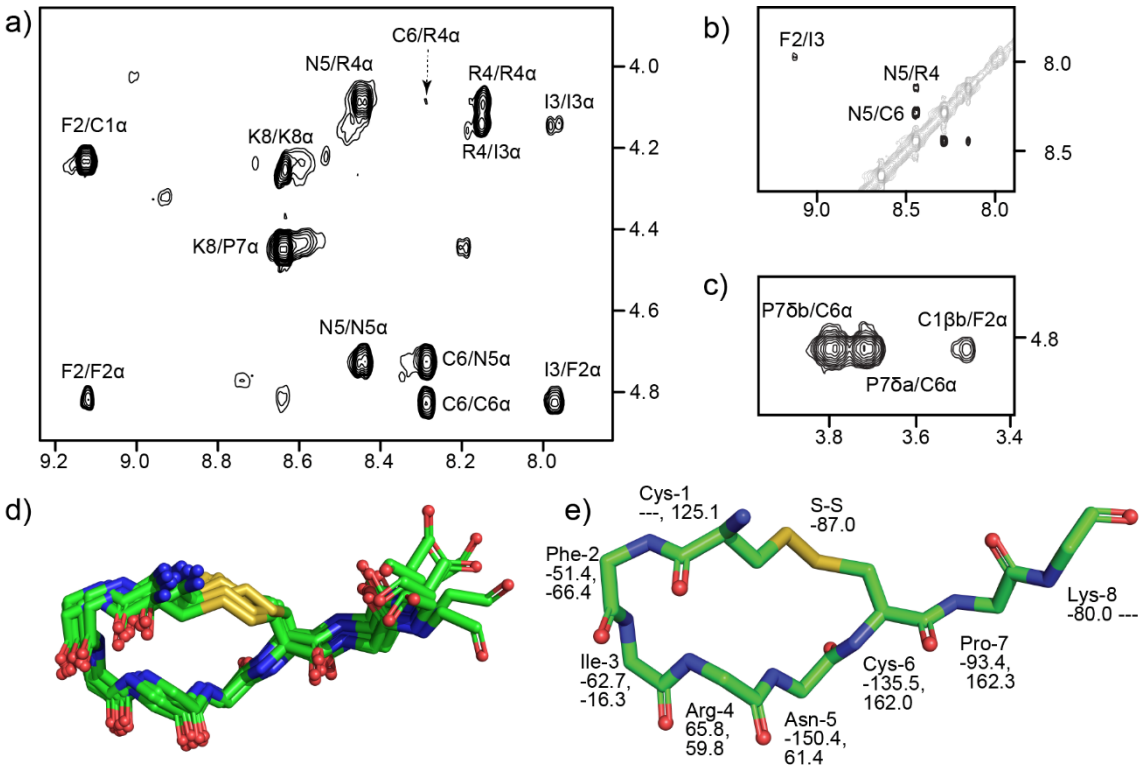

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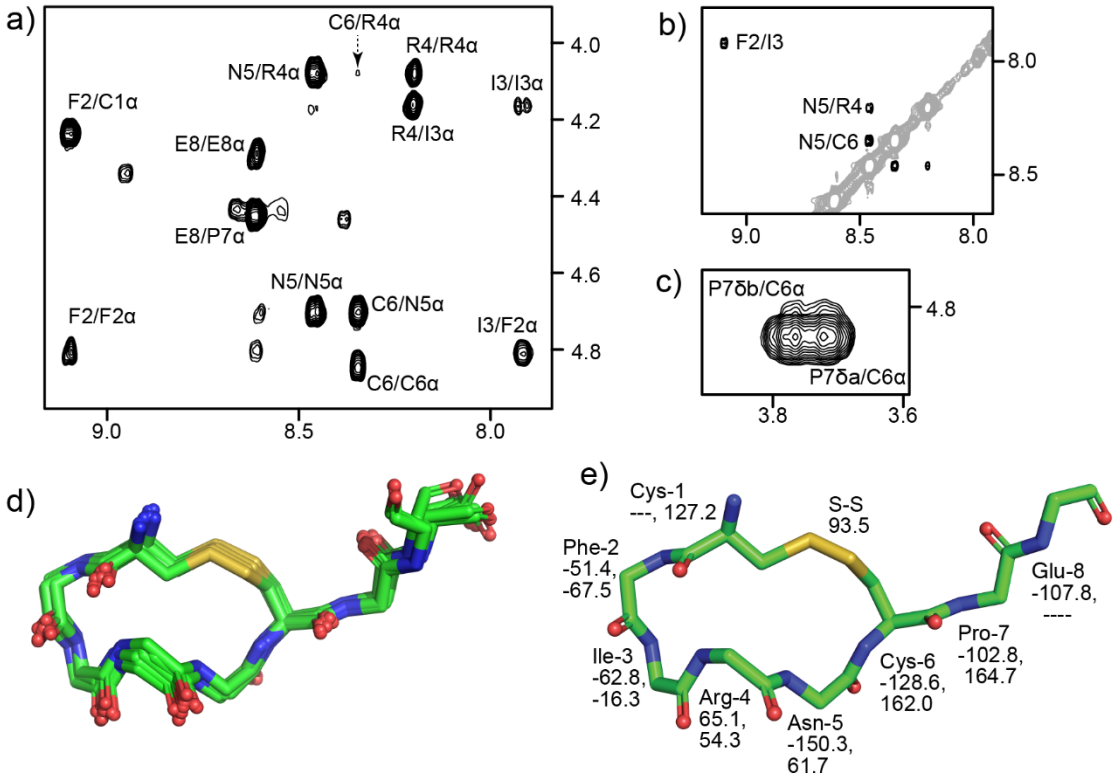

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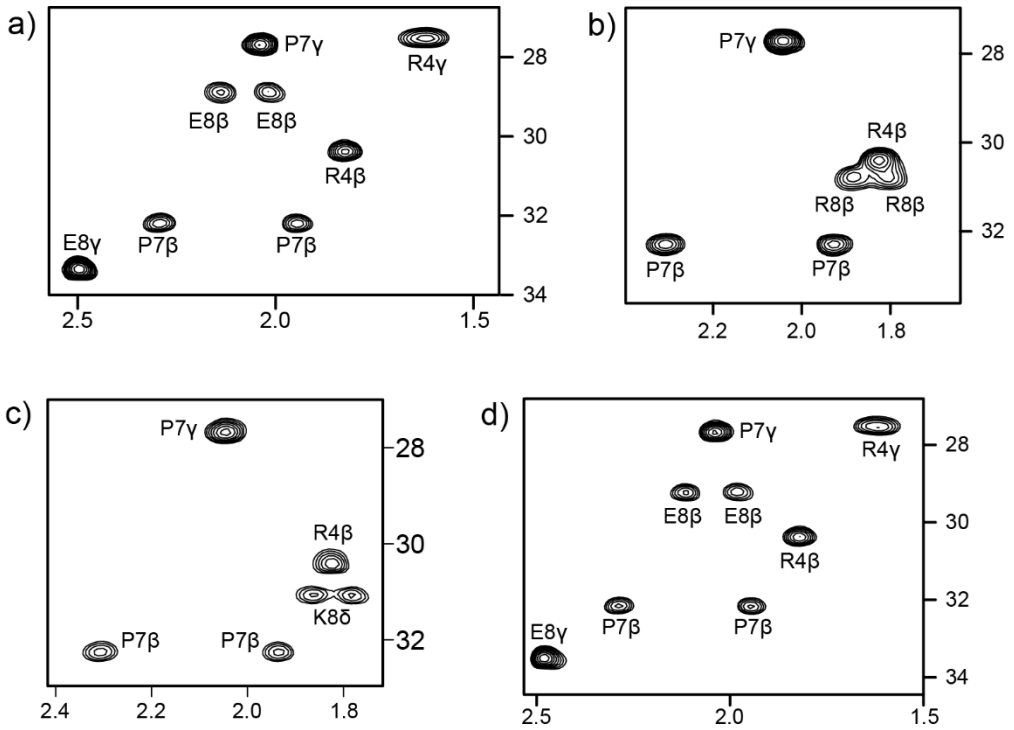
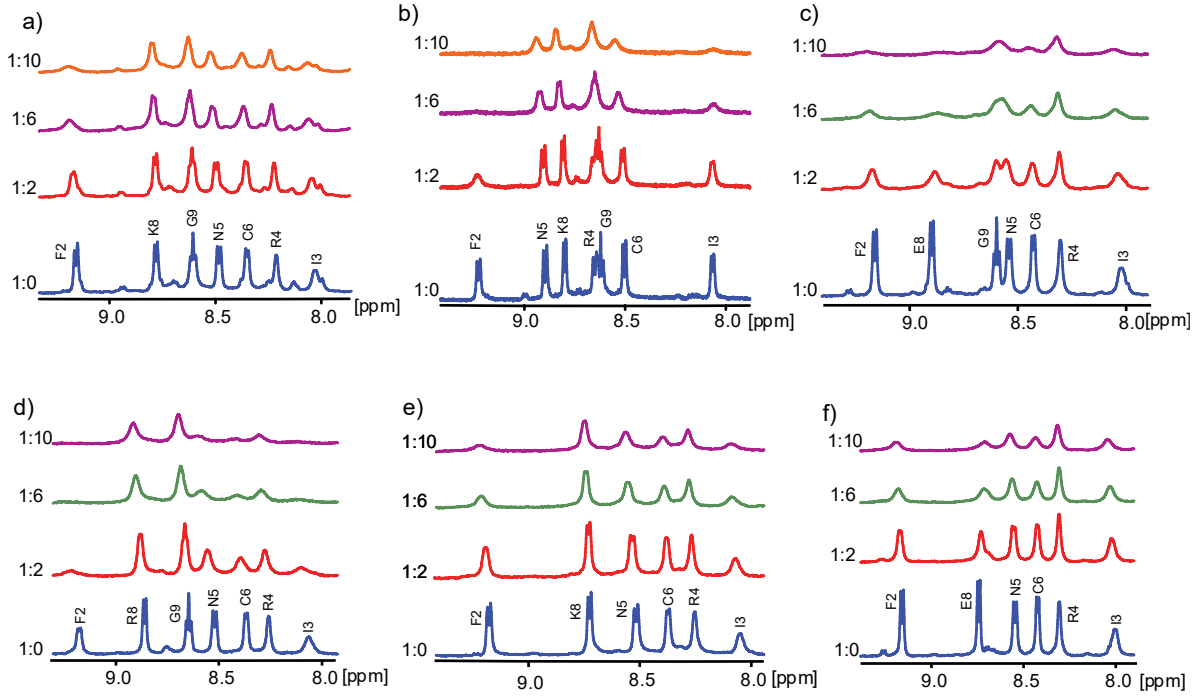
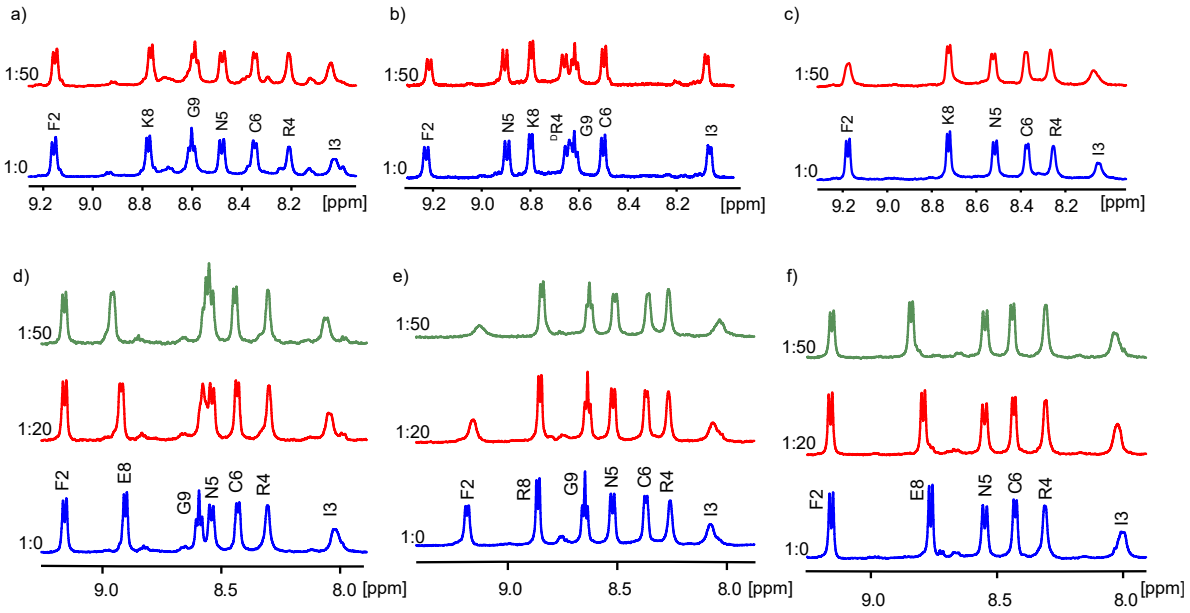


