The mechanism of patellamide macrocyclization revealed by study of the *Prochloron sp* PatG macrocyclase domain

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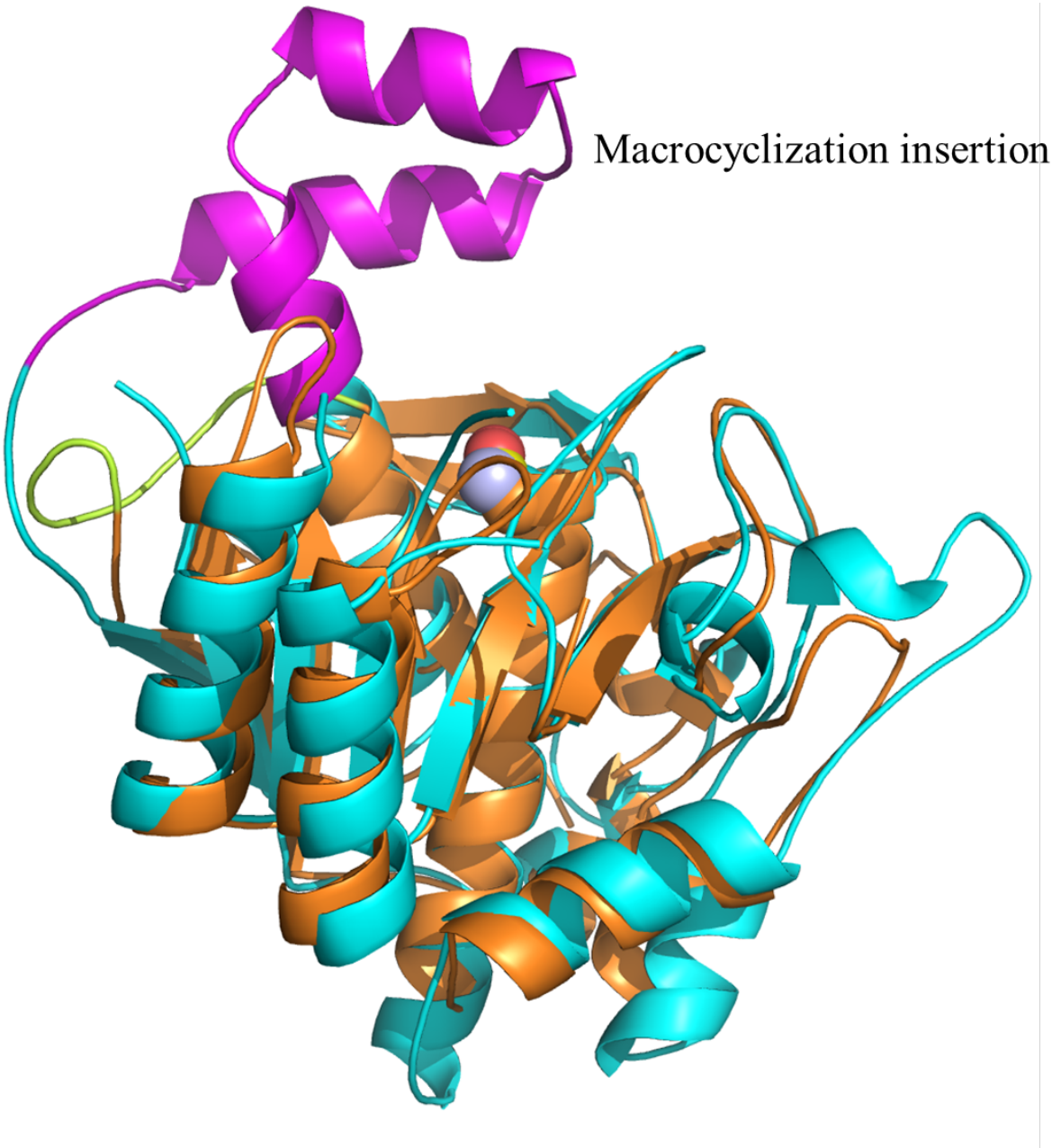
Figure S1 Superposition of PatGmac with AkP



(a) PatGmac (cyan) with macrocyclization insertion (magenta) superposed onto AkP (orange) (PDB 1DBI). The loop of AkP that contains the macrocyclization insertion in PatGmac is shown in yellow-green, active site serines are shown as spheres.

