Supporting information for

Molecular grafting onto a stable framework yields novel cyclic peptides for the treatment of multiple sclerosis

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Supplementary Methods

Antibodies and Recombinant Proteins - The mouse anti-MOG mAb (clone 8-18C5) was purified from hybridoma culture supernatants on Protein G-Sepharose 4 Fast Flow column (GE Healthcare) according to the manufacturer's instructions. Antiserum to $MOG_{35–55}$ peptide[1–2] was raised in rabbits by procedures similar to those described previously.[3–4] The extracellular domain of mouse MOG (amino acid residues 1–117 of the mature protein) (rMOG) was produced in the *E. coli* strain M15pREP4 using the pQE9 expression vector (Qiagen, Australia) to incorporate an amino-terminal histidine tag as per manufacturer's instructions. A clarified bacterial lysate containing rMOG was loaded onto a Ni-NTA Superflow (Qiagen, Australia) column under denaturing conditions (6 M Guanidine-HCl, 100 mM NaH_2PO_4, 10 mM Tris pH 8.0) as per the manufacturer's instructions using a BioLogic LP Chromatography System (Bio-Rad Laboratories, Australia). Bound protein was washed sequentially with Buffer A (8 M Urea 100 mM NaH_2PO_4, 10 mM Tris pH 8.0), Buffer A (at pH 6.3), 10 mM Tris pH 8.0/ 60% propan-2-ol (to remove endotoxin) and again with Buffer A. Refolding of the bound protein was carried out by applying a linear gradient of Buffer A containing 14 mM 2-mercaptoethanol (100%–0%) vs. Buffer B (100 mM NaH_2PO_4, 10 mM Tris pH 8.0, 2 mM reduced glutathione, 0.2 mM oxidised glutathione) (0%–100%). This was followed by a second linear gradient of Buffer B (100%–0%) vs. Buffer C (100 mM NaH_2PO_4, 10 mM Tris pH 8.0) (0%–100%). The bound protein was eluted using Buffer C containing 300 mM imidazole, then extensively dialysed against 50 mM NaCl/ 10 mM Tris pH 8.0. Protein concentration and purity were estimated using a Micro BCA assay (Bio-Rad Laboratories, Australia) and SDS-PAGE, respectively. The protein produced was verified as rMOG by Western blot analysis using antibodies specific for native MOG. Endotoxin levels

were determined using a *Limulus* Amebocyte Lysate assay (Associates of Cape Cod, Falmouth, MA).

Homology modelling - Homology models of the grafted cyclotides were generated using MODELLER 9v1.[5] The sequences of the grafted cyclotides were aligned to the sequence of kalata B1 (PDB ID: 1NB1) and to the sequence of $MOG_{35–55}$ (PDB ID: 1PY9). A series of models were generated using the standard modelling procedure of MODELLER 9v1. The model with the lowest MODELLER target energy function was selected for further analysis.

Supplementary References

1. Ichikawa, M.; Johns, T. G.; Adelmann, M.; Bernard, C. C., Antibody response in Lewis rats injected with myelin oligodendrocyte glycoprotein derived peptides. *Int. Immunol.* **1996,** *8*, 1667–74.

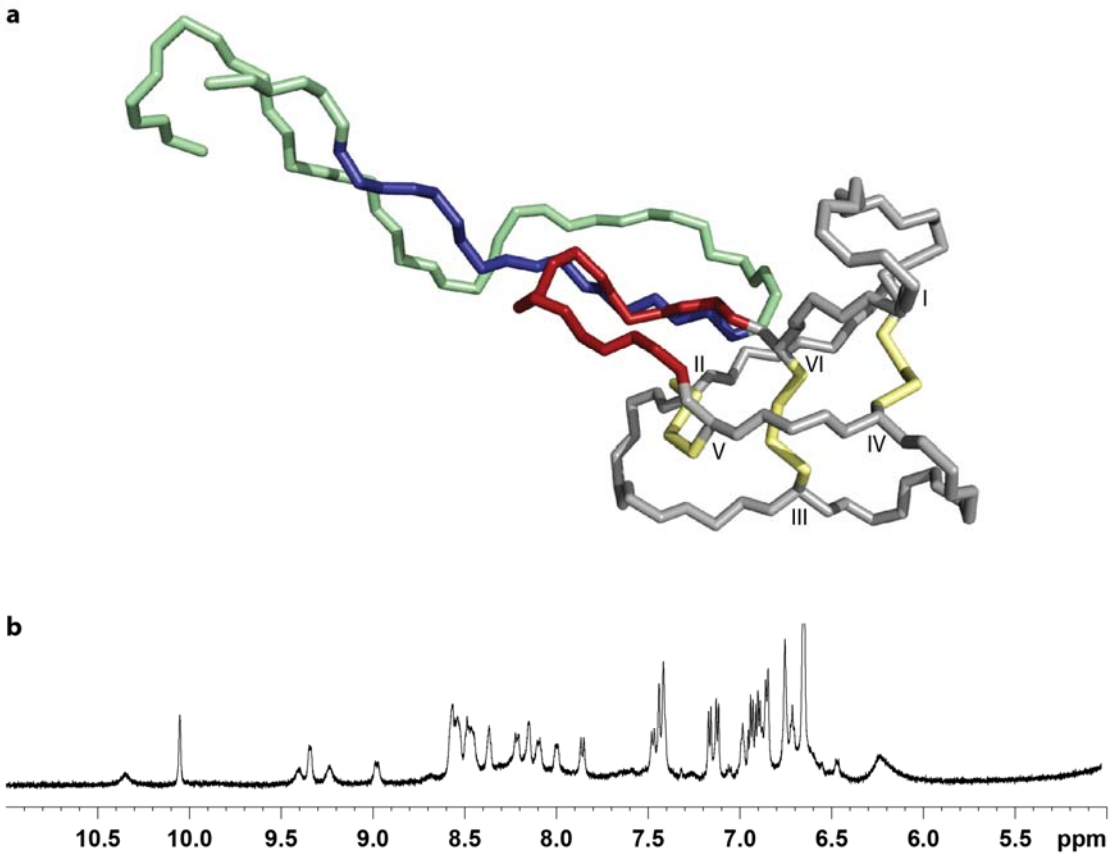
2. Ichikawa, M.; Johns, T. G.; Liu, J.; Bernard, C. C., Analysis of the fine B cell specificity during the chronic/relapsing course of a multiple sclerosis-like disease in Lewis rats injected with the encephalitogenic myelin oligodendrocyte glycoprotein peptide 35-55. *J. Immunol.* **1996,** *157*, 919–26.