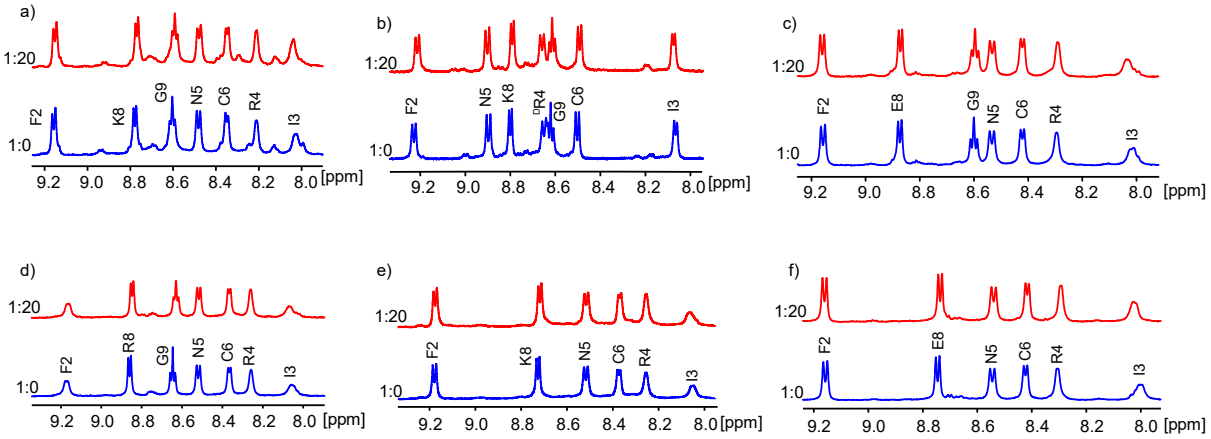
Supplementary Figure 26: Partial ^{1}H-^{13}C HSQC spectra indicating the assignment of ^{13}C chemical shifts of Pro7 Cβ and Cγ resonances of a) Mo1034, b) Mo1061, c) Tr-Mo976, and d) Tr-Mo977



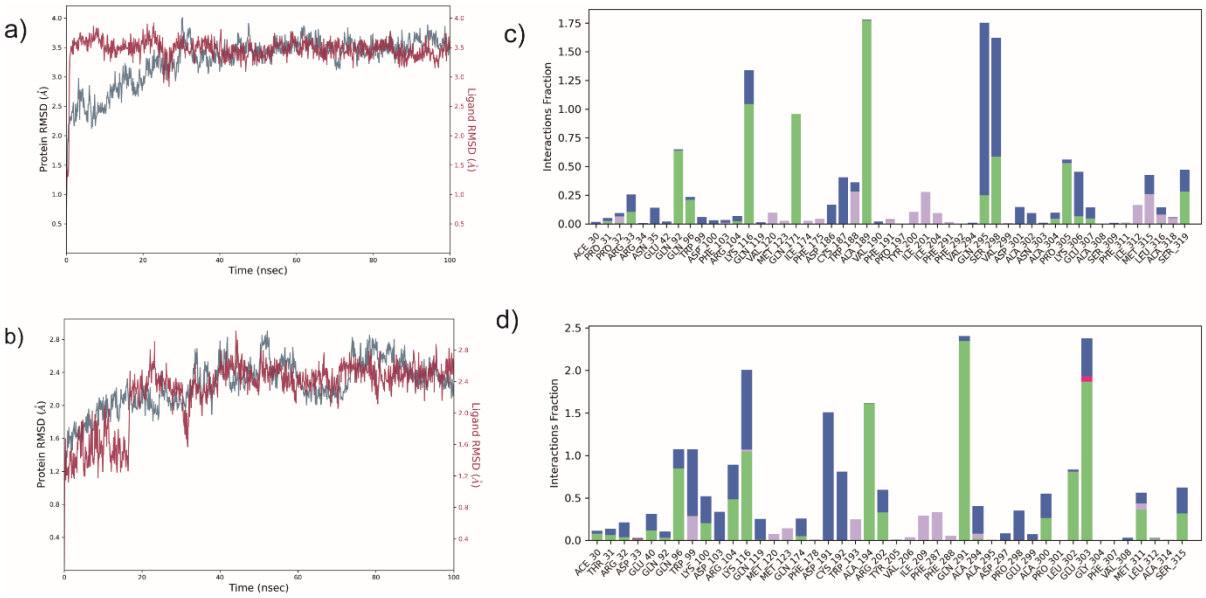
Supplementary Figure 27: Partial 500 MHz ^{1}H 1D NMR spectra of a) Mo1033, b) ^{D}R4-Mo1033, at 278 K and c) Mo1034, d) Mo1061, d) Tr-Mo976, and e) Tr-Mo977 in H_2O: D_2O in the presence of copper ions at 273 K.



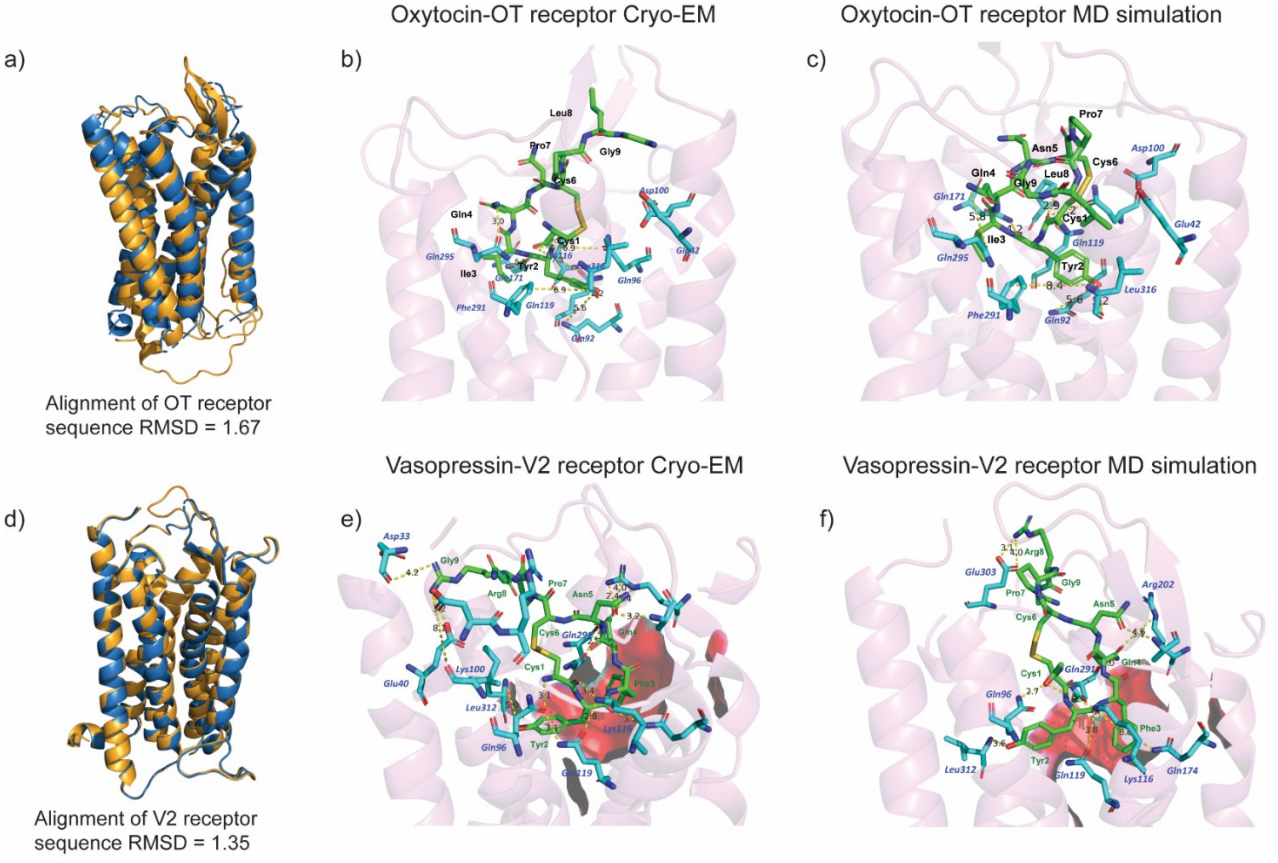
Supplementary Figure 28: Partial 500 MHz ^{1}H 1D NMR spectra of a) Mo1033, b) ^{D}R4-Mo1033, at 278 K and c) Tr-Mo976, d) Mo1034, d) Mo1061, and e) Tr-Mo977 in H_2O: D_2O in the presence of magnesium ions at 273 K.