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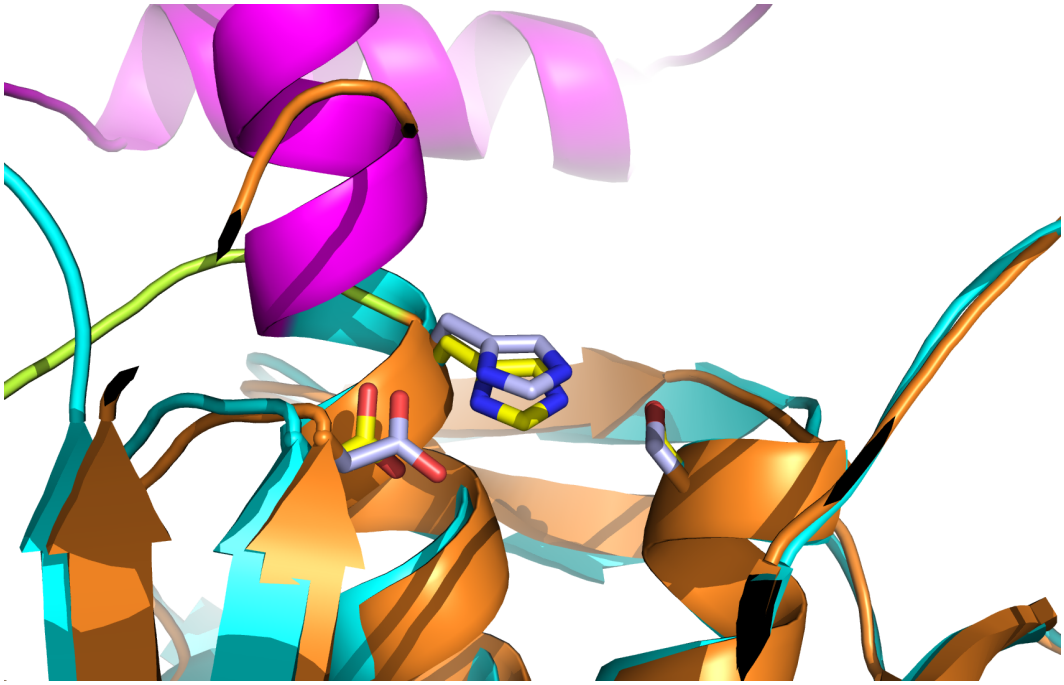
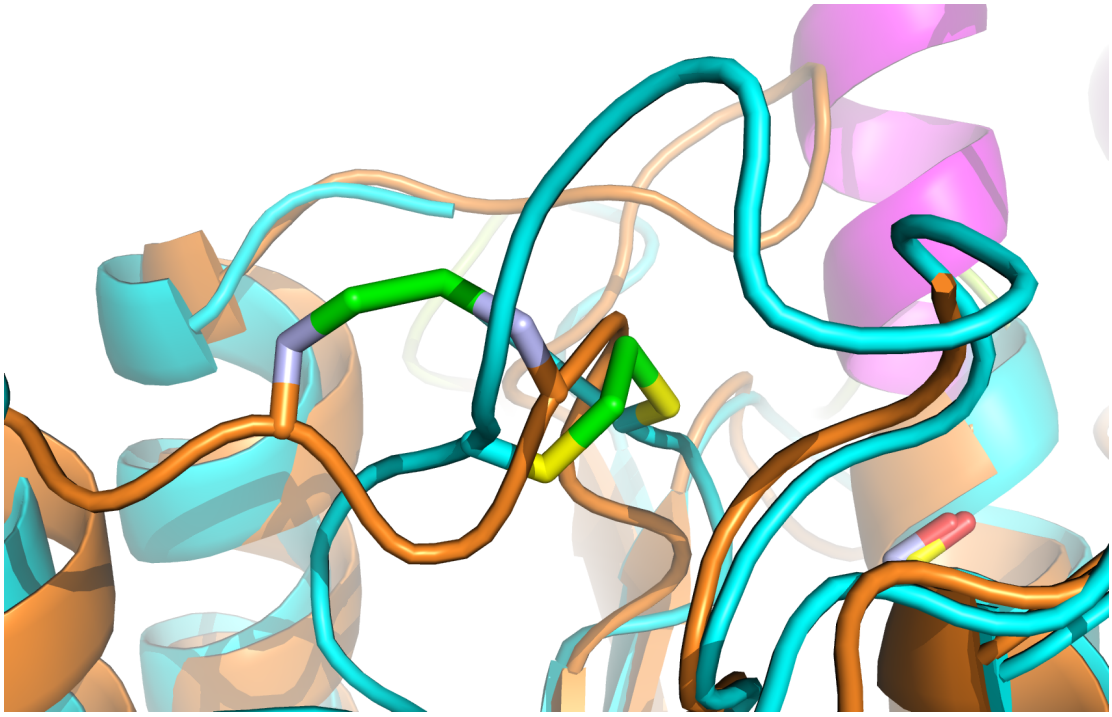