3. Bernard, C. C.; Townsend, E.; Randell, V. B.; Williamson, H. G., Do antibodies to myelin basic protein isolated from multiple sclerosis cross-react with measles and other common virus antigens? *Clin. Exp. Immunol.* **1983,** *52*, 98–106.

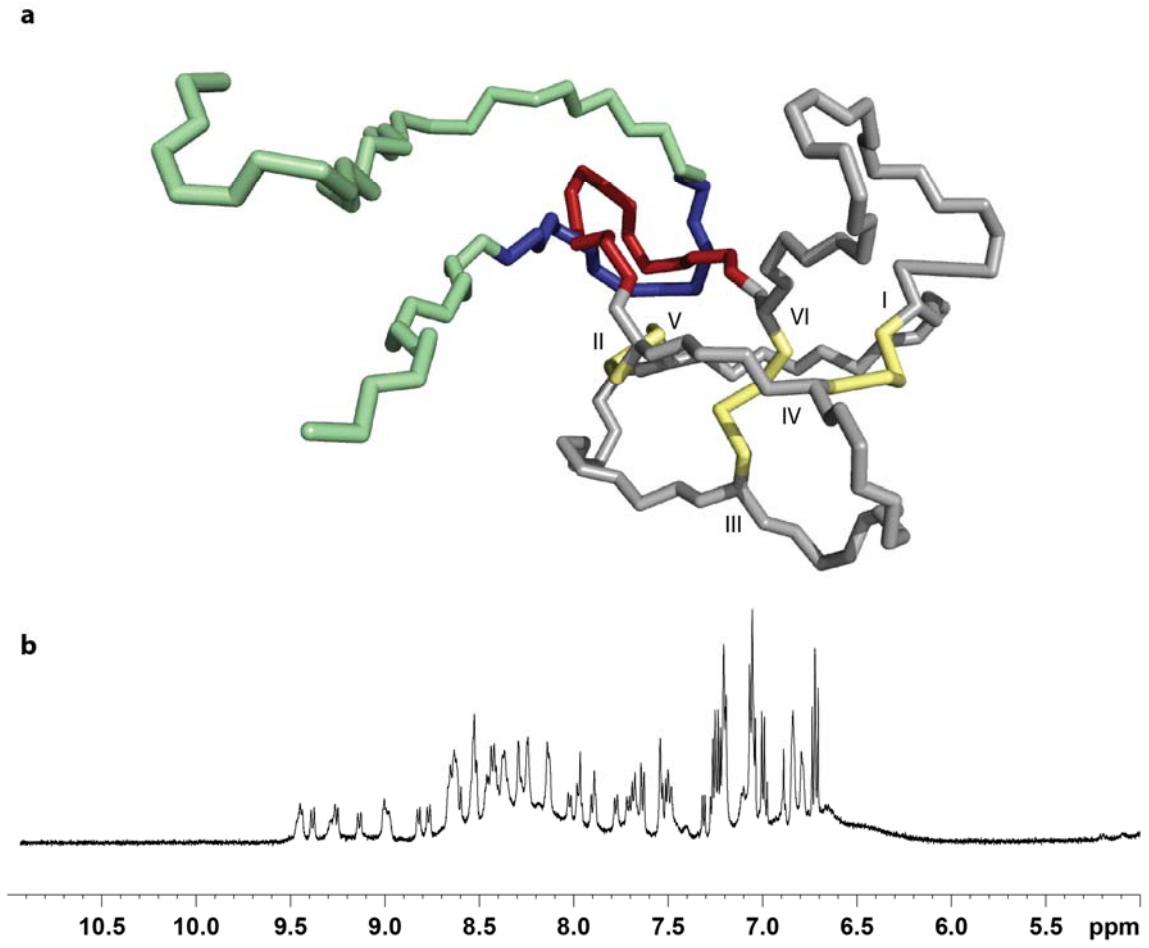
4. Pedersen, J. S.; Walker, M.; Toh, B. H.; De Aizpurua, H. J.; Lolait, S. J.; Bernard, C. C., Flow microfluorometry detects IgM autoantibody to oligodendrocytes in multiple sclerosis. *J. Neuroimmunol.* **1983,** *5*, 251–9.