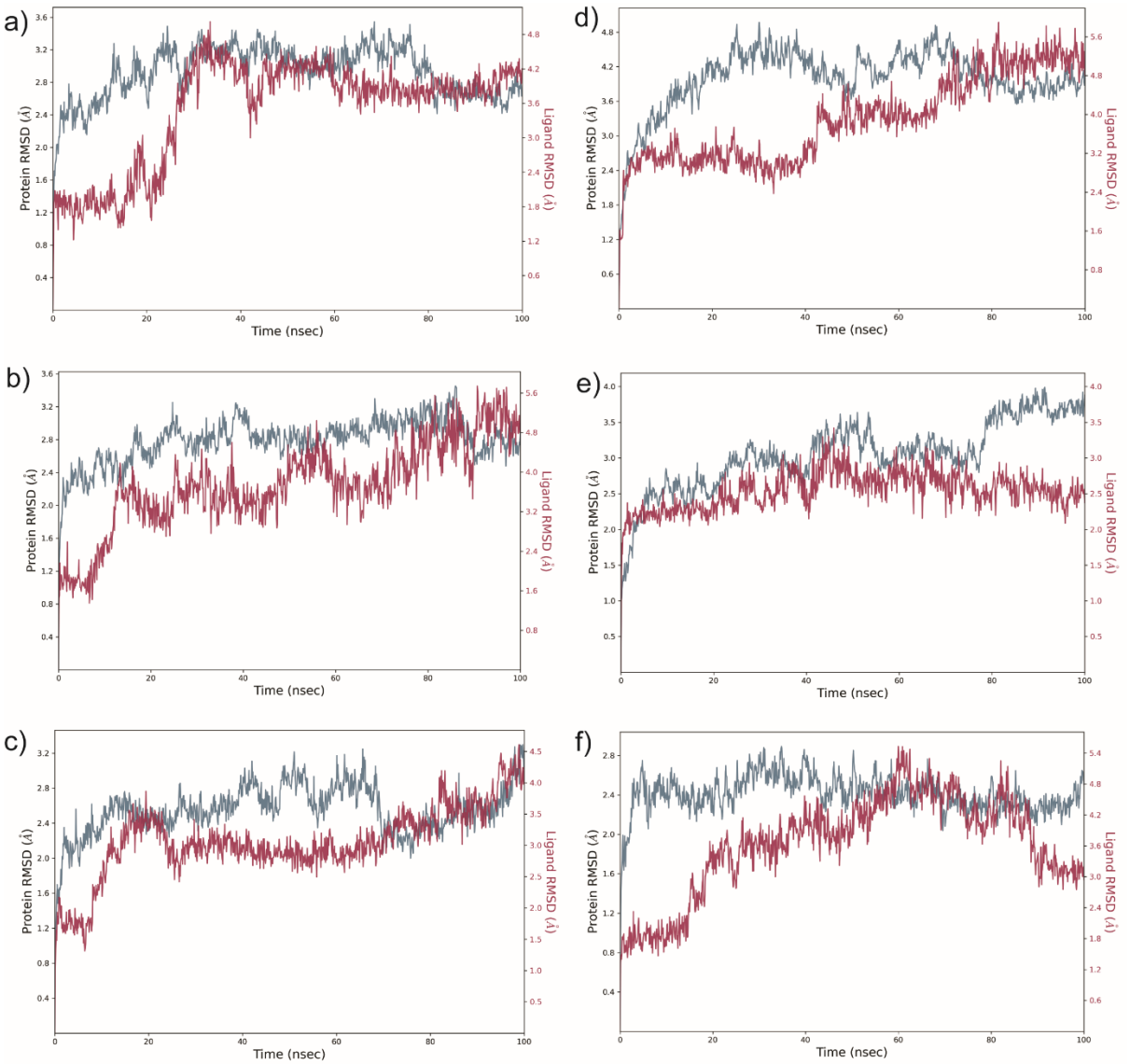
Supplementary Figure 29: Partial 500 MHz ^{1}H 1D NMR spectra of a) Mo1033, b) ^{D}R4-Mo1033, at 278 K and c) Mo1034, d) Mo1061, d) Tr-Mo976, and e) Tr-Mo977 in H_2O: D_2O in the presence of calcium ions at 273 K.



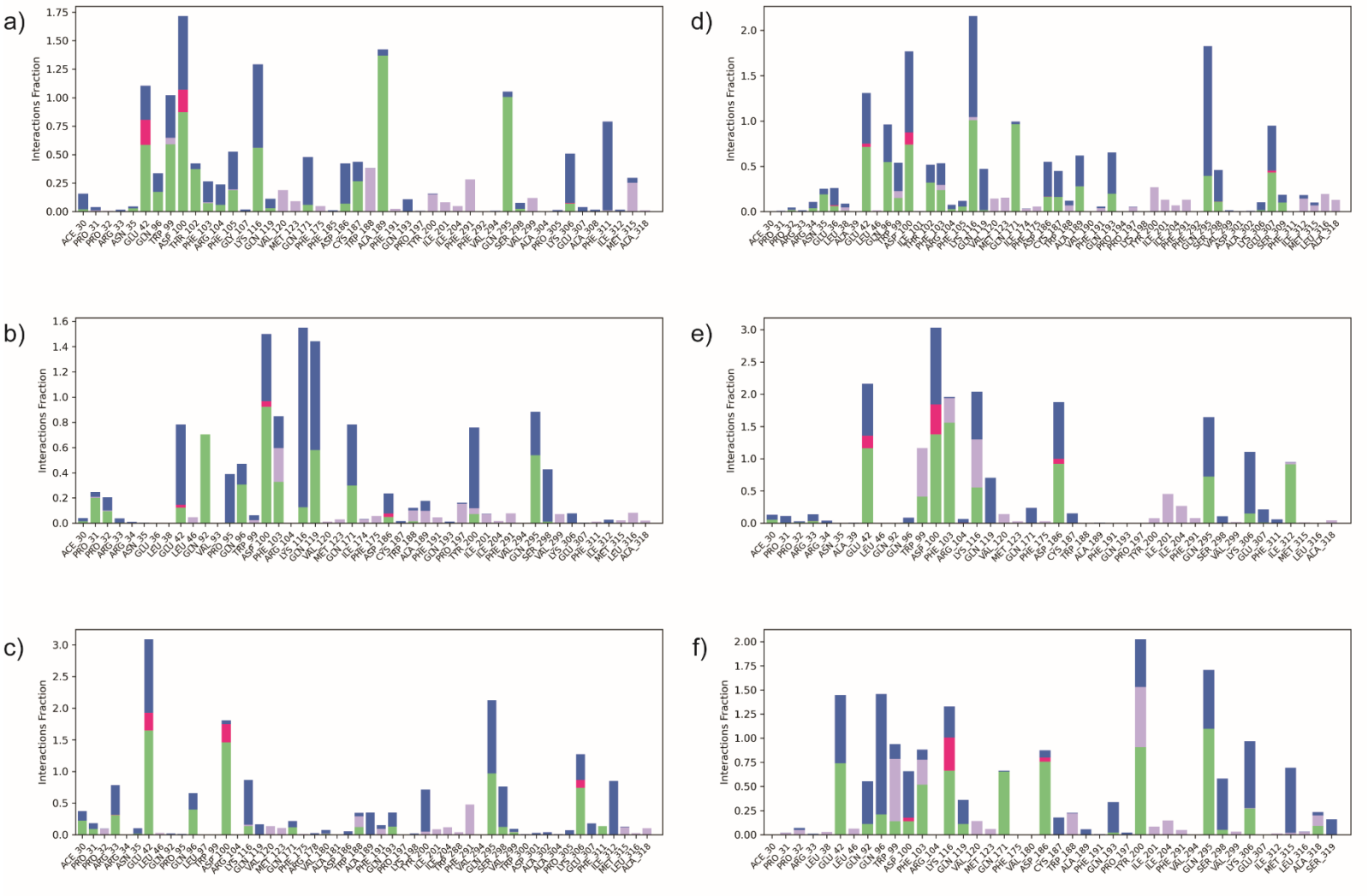
Supplementary Figure 30: RMSD of the atomic positions for the ligand a) oxytocin and, b) vasopressin (in red, Lig fit Prot) and the receptor oxytocin and vasopressin (Cα positions in blue) of the 100 ns molecular dynamics simulations using Desmond package. Protein-ligand contacts of c) OT receptor- oxytocin d) V2 receptor – vasopressin for a simulation time of 100 ns through hydrogen bond (green), hydrophobic (grey) and ionic interactions (pink), and water bridges (blue).



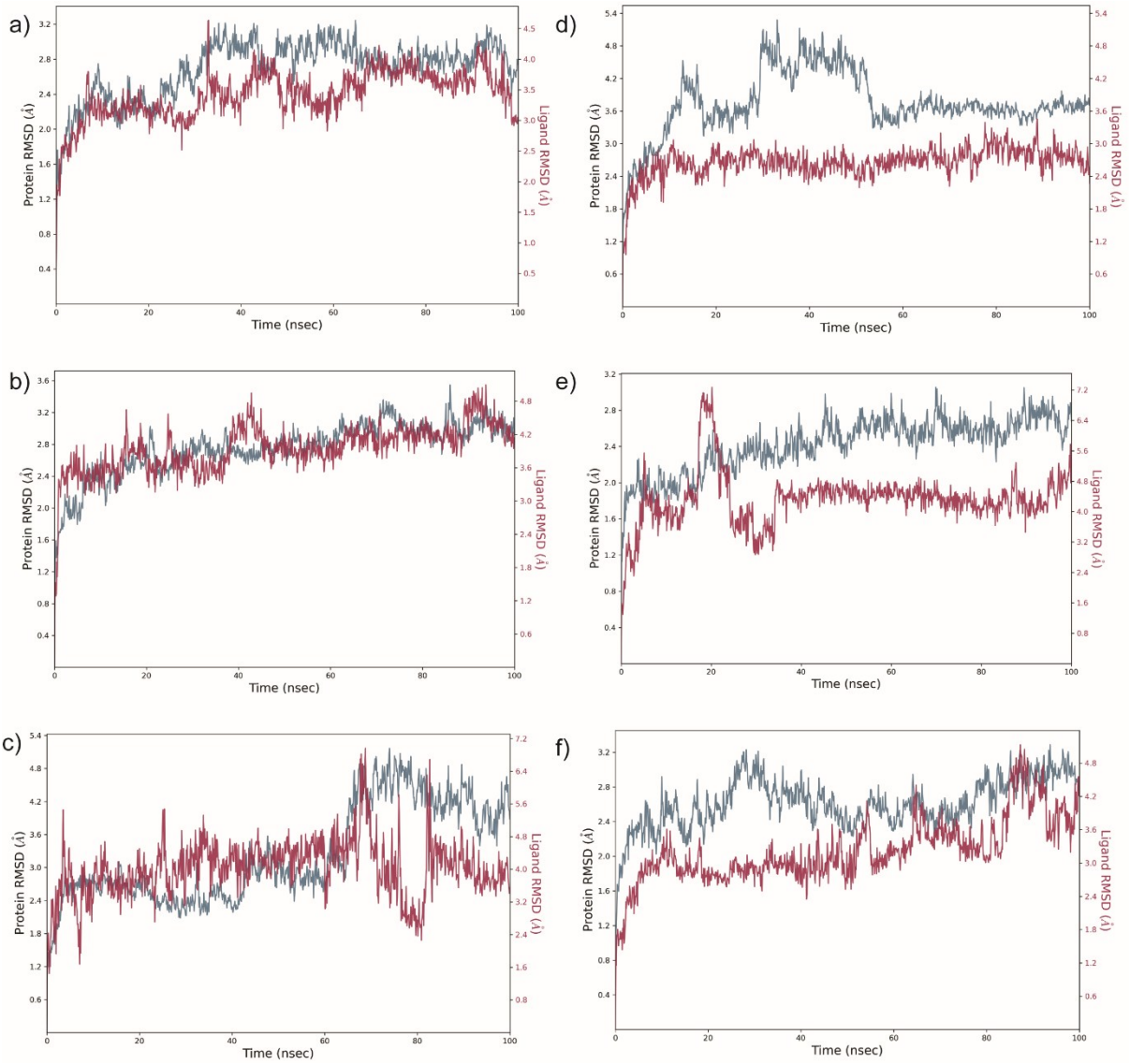
Supplementary Figure 31: The comparison of MD simulated and the cryo-EM interactions. a) Superposition of OT receptor cryo-EM (blue) and MD simulated (orange) structures; the seven transmembrane helices aligned over C^{α} atoms are shown as a cartoon (PDB ID: 7RYC) with an RMSD of 1.67 Å. b) Cryo-EM oxytocin-OT receptor interactions and c) MD simulation oxytocin-OT receptor interactions, the oxytocin ligand (carbon green), the interacting receptor residues (carbon cyan) are represented as sticks, and the transmembrane helices are represented as cartoon (violet). d) Superposition of V2 receptor cryo-EM (blue) and MD simulated (orange) structures; the seven transmembrane helices aligned over C^{α} atoms are shown as a cartoon (PDB ID: 7DW9). b) Cryo-EM vasopressin-V2 receptor interactions and c) MD simulation vasopressin-V2 receptor interactions, the vasopressin ligand (carbon green), the interacting receptor residues (carbon cyan) are represented as sticks, and the transmembrane helices are represented as cartoon (violet) and the hydrophobic cleft is shown as surface (red).