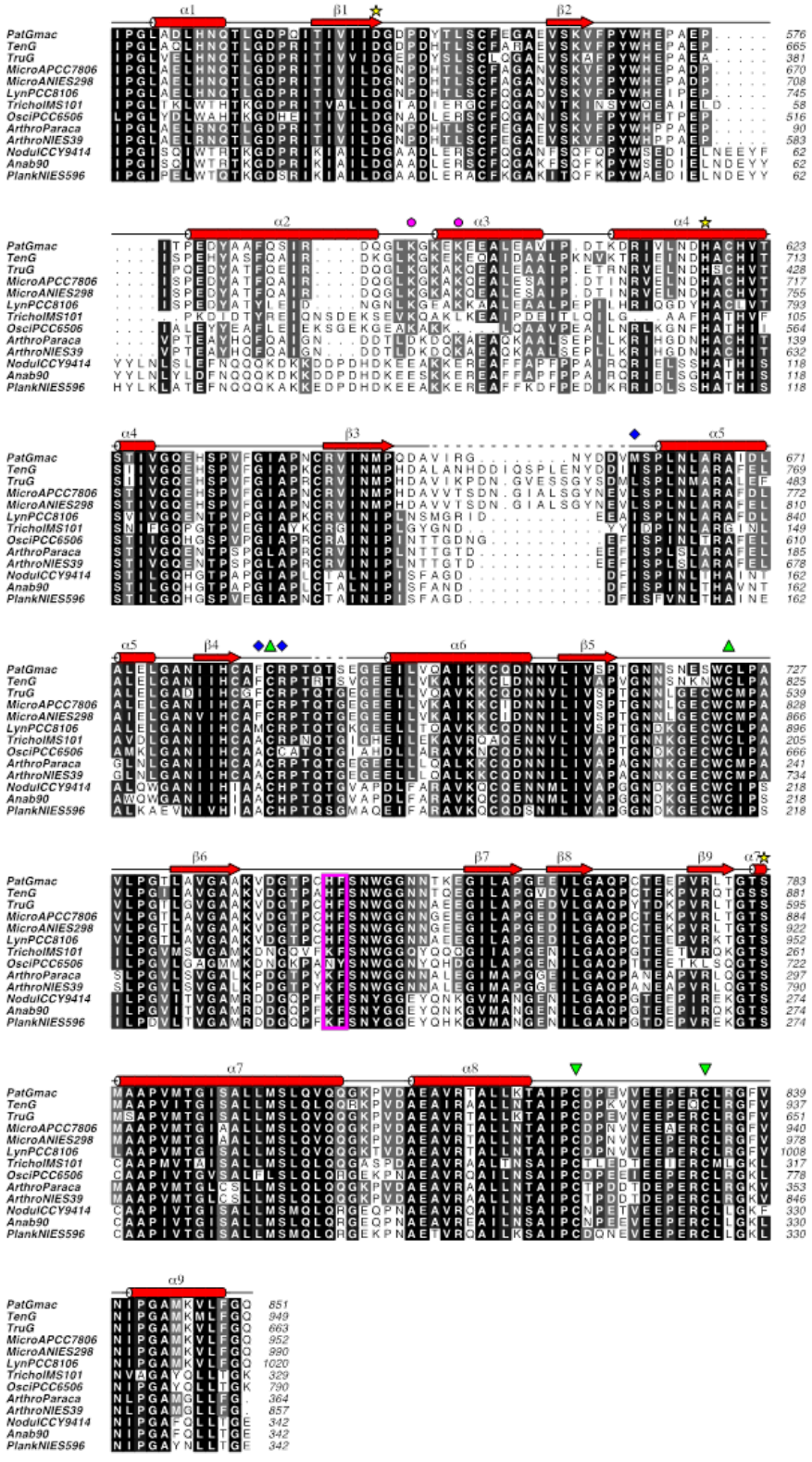