5. Eswar, N.; Webb, B.; Marti-Renom, M. A.; Madhusudhan, M. S.; Eramian, D.; Shen, M. Y.; Pieper, U.; Sali, A., Comparative protein structure modeling using MODELLER. *Curr. Protoc. Protein Sci.* **2007,** *Chapter 2*, Unit 2 9.

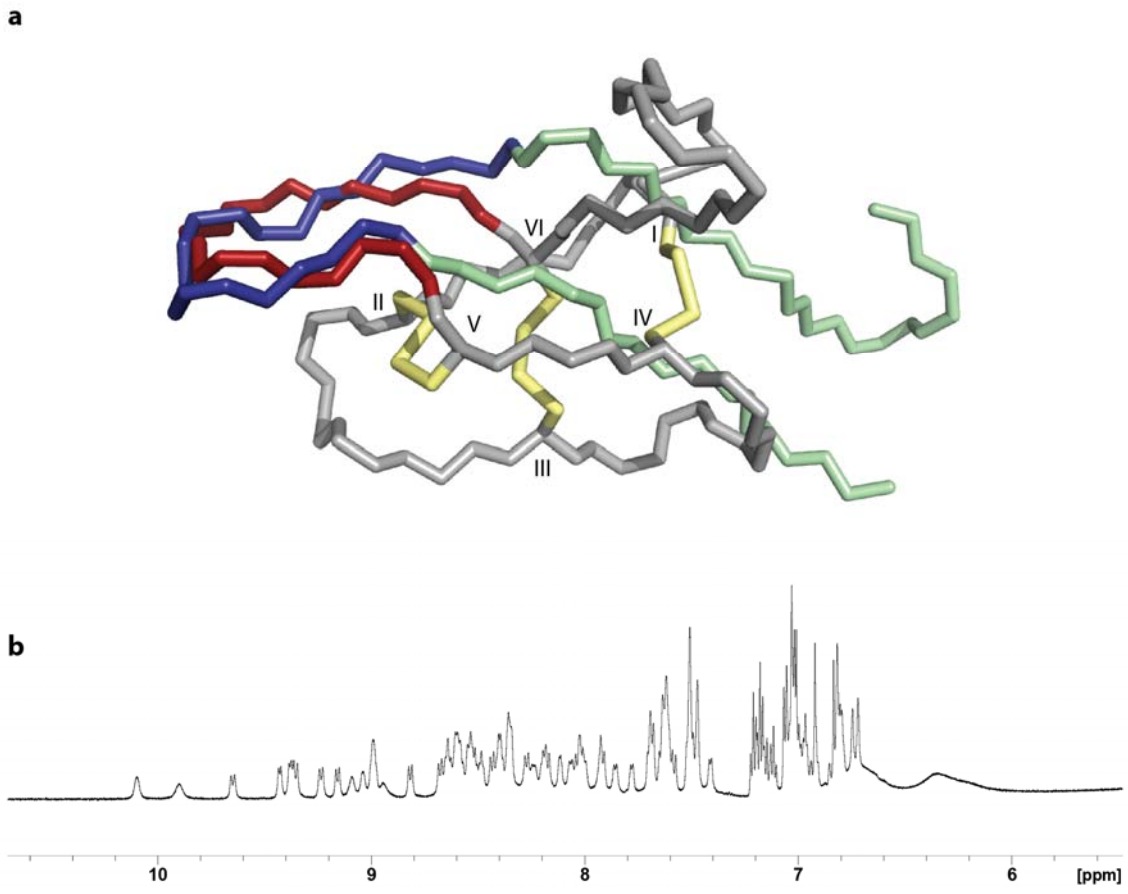
Supplementary Figures



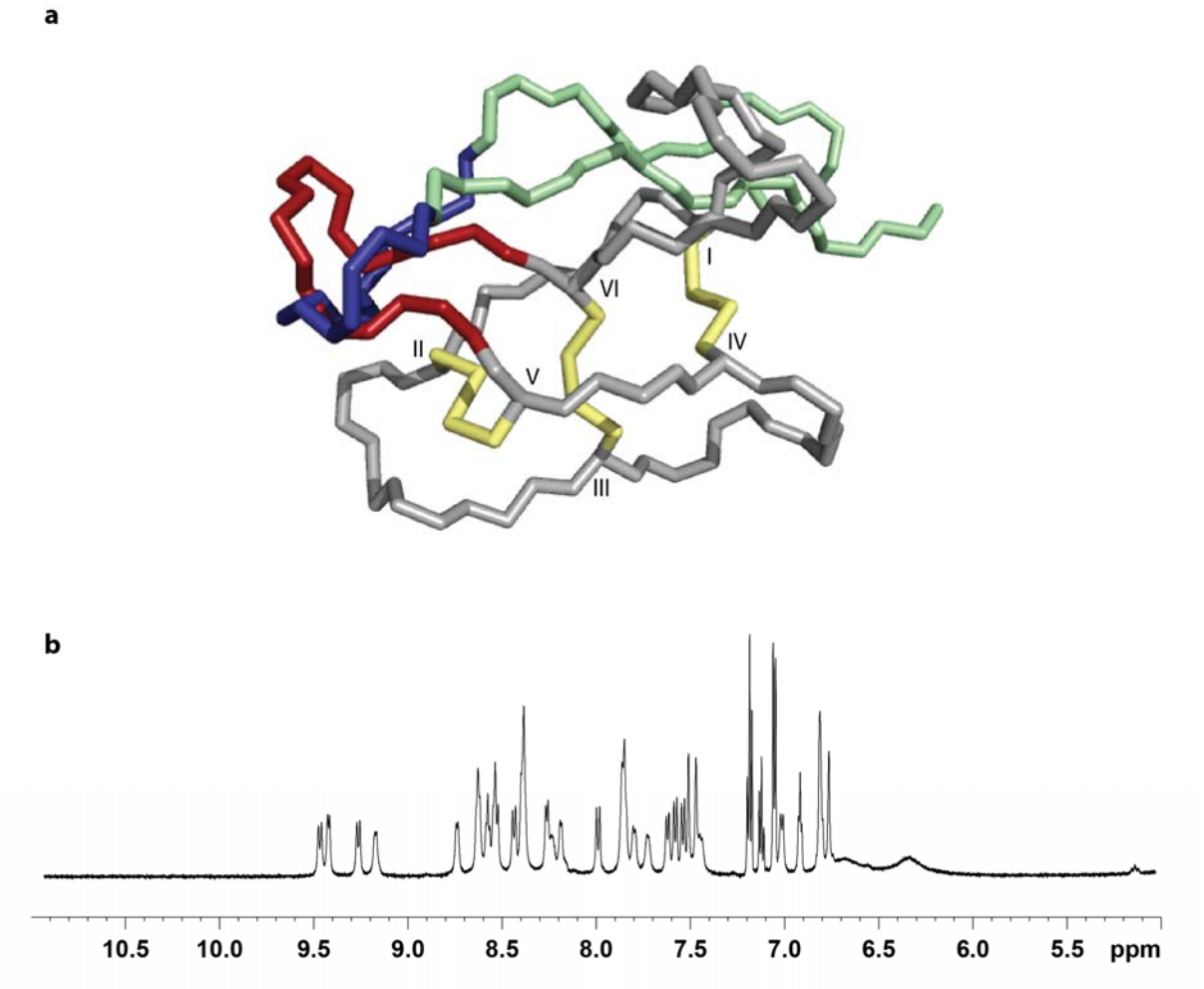
Supplementary Figure 1: Structural characterisation of MOG1. Panel (a) shows a homology model of the grafted cyclotide (gray) aligned to the $MOG_{35–55}$ epitope (green). The alignment was generated by comparing the structure of the region