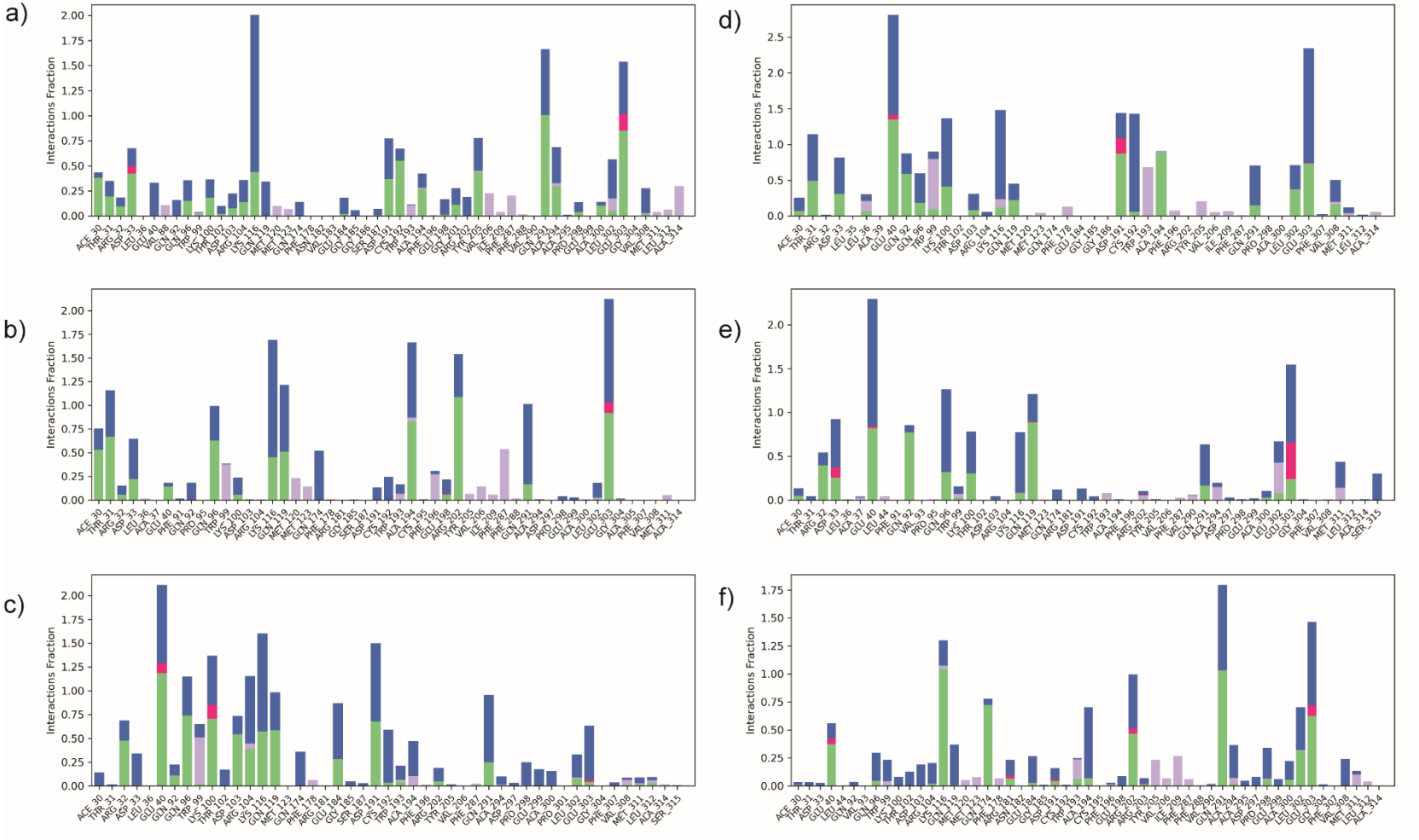
Supplementary Figure 32: RMSD of the atomic positions for the ligand a) Mo1033, b) ^{D}R4-Mo1033, c) Mo1034, d) Mo1061, e) Tr-Mo976, and f) Tr-Mo977 (in red, Lig fit Prot) and the receptor oxytocin (Cα positions in blue) of the 100 ns molecular dynamics simulations using Desmond package.



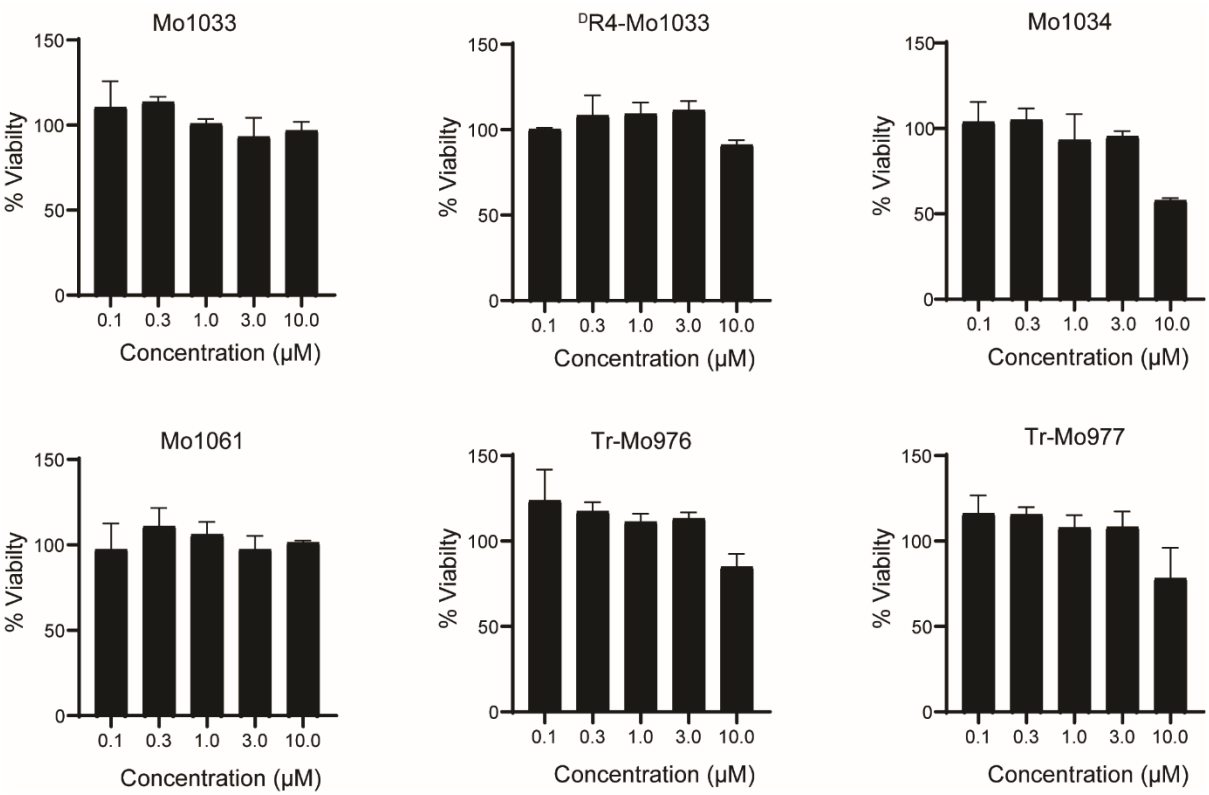
Supplementary Figure 33: Protein-ligand contacts of oxytocin receptor a) Mo1033, b) ^{D}R4-Mo1033, c) Mo1034, d) Mo1061, e) Tr-Mo976, and f) Tr-Mo977 for a simulation time of 100 ns through hydrogen bond (green), hydrophobic (grey) and ionic interactions (pink), and water bridges (blue).



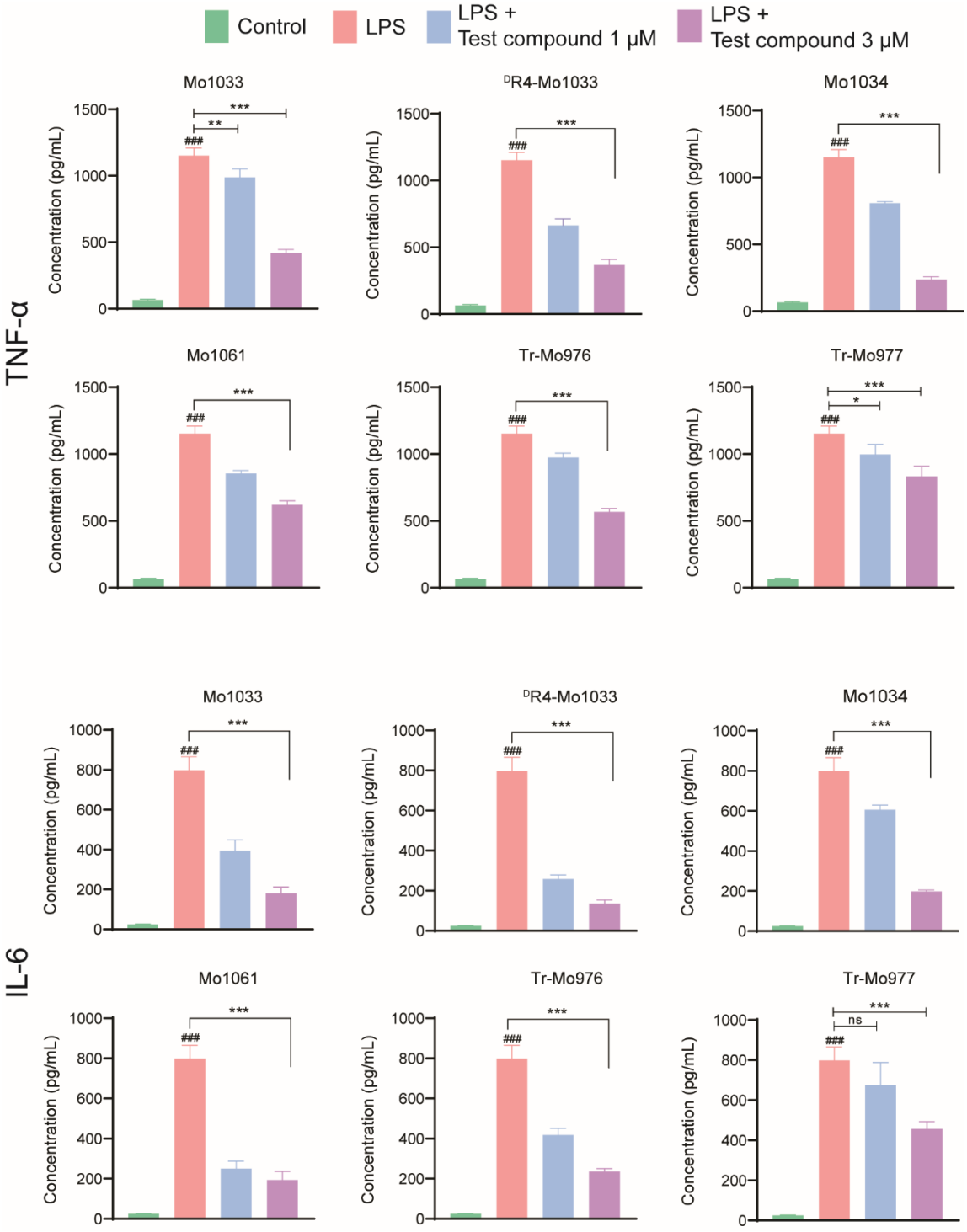
Supplementary Figure 34: RMSD of the atomic positions for the ligand a) Mo1033, b) ^{D}R4-Mo1033, c) Mo1034, d) Mo1061, e) Tr-Mo976, and f) Tr-Mo977 (in red, Lig fit Prot) and the vasopressin V2 (Cα positions in blue) of the 100 ns molecular dynamics simulations using Desmond package.