(b) Close-up of the active sites of PatGmac (cyan, magenta) and AkP (orange) with active site residues shown as sticks (PatGmac: yellow, AkP: light blue).



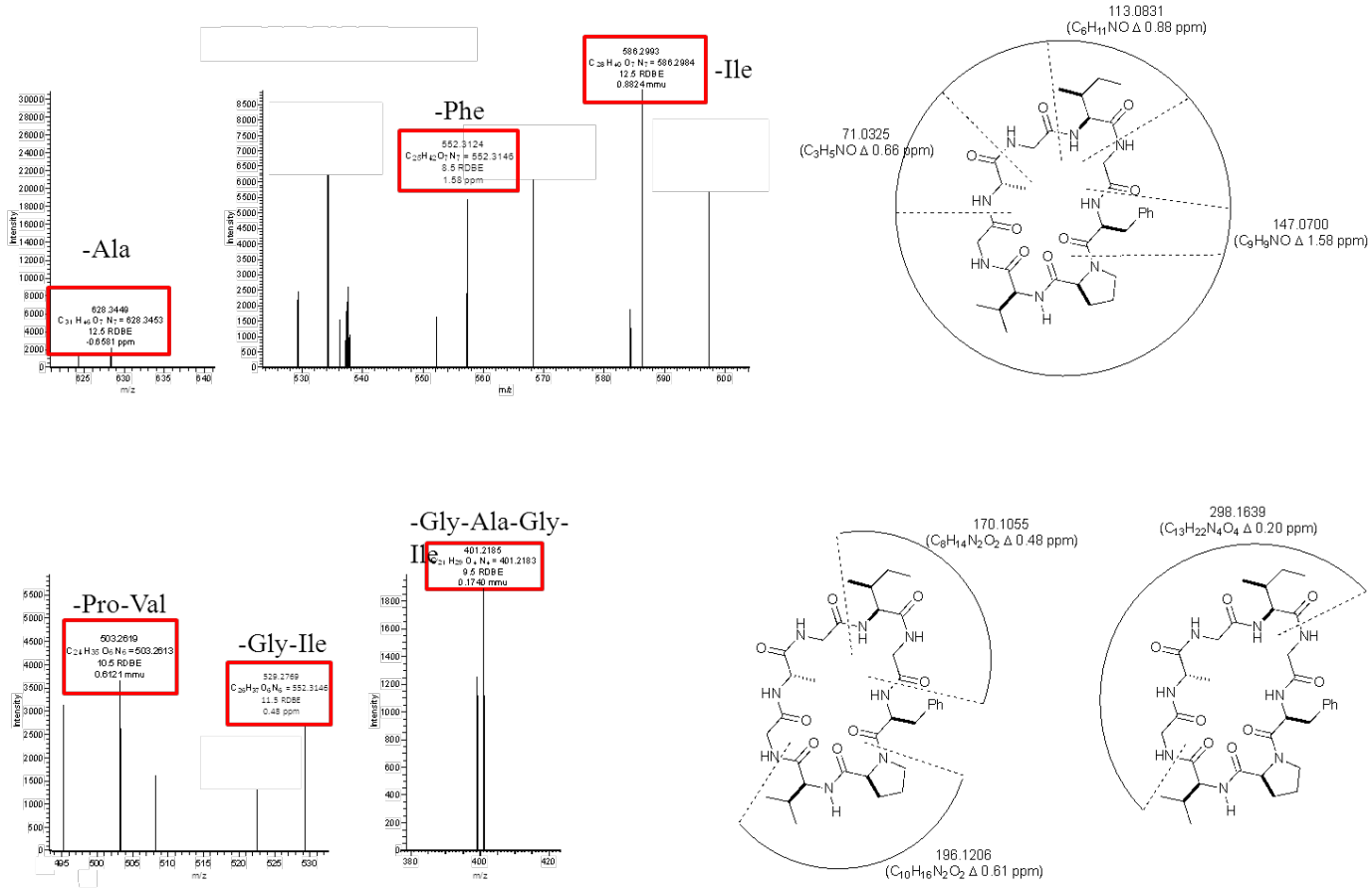
(c) Difference in PatGmac and AkP disulfide bonds. PatGmac Cys685 and Cys724 (yellow sticks) link two loops, while in AkP Cys137 and Cys139 (light blue sticks) form an intra-loop ring. Active site serines are shown as sticks in the background.