of $MOG_{35–55}$ (blue) to its predicted conformation (red) when grafted onto kalata B1. Panel (b) shows a region of the 1D ^{1}H NMR spectrum.



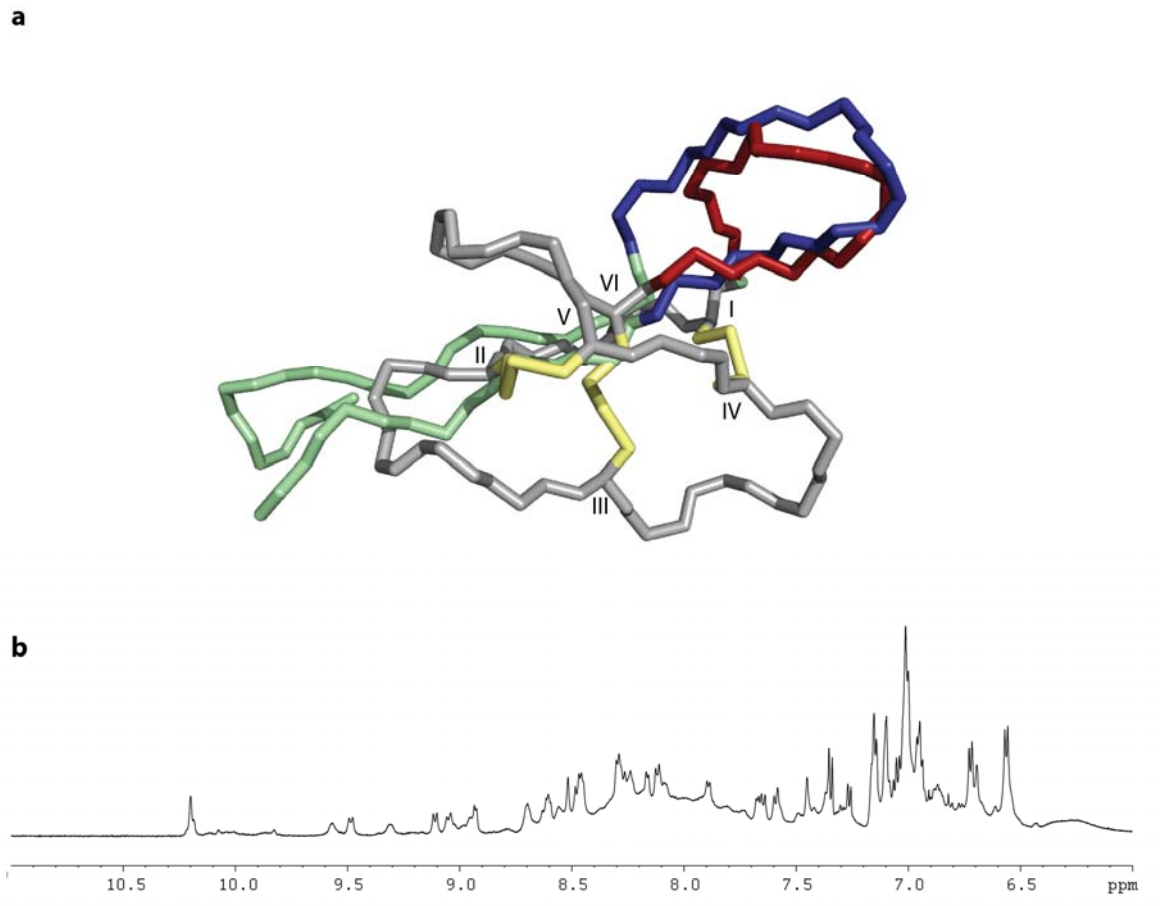
Supplementary Figure 2: Structural characterisation of MOG2. Panel (a) shows a homology model of the grafted cyclotide (gray) aligned to the $MOG_{35–55}$ epitope (green). The alignment was generated by comparing the structure of the region of $MOG_{35–55}$ (blue) to its predicted conformation (red) when grafted onto kalata B1. Panel (b) shows a region of the 1D ^{1}H NMR spectrum.