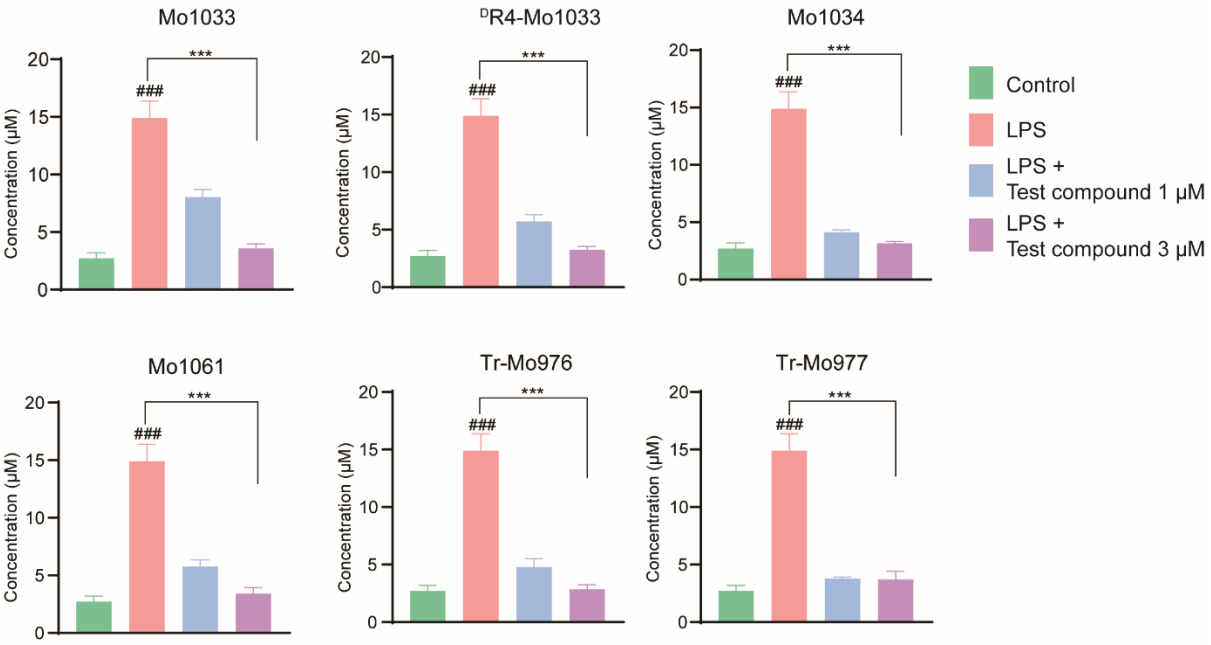
Supplementary Figure 35: Protein-ligand contacts of vasopressin V2 receptor a) Mo1033, b) ^{D}R4-Mo1033, c) Mo1034, d) Mo1061, e) Tr-Mo976, and f) Tr-Mo977 for a simulation time of 100 ns through hydrogen bond (green), hydrophobic (grey) and ionic interactions (pink), and water bridges (blue).



Supplementary Figure 36: The cytotoxicity of the conopressins was determined with the MTT assay after 24 H of treatment with different doses of conopressins. All the conopressins showed safety up to a concentration of 3 μM, while Mo1034, Tr-Mo976, and Tr-Mo977 showed ~20-40% toxicity at 10 μM concentration.



Supplementary Figure 37: Effects of conopressins on the production of TNF-α, and IL-6 in LPS-activated RAW 264.7 cells. Pro-inflammatory cytokine (TNF-α and IL-6) production was measured in the culture medium to determine the anti-inflammatory activity of the conopressins. LPS-activated RAW 264.7 cells were treated with the conopressins at the concentrations 1 and 3 µM. The proinflammatory cytokine production was significantly reduced as compared to the LPS treated group. The values are expressed as the means ± SD. ### $p < 0.001$, compared with the control group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with the LPS group, $n = 3$.



Supplementary Figure 38: Effects of conopressins on NO production in LPS-activated RAW 264.7 cells. NO production was measured in the culture medium to determine the anti-inflammatory activity of the above compounds. LPS-activated RAW 264.7 cells were treated with the above conopressins at concentrations 1 and 3 μM. NO production was significantly reduced compared to that of the LPS-treated group. The values are expressed as the means ± SD. ### $p < 0.001$, compared with the control group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with the LPS group, $n = 3$.

Supplementary Table 1: NMR parameters for Mo1033 in water.

Residue (temp 5°C)	**NH**	**H^{α}**	**H^{β}**	**Others**	**$^{3}J_{NH\text{-}C^{\alpha}H}$ (Hz)**	**dδ/dT (ppb/K)**
Cys 1	-	4.22	3.28 $H^{\beta}2$, 3.50 $H^{\beta}3$	-	-	-
Phe 2	9.15	4.82	3.08 $H^{\beta}2$, 3.27 $H^{\beta}3$	7.33 H^{δ}, 7.39 H^{ε}	7.43	6.45
Ile 3	8.01	4.15	1.95	1.09 $H^{\gamma}1$, 1.32 $H^{\gamma}2$, 0.90 H^{δ}	-	7.73
Arg 4	8.20	4.09	1.83	1.63 $H^{\gamma}1$, 3.22 H^{δ}, 7.30 H^{ε}, 6.52 $H^{\eta}a$, 6.95 $H^{\eta}b$	-	8.95
Asn 5	8.47	4.73	2.87	7.01 $H^{\delta}2a$, 7.75 $H^{\delta}2b$	8.16	7.10
Cys 6	8.34	4.83	2.97 $H^{\beta}2$, 3.25 $H^{\beta}3$	-	6.30	6.82
Pro 7	-	4.45	2.31	1.94 $H^{\gamma}1$, 2.04 $H^{\gamma}2$, 3.72 $H^{\delta}1$, 3.78 $H^{\delta}2$	-	-
Lys 8	8.77	4.26	1.83	1.48 H^{γ}, 1.70 H^{δ}, 3.01 H^{ε}, 7.61 H^{ζ}	6.30	10.50
Gly 9	8.59	3.92	-	7.19 and 7.56 amidated protons	6.21	9.90

Supplementary Table 2: List of experimental restraints used in the structure calculation of Mo1033 in water.

NOE restraints (Å)	
1 CYSS SG	6 CYSS SG 2.10
1 CYSS SG	6 CYSS CB 3.10
1 CYSS CB	6 CYSS SG 3.10
1 CYSS CB	6 CYSS CB 4.00
2 PHE H	1 CYSS HA 2.50
2 PHE HA	2 PHE H 5.00
2 PHE QB	2 PHE H 5.00
2 PHE QB	2 PHE QD 3.50
2 PHE HA	2 PHE QD 2.50
2 PHE HA	2 PHE QE 5.00
2 PHE H	2 PHE QD 5.00
3 ILE H	2 PHE HA 5.00
3 ILE H	2 PHE QB 5.00
3 ILE QD1	2 PHE QD 5.00
3 ILE QD1	2 PHE QE 5.00
3 ILE H	2 PHE H 5.00
3 ILE H	3 ILE HA 5.00
3 ILE H	3 ILE HB 5.00
3 ILE H	3 ILE QG2 5.00
3 ILE HA	3 ILE QG1 5.00
3 ILE HA	3 ILE QD1 5.00
3 ILE HB	3 ILE QG1 5.00
3 ILE HB	3 ILE QD1 3.50
3 ILE HA	4 ARG H 3.50
3 ILE HB	4 ARG H 5.00
3 ILE QG1	4 ARG H 5.00
3 ILE QD1	4 ARG H 5.00
3 ILE QG1	5 ASN H 5.00
3 ILE QD1	5 ASN H 5.00
4 ARG H	4 ARG HA 3.50
4 ARG H	4 ARG QB 3.50
4 ARG H	4 ARG QG 5.00
4 ARG HA	4 ARG QG 3.50
4 ARG HA	4 ARG QD 5.00
4 ARG QD	4 ARG HE 2.50
4 ARG QB	4 ARG HE 5.00
4 ARG QG	4 ARG HE 5.00
4 ARG HA	5 ASN H 2.50
4 ARG QB	5 ASN H 5.00
4 ARG QG	5 ASN H 5.00
4 ARG H	5 ASN H 5.00
4 ARG HA	6 CYSS H 5.00
5 ASN H	5 ASN HA 3.50
5 ASN H	5 ASN QB 3.50
5 ASN HA	5 ASN QB 2.50
5 ASN HA	5 ASN HD21 5.00
5 ASN HD21	5 ASN HD22 2.50
5 ASN QB	5 ASN HD21 2.50
5 ASN QB	6 CYSS H 5.00
5 ASN HA	6 CYSS H 2.50
5 ASN H	6 CYSS H 5.00
6 CYSS H	7 PROO QD 5.00
6 CYSS HA	7 PROO QD 2.50
6 CYSS QB	7 PROO QD 5.00
7 PROO HA	7 PROO QG 5.00
7 PROO QD	7 PROO QB 5.00
7 PROO QD	7 PROO HA 5.00
7 PROO HA	8 LYS H 2.50
7 PROO QG	8 LYS H 5.00
8 LYS HA	9 GLY H 2.50
8 LYS H	9 GLY H 5.00
9 GLY H	8 LYS QG 5.00
9 GLY H	8 LYS QB 5.00
9 GLY H	9 GLY QA 2.50
2 PHE HA	1 CYSS QB 4.10
3 ILE QD1	2 PHE QD 4.10
3 ILE QD1	2 PHE QE 4.10
3 ILE QG1	2 PHE QD 4.10
3 ILE QG1	2 PHE QE 4.10
3 ILE H	3 ILE QG1 4.10
3 ILE H	3 ILE QD1 4.10
3 ILE HA	3 ILE QD1 3.10
3 ILE HA	4 ARG H 3.10
3 ILE HB	4 ARG H 4.10
3 ILE QG1	4 ARG H 4.10
3 ILE QD1	4 ARG H 4.10
3 ILE QG1	5 ASN H 4.10
3 ILE QD1	5 ASN H 4.10
4 ARG H	4 ARG QG 4.10
4 ARG QB	5 ASN H 4.10
4 ARG QG	5 ASN H 4.10
4 ARG HA	6 CYSS H 4.10
5 ASN QB	6 CYSS H 4.10
6 CYSS H	7 PROO QD 4.10
7 PROO HA	7 PROO QG 3.70
8 LYS H	9 GLY H 4.10
Dihedral angle restraints (deg)	
5 ASN PHI	-150.0 -90.0