Figure S2 Sequence alignment of PatGmac with its homologs

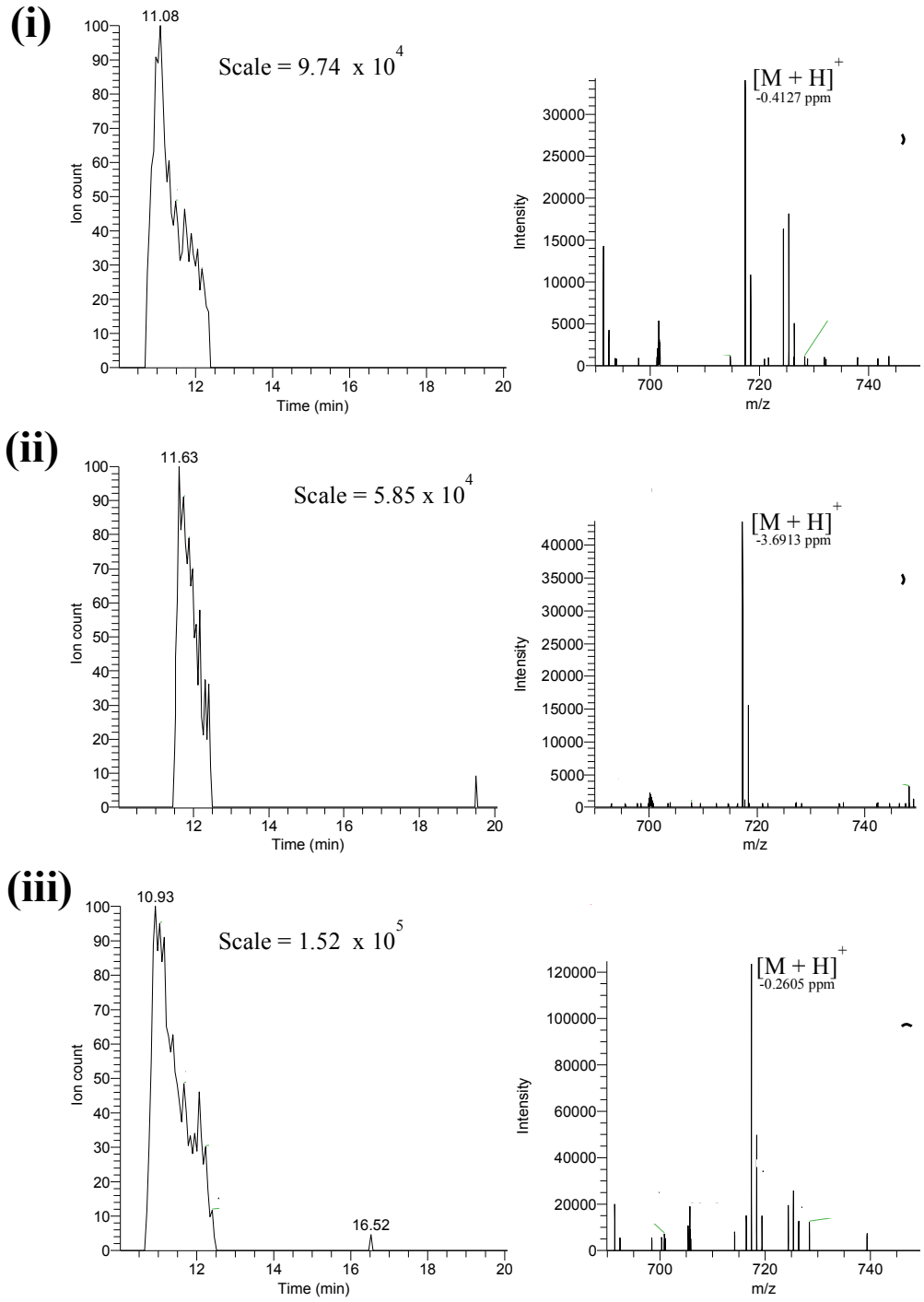


Secondary structure elements are shown in red. Active site residues are indicated by yellow stars, cysteines involved in disulfide bonding as green triangles (matching directions represent disulfide pairs), residues blocking the S3 and S4 sites as blue diamonds, lysines forming salt-bridges with the substrate as purple circles and His and Phe residues involved in substrate binding are marked by a magenta box.

Figure S3 MS analysis of macrocyclization reactions



(a) Fragmentation pattern of cyclo[VGAGIGFP].



(b) LC-MS of macrocyclization reactions with PatGmacΔ1 (i), PatGmac K598D (ii) and PatGmac triple mutant R589D K594D K598D (iii). Only linear product is observed (curved lines). The error between observed and calculated mass is shown below the $[M + H]^+$ species.

Figure S4 Relative reaction rates of PatGmac and VGAGIGFPAYDG in different buffers and temperatures as determined by LC-MS