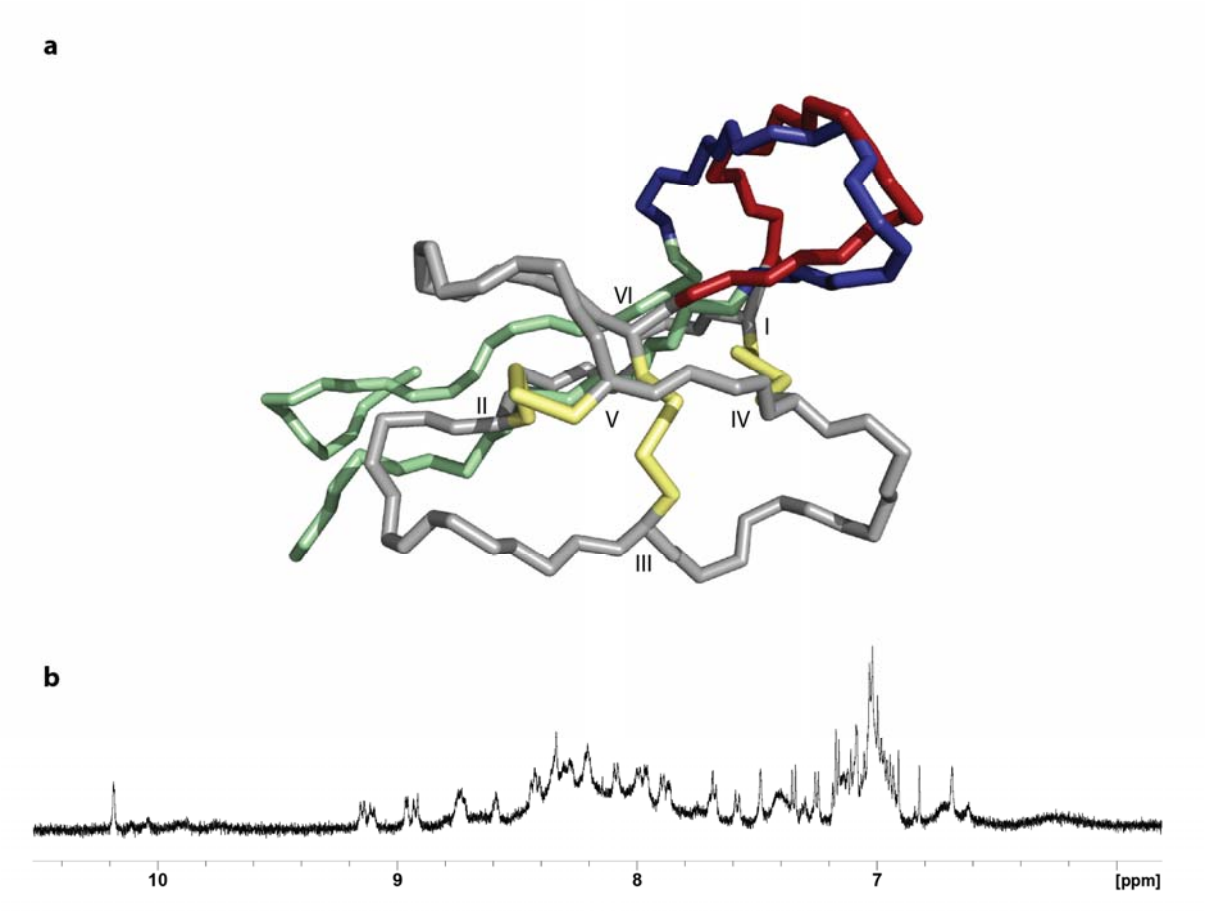
Supplementary Figure 3: Structural characterisation of MOG3. Panel (a) shows a homology model of the grafted cyclotide (gray) aligned to the $MOG_{35–55}$ epitope (green). The alignment was generated by comparing the structure of the region of $MOG_{35–55}$ (blue) to its predicted conformation (red) when grafted onto kalata B1. Panel (b) shows a region of the 1D ^{1}H NMR spectrum.