Supplementary Table 3: Comparative dihedral angles of NMR derived and AlphaFold predicted structures

Residue	**NMR derived structure (φ, ψ)**	**model_0 (φ, ψ)**	**model_1 (φ, ψ)**	**model_2 (φ, ψ)**	**model_3 (φ, ψ)**	**model_4 (φ, ψ)**
Phe2	-79.7, -64.1	-85.5, 172.4	-106.5, 167.2	-95.7, -177.7	-83.7, 155.2	-72.8, 156.8
Ile3	-64.1, -14.9	-66.9, 101.6	-64.3, -30.2	-69.5, -69.1	-69.2, -25.5	-64.2, -33.8
Arg4	66.8, 51.6	73.1, 23.2	-111.7, -1.8	-97.6, 16.5	-82.9, -30.6	-99.0, -12.4
Asn5	-150.4, 60.3	-144.7, 93.0	-138.1, 71.3	-142.0, 82.9	-151.7, 108.6	-144.5, 102.3
Cys6	-129.1, 162.0	-114.5, 84.2	-122.2, 115.9	-116.3, 95.9	-128.5, 84.0	-111.9, 91.1
Pro7	-92.9, 146.8	-74.1, 100.6	-68.3, 119.1	-76.3, 104.8	-74.5, 137.6	-70.1, 129.8
Lys8	-69.3, 85.4	-83.9, 125.5	-94.1, 28.2	-71.5, -22.1	-97.1, -2.1	-89.5, -13.9
Disulfide dihedral	-84.6	39.4	59.0	-68.6	-71.8	55.8

Supplementary Table 4: NMR parameters for ^{D}R4-Mo1033 in water.

Residue	**NH**	**H^{α}**	**H^{β}**	**Others**	**$^{3}J_{NH\text{-}C^{\alpha}H}$ (Hz)**	**dδ/dT (ppb/K)**
Cys 1	-	4.24	3.23 $H^{\beta}2$, 3.35 $H^{\beta}3$	-	-	-
Phe 2	9.21	4.79	3.12 $H^{\beta}2$, 3.19 $H^{\beta}3$	7.33 H^{δ}, 7.37 H^{ε}	7.72	7.55
Ile 3	8.05	4.12	1.79	1.43 $H^{\gamma}1$, 1.13 $H^{\gamma}2$, 0.88 H^{δ}	6.33	9.13
D-Arg 4	8.64	4.36	1.89	1.59 $H^{\gamma}1$, 1.69 $H^{\gamma}2$, 3.21 H^{δ}, 7.28 H^{ε}, 6.50 $H^{\eta}a$, 6.93 $H^{\eta}b$	8.18	10.20
Asn 5	8.88	4.85	2.78 $H^{\beta}2$, 2.70 $H^{\beta}3$	7.67 $H^{\delta}2a$, 6.89 $H^{\delta}2b$	8.35	7.93
Cys 6	8.50	4.96	3.19 $H^{\beta}2$, 2.84 $H^{\beta}3$	-	7.53	8.30
Pro 7	-	4.44	2.31	1.93 $H^{\gamma}1$, 2.03 $H^{\gamma}2$, 3.79 $H^{\delta}1$, 3.72 $H^{\delta}2$	-	-
Lys 8	8.79	4.26	1.83	1.70 H^{γ}, 1.48 H^{δ}, 3.01 H^{ε}, 7.61 H^{ζ}	6.42	10.55
Gly 9	8.61	3.92	-	7.56 7.20 amidated protons	6.28	9.90

Supplementary Table 5: List of experimental restraints used in the structure calculation of ^{D}R4-Mo1033 in water.

NOE restraints (Å)	
1 CYSS SG	6 CYSS SG 2.10
1 CYSS SG	6 CYSS CB 3.10
1 CYSS CB	6 CYSS SG 3.10
1 CYSS CB	6 CYSS CB 4.10
2 PHE H	1 CYSS HA 2.50
2 PHE H	2 PHE HA 3.50
2 PHE H	2 PHE QB 5.00
2 PHE QB	2 PHE QD 3.50
2 PHE HA	2 PHE QD 2.50
2 PHE HA	2 PHE QB 3.50
2 PHE QE	3 ILE QD1 5.00
2 PHE QD	3 ILE QD1 3.50
2 PHE QD	3 ILE QG2 5.00
2 PHE QD	3 ILE QG1 5.00
3 ILE H	2 PHE HA 3.50
3 ILE H	2 PHE H 5.00
3 ILE H	2 PHE QB 5.00
3 ILE HA	3 ILE H 3.50
3 ILE H	3 ILE HB 2.50
3 ILE H	3 ILE QG1 5.00
3 ILE H	3 ILE QG2 5.00
3 ILE H	3 ILE QD1 5.00
3 ILE HA	3 ILE QG1 5.00
4 DAR H	3 ILE HA 2.50
4 DAR H	3 ILE HB 5.00
4 DAR H	3 ILE QD1 5.00
5 ASN H	3 ILE HA 5.00
5 ASN H	4 DAR HA 2.50
5 ASN H	4 DAR H 5.00
5 ASN H	4 DAR QB 5.00
5 ASN HA	5 ASN H 3.50
5 ASN H	5 ASN QB 3.50
5 ASN HD21	5 ASN QB 3.50
6 CYSS H	5 ASN HA 2.50
6 CYSS H	5 ASN H 3.50
6 CYSS H	6 CYSS HA 3.50
6 CYSS H	6 CYSS QB 3.50
6 CYSS HA	7 PROO QD 2.50
7 PROO QD	6 CYSS QB 5.00
7 PROO HA	7 PROO QG 5.00
7 PROO QD	7 PROO QB 5.00
8 LYS H	7 PROO HA 2.50
8 LYS H	7 PROO QG 5.00
8 LYS H	8 LYS HA 3.50
8 LYS H	8 LYS QG 3.50
8 LYS H	8 LYS QB 3.50
8 LYS H	8 LYS QD 5.00
8 LYS H	9 GLY H 5.00
9 GLY H	8 LYS HA 2.50
9 GLY H	8 LYS QB 5.00
9 GLY H	9 GLY QA 2.50
3 ILE H	2 PHE H 3.70
3 ILE H	2 PHE QB 3.70
4 DAR H	3 ILE HB 3.70
5 ASN H	3 ILE HA 3.70
5 ASN H	4 DAR H 3.70
6 CYSS H	6 CYSS HA 3.10
7 PROO HA	7 PROO QB 2.10
7 PROO HA	7 PROO QG 3.70
8 LYS H	7 PROO QG 3.70
8 LYS H	9 GLY H 3.70
9 GLY H	8 LYS QB 3.70
Dihedral angle restraints (deg)	
2 PHE PHI	-160.0 -80.0
4 DAR PHI	+90.0 +150.0
5 ASN PHI	-150.0 -90.0

Supplementary Table 6: NMR parameters for Mo1034 in water

Residue	**NH**	**H^{α}**	**H^{β}**	**Others**	**$^{3}J_{NH\text{-}C^{\alpha}H}$ (Hz)**	**dδ/dT (ppb/K)**
Cys 1	-	4.23	3.29 $H^{\beta}2$, 3.49 $H^{\beta}3$	-	-	-
Phe 2	9.10	4.81	3.07 $H^{\beta}2$, 3.26 $H^{\beta}3$	7.33 H^{δ}, 7.39 H^{ε}, 7.32 H^{ζ}	7.01	5.68
Ile 3	7.93	4.15	1.94	1.07 $H^{\gamma}1$, 1.30 $H^{\gamma}2$, 0.89 H^{δ}	6.27	6.31
Arg 4	8.19	4.08	1.83	1.63 $H^{\gamma}1$, 3.21 H^{δ}, 7.27 H^{ε}, 6.52 $H^{\eta}a$, 6.88 $H^{\eta}b$	-	7.81
Asn 5	8.44	4.70	2.85	6.97 $H^{\delta}2a$, 7.70 $H^{\delta}2b$	7.51	6.82
Cys 6	8.34	4.85	3.01 $H^{\beta}2$, 3.27 $H^{\beta}3$	-	6.08	5.82
Pro 7	-	4.44	2.03 $H^{\beta}2$, 1.95 $H^{\beta}3$	2.04 H^{γ}, 3.72 $H^{\delta}1$, 3.76 $H^{\delta}2$	-	-
Glu 8	8.73	4.32	2.02 $H^{\beta}2$, 2.14 $H^{\beta}3$	2.50 H^{γ}	6.34	9.34
Gly 9	8.51	3.89 $H^{\alpha}2$, 3.94 $H^{\alpha}3$	-	7.16, 7.50 amidated protons	5.99	7.89

Supplementary Table 7: List of experimental restraints used in the structure calculation of Mo1034 in water.