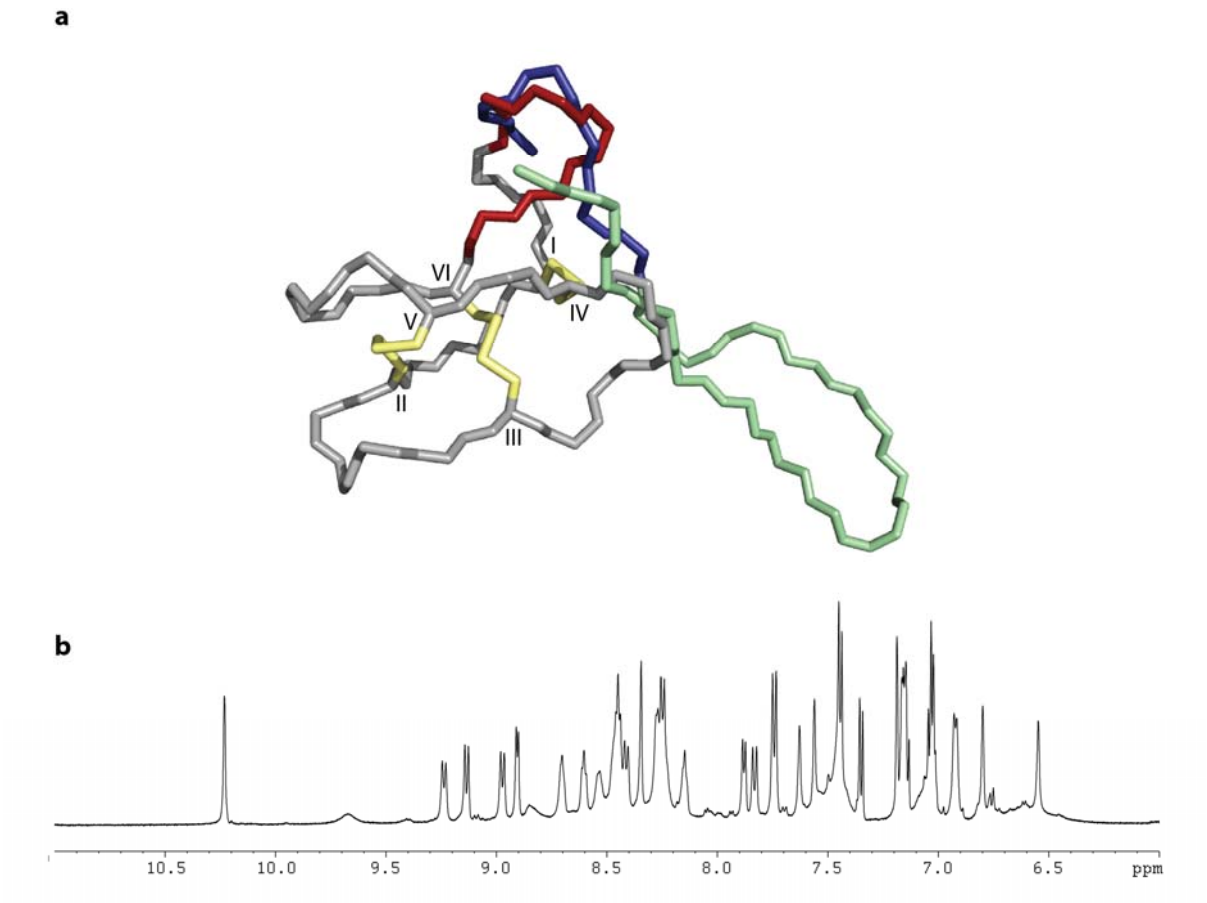
Supplementary Figure 4: Structural characterisation of MOG4. Panel (a) shows a homology model of the grafted cyclotide (gray) aligned to the $MOG_{35–55}$ epitope (green). The alignment was generated by comparing the structure of the region of $MOG_{35–55}$ (blue) to its predicted conformation (red) when grafted onto kalata B1. Panel (b) shows a region of the 1D ^{1}H NMR spectrum.



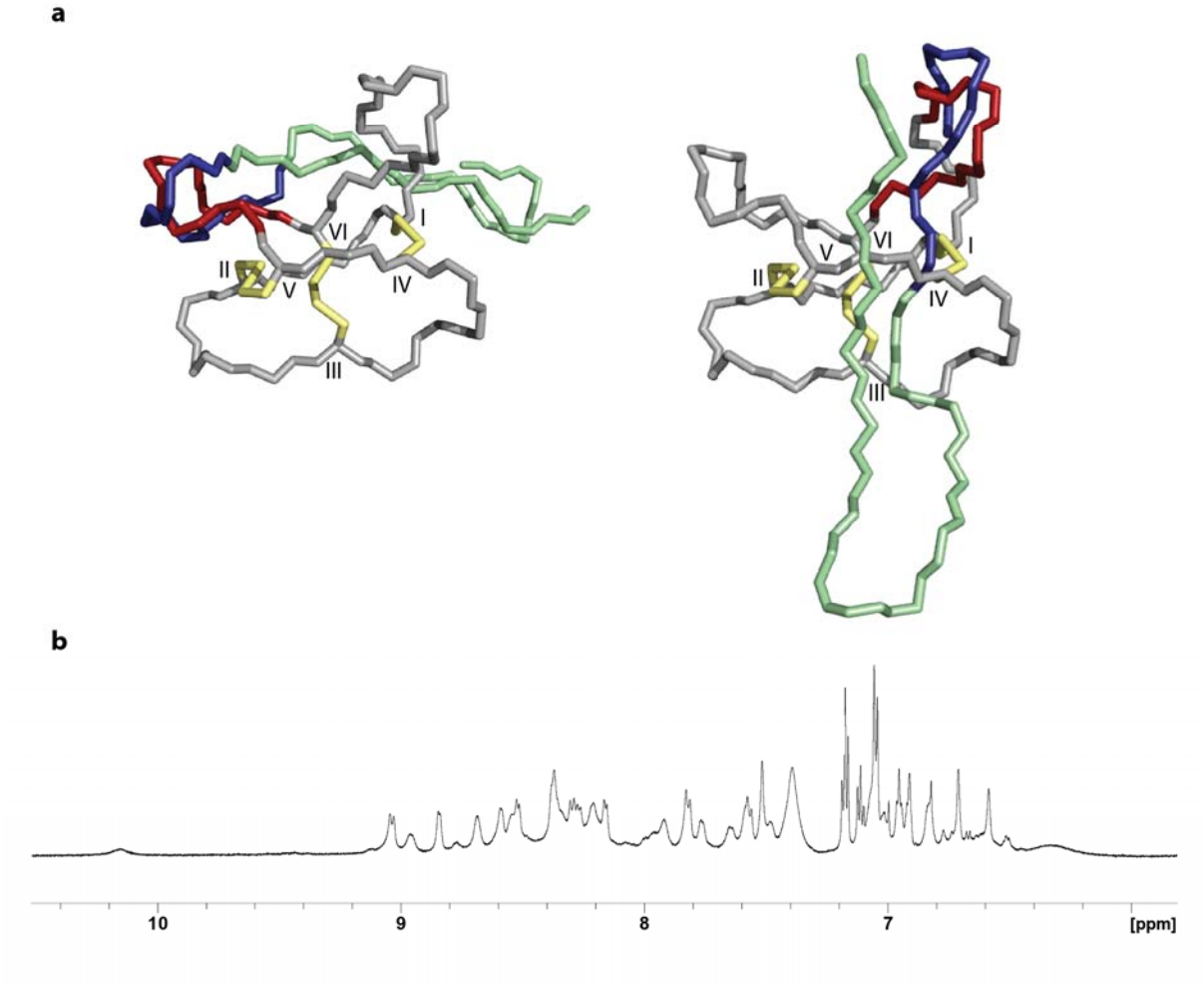
Supplementary Figure 5: Structural characterisation of MOG9. Panel (a) shows a homology model of the grafted cyclotide (gray) aligned to the $MOG_{35–55}$ epitope (green). The alignment was generated by comparing the structure of the region of $MOG_{35–55}$ (blue) to its predicted conformation (red) when grafted onto kalata B1. Panel (b) shows a region of the 1D ^{1}H NMR spectrum.



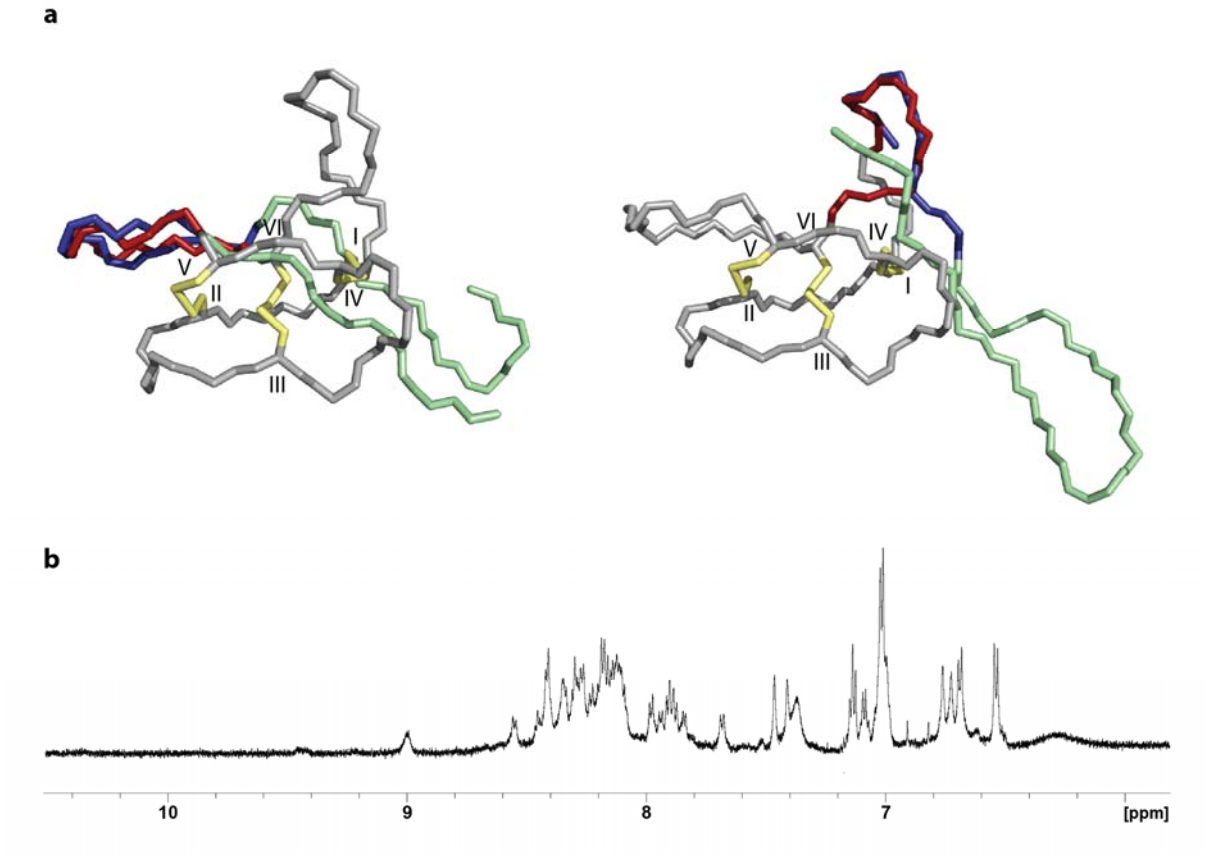
Supplementary Figure 6: Structural characterisation of MOG10. Panel (a) shows a homology model of the grafted cyclotide (gray) aligned to the $MOG_{35–55}$ epitope (green). The alignment was generated by comparing the structure of the region of $MOG_{35–55}$ (blue) to its predicted conformation (red) when grafted onto kalata B1. Panel (b) shows a region of the 1D ^{1}H NMR spectrum.