NOE restraints (Å)	
1 CYSS SG 6 CYSS SG 2.10	5 ASN HA 5 ASN QB 2.50
1 CYSS SG 6 CYSS CB 3.10	5 ASN HA 5 ASN HD21 5.00
1 CYSS CB 6 CYSS SG 3.10	5 ASN HD21 5 ASN HD22 2.50
1 CYSS CB 6 CYSS CB 4.00	5 ASN QB 5 ASN HD21 2.50
2 PHE H 1 CYSS HA 2.50	5 ASN QB 6 CYSS H 5.00
2 PHE HA 2 PHE H 3.50	5 ASN HA 6 CYSS H 2.50
2 PHE QB 2 PHE H 3.50	5 ASN H 6 CYSS H 5.00
2 PHE QB 2 PHE QD 3.50	6 CYSS H 7 PROO QD 5.00
2 PHE HA 2 PHE QD 2.50	6 CYSS HA 7 PROO QD 2.50
2 PHE HA 2 PHE QE 5.00	6 CYSS QB 7 PROO QD 5.00
2 PHE H 2 PHE QD 5.00	7 PROO HA 7 PROO QG 5.00
3 ILE H 2 PHE QB 5.00	7 PROO QD 7 PROO QB 5.00
3 ILE QD1 2 PHE QD 5.00	7 PROO QD 7 PROO HA 5.00
3 ILE QD1 2 PHE QE 5.00	8 GLU H 7 PROO HA 2.50
3 ILE H 2 PHE H 5.00	8 GLU H 7 PROO QB 5.00
3 ILE H 3 ILE HA 5.00	8 GLU H 9 GLY H 5.00
3 ILE H 3 ILE HB 5.00	8 GLU HA 9 GLY H 2.50
3 ILE H 3 ILE QG2 5.00	8 GLU QG 9 GLY H 5.00
3 ILE HA 3 ILE QG1 5.00	8 GLU QB 9 GLY H 5.00
3 ILE HA 3 ILE QD1 5.00	
3 ILE HB 3 ILE QG1 5.00	3 ILE HA 4 ARG H 3.10
3 ILE HB 3 ILE QD1 3.50	3 ILE HB 4 ARG H 4.10
3 ILE HA 4 ARG H 3.50	3 ILE QG1 4 ARG H 4.10
3 ILE HB 4 ARG H 5.00	3 ILE QD1 4 ARG H 4.10
3 ILE QG1 4 ARG H 5.00	3 ILE QG1 5 ASN H 4.10
3 ILE QD1 4 ARG H 5.00	3 ILE QD1 5 ASN H 4.10
3 ILE QG1 5 ASN H 5.00	4 ARG QB 5 ASN H 4.10
3 ILE QD1 5 ASN H 5.00	4 ARG QG 5 ASN H 4.10
4 ARG H 4 ARG HA 3.50	4 ARG HA 6 CYSS H 4.10
4 ARG H 4 ARG QB 3.50	5 ASN QB 6 CYSS H 4.10
4 ARG H 4 ARG QG 5.00	6 CYSS H 6 CYSS QB 3.10
4 ARG HA 4 ARG QG 3.50	6 CYSS H 7 PROO QD 4.10
4 ARG HA 4 ARG QD 5.00	7 PROO HA 7 PROO QG 3.70
#4 ARG QG 4 ARG QD 2.50	
4 ARG QD 4 ARG HE 2.50	**Dihedral angle restraints (deg)**
4 ARG QB 4 ARG HE 5.00	5 ASN PHI -150.0 -90.0
4 ARG QG 4 ARG HE 5.00	
4 ARG QB 5 ASN H 5.00	
4 ARG QG 5 ASN H 5.00	
4 ARG H 5 ASN H 5.00	
4 ARG HA 6 CYSS H 5.00	
5 ASN H 5 ASN HA 3.50	
5 ASN H 5 ASN QB 3.50	

Supplementary Table 8: NMR parameters for Mo1061 in water

Residue	**NH**	**H^{α}**	**H^{β}**	**Others**	**$^{3}J_{NH\text{-}C^{\alpha}H}$ (Hz)**	**dδ/dT (ppb/K)**
Cys 1	-	4.23	3.50H^{β}3, 3.28 H^{β}2	-	-	-
Phe 2	9.12	4.82	3.07H^{β}2, 3.26H^{β}3	7.34 H^{δ}, 7.39 H^{ε}, 7.33 H^{ζ}	7.55	5.32
Ile 3	7.98	4.13	1.94	1.31 H^{γ}2, 1.08 H^{γ}1	-	6.43
Arg 4	8.14	4.09	1.82	1.64 H^{γ}, 3.22 H^{δ}, 7.28 H^{ε}	-	7.48
Asn 5	8.43	4.72	2.86	6.98 H^{δ}2a, 7.71 H^{δ}2b	8.23	5.90
Cys 6	8.29	4.83	3.25H^{β}3, 2.97H^{β}2	-	6.46	5.65
Pro 7	-	4.45	2.31	1.93 H^{γ}1, 2.04 H^{γ}2, 3.71 H^{δ}1 3.78$^{\delta}$2	-	-
Arg 8	8.74	4.29	1.89H^{β}2, 1.80H^{β}3	1.68 H^{γ}, 3.22 H^{δ}, 7.24H^{ε}	6.62	8.77
Gly 9	8.53	3.92	-	7.15 and 7.53 amidated protons	6.12	7.70

Supplementary Table 9: List of experimental restraints used in the structure calculation of Mo1061 in water.

NOE restraints (Å)	
1 CYSS SG 6 CYSS SG 2.10	5 ASN QB 5 ASN HD21 2.50
1 CYSS SG 6 CYSS CB 3.10	5 ASN QB 6 CYSS H 5.00
1 CYSS CB 6 CYSS SG 3.10	5 ASN HA 6 CYSS H 2.50
1 CYSS CB 6 CYSS CB 4.00	5 ASN H 6 CYSS H 5.00
2 PHE H 1 CYSS HA 2.50	6 CYSS H 7 PROO QD 5.00
2 PHE HA 2 PHE H 3.50	6 CYSS HA 7 PROO QD 2.50
2 PHE QB 2 PHE H 3.50	6 CYSS QB 7 PROO QD 5.00
2 PHE QB 2 PHE QD 3.50	7 PROO HA 7 PROO QG 5.00
2 PHE HA 2 PHE QD 2.50	7 PROO QD 7 PROO QB 5.00
2 PHE HA 2 PHE QE 5.00	7 PROO QD 7 PROO HA 5.00
2 PHE H 2 PHE QD 5.00	8 ARG H 7 PROO HA 2.50
3 ILE H 2 PHE QB 5.00	8 ARG H 9 GLY H 5.00
3 ILE QD1 2 PHE QD 5.00	8 ARG HA 9 GLY H 2.50
3 ILE QD1 2 PHE QE 5.00	8 ARG QG 9 GLY H 5.00
3 ILE H 2 PHE H 5.00	8 ARG QB 9 GLY H 5.00
3 ILE H 3 ILE HA 5.00	9 GLY H 9 GLY QA 2.50
3 ILE H 3 ILE HB 5.00	
3 ILE H 3 ILE QG2 5.00	3 ILE QG1 2 PHE QD 4.10
3 ILE HA 3 ILE QG1 5.00	3 ILE QG1 2 PHE QE 4.10
3 ILE HA 3 ILE QD1 5.00	3 ILE H 3 ILE QG1 4.10
3 ILE HB 3 ILE QG1 5.00	3 ILE H 3 ILE QD1 4.10
3 ILE HB 3 ILE QD1 3.50	3 ILE HA 3 ILE QD1 3.10
3 ILE HA 4 ARG H 3.50	3 ILE HA 4 ARG H 3.10
3 ILE HB 4 ARG H 5.00	3 ILE HB 4 ARG H 4.10
3 ILE QG1 4 ARG H 5.00	3 ILE QG1 4 ARG H 4.10
3 ILE QD1 4 ARG H 5.00	3 ILE QD1 4 ARG H 4.10
3 ILE QG1 5 ASN H 5.00	3 ILE QG1 5 ASN H 4.10
3 ILE QD1 5 ASN H 5.00	3 ILE QD1 5 ASN H 4.10
4 ARG H 4 ARG HA 3.50	4 ARG QB 5 ASN H 4.10
4 ARG H 4 ARG QB 3.50	4 ARG QG 5 ASN H 4.10
4 ARG H 4 ARG QG 5.00	4 ARG HA 6 CYSS H 4.10
4 ARG HA 4 ARG QG 3.50	5 ASN QB 6 CYSS H 4.10
4 ARG HA 4 ARG QD 5.00	6 CYSS H 6 CYSS QB 3.10
4 ARG QD 4 ARG HE 2.50	6 CYSS H 7 PROO QD 4.10
4 ARG QB 4 ARG HE 5.00	7 PROO HA 7 PROO QG 3.70
4 ARG QG 4 ARG HE 5.00	8 ARG QB 9 GLY H 4.10
4 ARG QB 5 ASN H 5.00	8 ARG H 9 GLY H 4.10
4 ARG QG 5 ASN H 5.00	
4 ARG H 5 ASN H 5.00	**Dihedral angle restraints (deg)**
4 ARG HA 6 CYSS H 5.00	5 ASN PHI -150.0 -90.0
5 ASN H 5 ASN HA 3.50	
5 ASN H 5 ASN QB 3.50	
5 ASN HA 5 ASN QB 2.50	
5 ASN HA 5 ASN HD21 5.00	
5 ASN HD21 5 ASN HD22 2.50	

Supplementary Table 10: NMR parameters for Tr-Mo976 in water.

Residue	**NH**	**H^{α}**	**H^{β}**	**Others**	**$^{3}J_{NH\text{-}C^{\alpha}H}$ (Hz)**	**dδ/dT (ppb/K)**
Cys 1	-	4.23	3.50 $H^{\beta}2$, 3.29 $H^{\beta}3$	-	-	-
Phe 2	9.12	4.83	3.27 $H^{\beta}2$, 3.07 $H^{\beta}3$	7.33 H^{δ}, 7.39 H^{ε}	7.46	6.00
Ile 3	7.97	4.14	1.94	1.31 $H^{\gamma}1$, 1.31 $H^{\gamma}2$, 1.08 H^{δ}	6.42	9.20
Arg 4	8.15	4.09	1.82	1.64 H^{γ}, 3.22 H^{δ}, 7.28 H^{ε}	3.76	7.93
Asn 5	8.44	4.72	2.86	7.71 $H^{\delta}2a$, 6.98 $H^{\delta}2b$	8.31	6.27
Cys 6	8.28	4.83	3.25 $H^{\beta}2$, 2.97 $H^{\beta}3$	-	6.44	6.00
Pro 7	-	4.45	1.94 $H^{\beta}2$, 2.31 $H^{\beta}3$	2.05 H^{γ}, 3.79 $H^{\delta}1$, 3.72 $H^{\delta}2$	-	-
Lys 8	8.64	4.26	1.86 $H^{\beta}2$, 1.78 $H^{\beta}3$	1.69 H^{γ}, 1.71 H^{δ}, 3.22 H^{ε}, 7.23 H^{ζ}	6.90	9.20

Supplementary Table 11: List of experimental restraints used in the structure calculation of Tr-Mo976 in water.