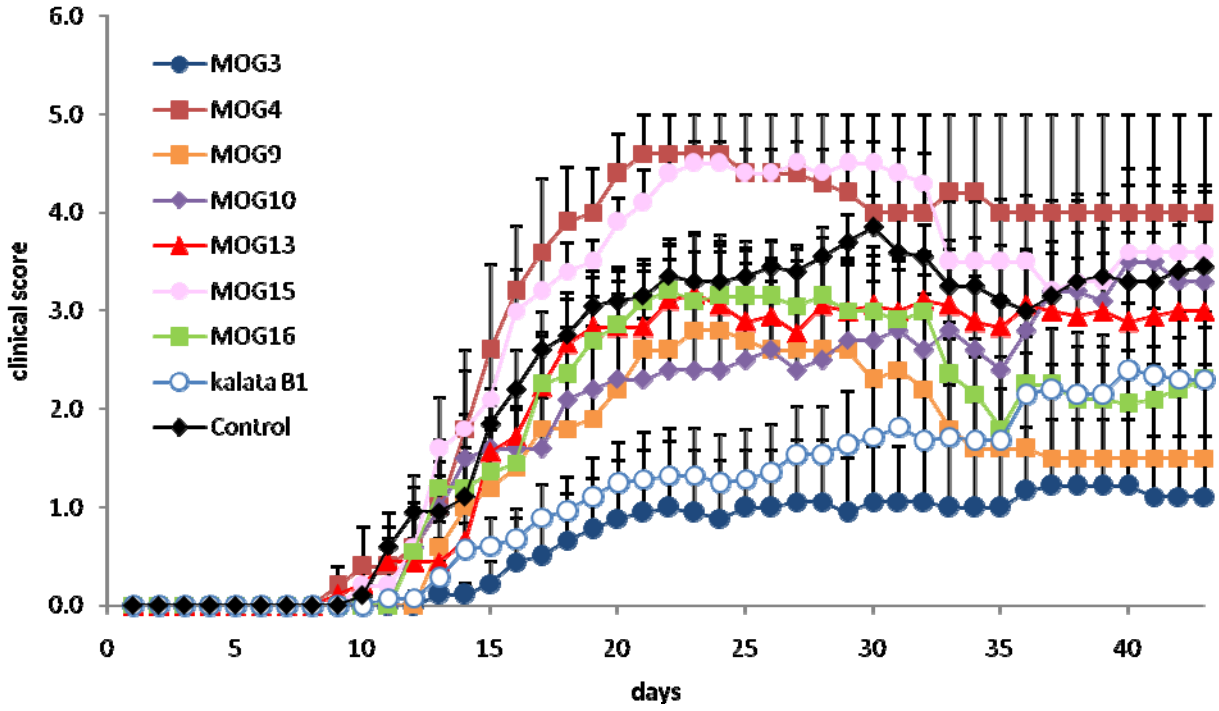
Supplementary Figure 7: Structural characterisation of MOG13. Panel (a) shows a homology model of the grafted cyclotide (gray) aligned to the $MOG_{35–55}$ epitope (green). The alignment was generated by comparing the structure of the region of $MOG_{35–55}$ (blue) to its predicted conformation (red) when grafted onto kalata B1. Panel (b) shows a region of the 1D ^{1}H NMR spectrum.



Supplementary Figure 8: Structural characterisation of MOG15. Panel (a) shows a homology model of the grafted cyclotide (gray) aligned to the $MOG_{35–55}$ epitope (green). The alignment was generated by comparing the structure of the region of $MOG_{35–55}$ (blue) to its predicted conformation (red) when grafted onto kalata B1. Panel (b) shows a region of the 1D ^{1}H NMR spectrum.



Supplementary Figure 9: Structural characterisation of MOG16. Panel (a) shows a homology model of the grafted cyclotide (gray) aligned to the $MOG_{35–55}$ epitope (green). The alignment was generated by comparing the structure of the region of $MOG_{35–55}$ (blue) to its predicted conformation (red) when grafted onto kalata B1. Panel (b) shows a region of the 1D ^{1}H NMR spectrum.



Supplementary Figure 10: Clinical score of EAE mice after vaccination with MOG-grafted peptides. The key shown in the upper left hand side of the figure describes the peptides that were tested. The graph shows the mean clinical score and the standard error of the mean.