NOE restraints (Å)	
1 CYSS SG 6 CYSS SG 2.10	5 ASN H 5 ASN HA 3.50
1 CYSS SG 6 CYSS CB 3.10	5 ASN H 5 ASN QB 3.50
1 CYSS CB 6 CYSS SG 3.10	5 ASN HA 5 ASN QB 2.50
1 CYSS CB 6 CYSS CB 4.00	5 ASN HA 5 ASN HD21 5.00
2 PHE H 1 CYSS HA 2.50	5 ASN HD21 5 ASN HD22 2.50
2 PHE HA 2 PHE H 5.00	5 ASN QB 5 ASN HD21 2.50
2 PHE QB 2 PHE H 5.00	5 ASN QB 6 CYSS H 5.00
2 PHE QB 2 PHE QD 3.50	5 ASN HA 6 CYSS H 2.50
2 PHE HA 2 PHE QD 2.50	5 ASN H 6 CYSS H 5.00
2 PHE HA 2 PHE QE 5.00	6 CYSS H 7 PROO QD 5.00
2 PHE H 2 PHE QD 5.00	6 CYSS HA 7 PROO QD 2.50
3 ILE H 2 PHE HA 5.00	6 CYSS QB 7 PROO QD 5.00
3 ILE H 2 PHE QB 5.00	7 PROO HA 7 PROO QG 5.00
3 ILE QD1 2 PHE QD 5.00	7 PROO QD 7 PROO QB 5.00
3 ILE QD1 2 PHE QE 5.00	7 PROO QD 7 PROO HA 5.00
3 ILE H 2 PHE H 5.00	7 PROO HA 8 LYS H 2.50
3 ILE H 3 ILE HA 5.00	7 PROO QG 8 LYS H 5.00
3 ILE H 3 ILE HB 5.00	
3 ILE H 3 ILE QG2 5.00	2 PHE HA 1 CYSS QB 4.10
3 ILE HA 3 ILE QG1 5.00	3 ILE QD1 2 PHE QD 4.10
3 ILE HA 3 ILE QD1 5.00	3 ILE QD1 2 PHE QE 4.10
3 ILE HB 3 ILE QG1 5.00	3 ILE QG1 2 PHE QD 4.10
3 ILE HB 3 ILE QD1 3.50	3 ILE QG1 2 PHE QE 4.10
3 ILE HA 4 ARG H 3.50	3 ILE H 3 ILE QG1 4.10
3 ILE HB 4 ARG H 5.00	3 ILE H 3 ILE QD1 4.10
3 ILE QG1 4 ARG H 5.00	3 ILE HA 3 ILE QD1 3.10
3 ILE QD1 4 ARG H 5.00	3 ILE HA 4 ARG H 3.10
3 ILE QG1 5 ASN H 5.00	3 ILE HB 4 ARG H 4.10
3 ILE QD1 5 ASN H 5.00	3 ILE QG1 4 ARG H 4.10
4 ARG H 4 ARG HA 3.50	3 ILE QD1 4 ARG H 4.10
4 ARG H 4 ARG QB 3.50	3 ILE QG1 5 ASN H 4.10
4 ARG H 4 ARG QG 5.00	3 ILE QD1 5 ASN H 4.10
4 ARG HA 4 ARG QG 3.50	4 ARG H 4 ARG QG 4.10
4 ARG HA 4 ARG QD 5.00	4 ARG QB 5 ASN H 4.10
4 ARG QD 4 ARG HE 2.50	4 ARG QG 5 ASN H 4.10
4 ARG QB 4 ARG HE 5.00	4 ARG HA 6 CYSS H 4.10
4 ARG QG 4 ARG HE 5.00	5 ASN QB 6 CYSS H 4.10
4 ARG HA 5 ASN H 2.50	6 CYSS H 7 PROO QD 4.10
4 ARG QB 5 ASN H 5.00	7 PROO HA 7 PROO QG 3.70
4 ARG QG 5 ASN H 5.00	
4 ARG H 5 ASN H 5.00	**Dihedral angle restraints (deg)**
4 ARG HA 6 CYSS H 5.00	5 ASN PHI -150.0 -90.0

Supplementary Table 12: NMR parameters for Tr-Mo977 in water

Residue	**NH**	**H^{α}**	**H^{β}**	**Others**	**$^{3}J_{NH\text{-}C^{\alpha}H}$ (Hz)**	**dδ/dT (ppb/K)**
Cys 1	-	4.24	3.29 $H^{\beta}2$, 3.50 $H^{\beta}3$	-	-	-
Phe 2	9.10	4.82	3.07 $H^{\beta}2$, 3.27 $H^{\beta}3$	7.33 H^{δ}, 7.39 H^{ε}, 7.32 H^{ζ}	7.55	5.15
Ile 3	7.91	4.16	1.94	1.07 $H^{\gamma}1$, 1.31 $H^{\gamma}2$, 0.89 H^{δ}	7.14	6.00
Arg 4	8.2	4.08	1.83	1.63 $H^{\gamma}1$, 3.22 H^{δ}, 7.28 H^{ε}, 6.53 $H^{\eta}a$, 6.88 $H^{\eta}b$	3.99	6.95
Asn 5	8.46	4.70	2.85	6.96 $H^{\delta}2a$, 7.70 $H^{\delta}2b$	7.98	5.98
Cys 6	8.34	4.85	3.01$H^{\beta}2$, 3.29 $H^{\beta}3$	-	6.76	5.33
Pro 7	-	4.45	2.30	1.96 $H^{\gamma}1$, 2.05 $H^{\gamma}2$, 3.72 $H^{\delta}1$, 3.77 $H^{\delta}2$	-	-
Glu 8	8.61	4.29	1.99 $H^{\beta}2$, 2.12 $H^{\beta}3$	2.49 H^{γ}	6.88	8.20

Supplementary Table 13: List of experimental restraints used in the structure calculation of Tr-Mo977 in water.

NOE restraints (Å)	
1 CYSS SG 6 CYSS SG 2.10	5 ASN HA 5 ASN HD21 5.00
1 CYSS SG 6 CYSS CB 3.10	5 ASN HD21 5 ASN HD22 2.50
1 CYSS CB 6 CYSS SG 3.10	5 ASN QB 5 ASN HD21 2.50
1 CYSS CB 6 CYSS CB 4.00	5 ASN QB 6 CYSS H 5.00
2 PHE H 1 CYSS HA 2.50	5 ASN HA 6 CYSS H 2.50
2 PHE HA 2 PHE H 3.50	5 ASN H 6 CYSS H 5.00
2 PHE QB 2 PHE H 3.50	6 CYSS H 6 CYSS HA 3.50
2 PHE QB 2 PHE QD 3.50	6 CYSS H 6 CYSS QB 5.00
2 PHE HA 2 PHE QD 2.50	6 CYSS H 7 PROO QD 5.00
2 PHE HA 2 PHE QE 5.00	6 CYSS HA 7 PROO QD 2.50
2 PHE H 2 PHE QD 5.00	6 CYSS QB 7 PROO QD 5.00
3 ILE H 2 PHE QB 5.00	7 PROO HA 7 PROO QG 5.00
3 ILE QD1 2 PHE QD 5.00	7 PROO QD 7 PROO QB 5.00
3 ILE QD1 2 PHE QE 5.00	7 PROO QD 7 PROO HA 5.00
3 ILE H 2 PHE H 5.00	8 GLU H 7 PROO HA 2.50
3 ILE H 3 ILE HA 5.00	8 GLU H 7 PROO QB 5.00
3 ILE H 3 ILE HB 5.00	
3 ILE H 3 ILE QG2 5.00	3 ILE QG1 2 PHE QD 4.10
3 ILE HA 3 ILE QG1 5.00	3 ILE QG1 2 PHE QE 4.10
3 ILE HA 3 ILE QD1 5.00	3 ILE H 3 ILE QG1 4.10
3 ILE HB 3 ILE QG1 5.00	3 ILE H 3 ILE QD1 4.10
3 ILE HB 3 ILE QD1 3.50	3 ILE HA 3 ILE QD1 3.10
3 ILE HA 4 ARG H 3.50	3 ILE HA 4 ARG H 3.10
3 ILE HB 4 ARG H 5.00	3 ILE HB 4 ARG H 4.10
3 ILE QG1 4 ARG H 5.00	3 ILE QG1 4 ARG H 4.10
3 ILE QD1 4 ARG H 5.00	3 ILE QD1 4 ARG H 4.10
3 ILE QG1 5 ASN H 5.00	3 ILE QG1 5 ASN H 4.10
3 ILE QD1 5 ASN H 5.00	3 ILE QD1 5 ASN H 4.10
4 ARG H 4 ARG HA 3.50	4 ARG QB 5 ASN H 4.10
4 ARG H 4 ARG QB 3.50	4 ARG QG 5 ASN H 4.10
4 ARG H 4 ARG QG 5.00	4 ARG HA 6 CYSS H 4.10
4 ARG HA 4 ARG QG 3.50	5 ASN QB 6 CYSS H 4.10
4 ARG HA 4 ARG QD 5.00	5 ASN HA 6 CYSS H 2.10
4 ARG QD 4 ARG HE 2.50	6 CYSS H 6 CYSS HA 3.10
4 ARG QB 4 ARG HE 5.00	6 CYSS H 6 CYSS QB 3.10
4 ARG QG 4 ARG HE 5.00	6 CYSS H 7 PROO QD 4.10
4 ARG QB 5 ASN H 5.00	7 PROO QB 7 PROO HA 2.10
4 ARG QG 5 ASN H 5.00	7 PROO HA 7 PROO QG 3.70
4 ARG H 5 ASN H 5.00	
4 ARG HA 6 CYSS H 5.00	**Dihedral angle restraints (deg)**
5 ASN H 5 ASN HA 3.50	5 ASN PHI -150.0 -90.0
5 ASN H 5 ASN QB 3.50	
5 ASN HA 5 ASN QB 2.50	

Supplementary Table 14: Comparative dihedral angles of oxytocin, vasopressin, and conopressins

Peptide	**Tyr/Phe** (φ, ψ)	**Ile/Phe** (φ, ψ)	**Gln/Arg** (φ, ψ)	**Asn** (φ, ψ)	**Cys** (φ, ψ)
CYIQNCPLG Oxytocin	-79, 123	-38, -44	-61, -37	-125, 126	-63, -137
CYFQNCPRG Vasopressin	-71, 129	-26, -61	-77, 1	-105, -27	-61, ---
CFI^{L}RNCPKG Mo1033	-51.3, -66.4	-62.8, -16.2	65.7, 59.8	-150.4, 61.4	-135.5, 162.0
CFI^{D}RNCPKG ^{D}R4-Mo1033	-80.0, 132.8	-68.8, 143.4	136.2, -93.0	-127.9, 64.6	-118.7, 71.6
CFIRNCPEG Mo1034	-51.4, -66.9	-63, -16.1	65.4, 55.9	-150.3, 62.4	-135.8, 162
CFIRNCPRG Mo1061	-51.4, -66.9	-62.9, -16.2	65.5, 55.9	-150.3, 62.3	-135.7, 162
CFIRNCPK Tr-Mo976	-51.2, -66.4	-62.7, -16.3	65.8, 59.8	-150.4, 61.4	-135.5, 162
CFIRNCPE Tr-Mo977	-51.4, -67.5	-62.8, -16.3	65.1, 54.3	-150.3, 61.7	-128.6, 162

Supplementary Table 15: Docking score of receptor-ligand complex for OT and V2 receptors with oxytocin, vasopressin and conopressin analogues.

Title	**Docking score (kcal/mol)**
Oxytocin receptor + Oxytocin	-10.72
Oxytocin receptor + Mo1033	-9.39
Oxytocin receptor + ^{D}R4-Mo1033	-10.75
Oxytocin receptor + Mo1034	-10.52
Oxytocin receptor + Mo1061	-10.98
Oxytocin receptor + Tr-Mo976	-9.07
Oxytocin receptor + Tr-Mo977	-9.14
V2 receptor + Vasopressin	-14.32
V2 receptor + Mo1033	-10.47
V2 receptor + ^{D}R4-Mo1033	-9.97
V2 receptor + Mo1034	-9.52
V2 receptor + Mo1061	-10.68
V2 receptor + Tr-Mo976	-9.14
V2 receptor + Tr-Mo977	-9.49

Supplementary Table 16: The IC_{50} values for conopressins

Compound	**IC_{50} µM**
Mo1033	37.01 ±3.00
^{D}R4-Mo1033	32.8 ±3.29
Mo1034	12.8 ±0.18
Mo1061	43.8 ±1.07
Tr-Mo976	22.9 ±3.06
Tr-Mo977	21.0 ±2.12