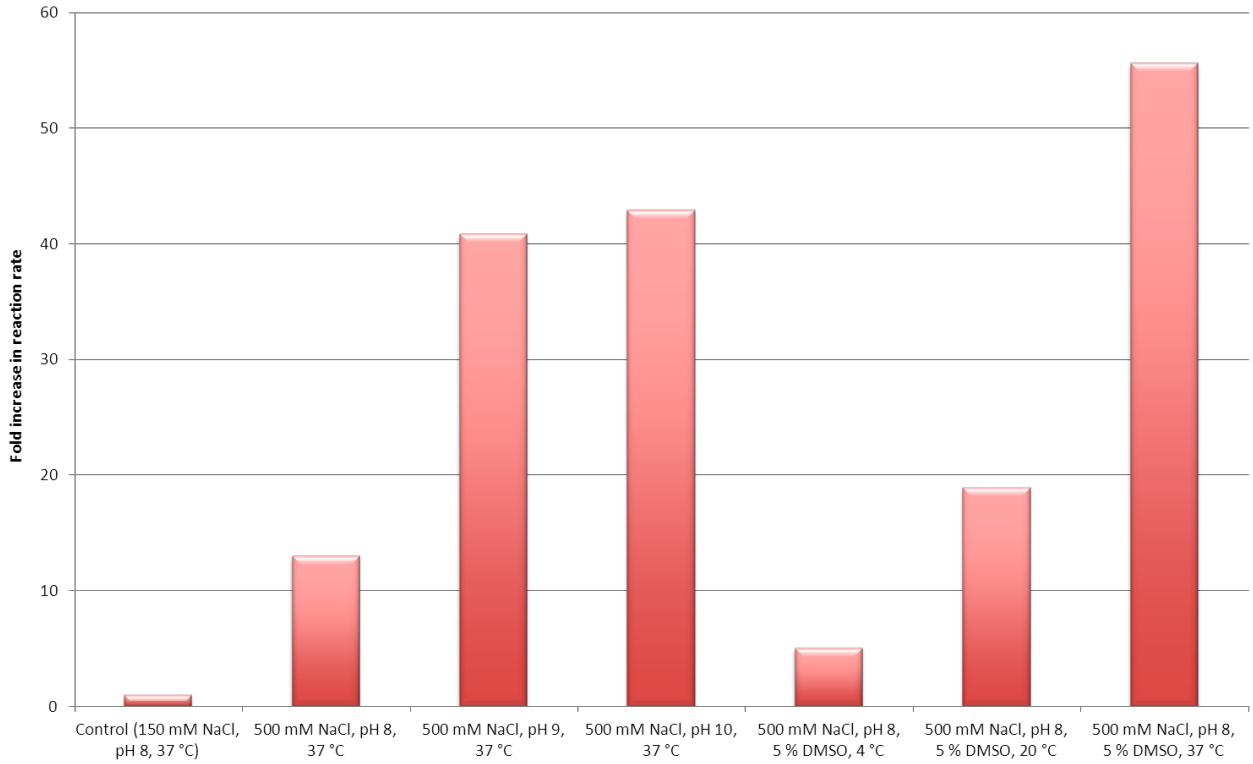


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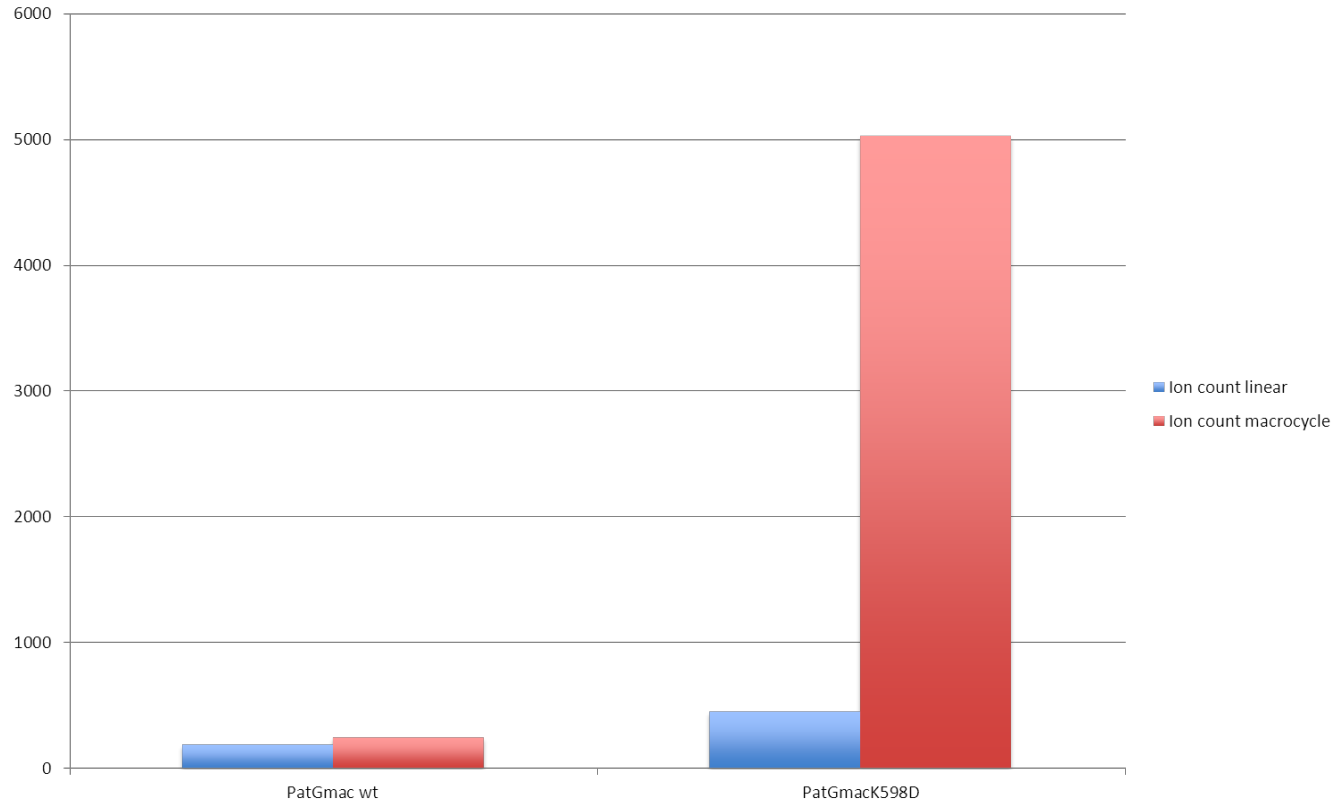
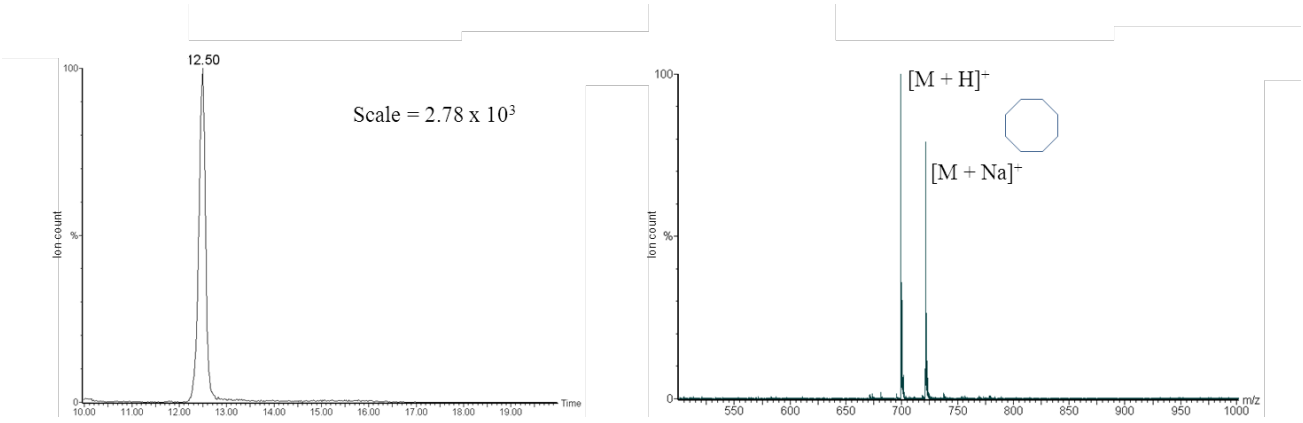
Figure S5 Macrocyclization of VGAGIGFPAYRG



(a) Ion counts of VGAGIGFPAYRG processed by PatGmac wild-type and PatGmac K598D for linear and macrocyclized products as determined by LC-MS.



(b) LC-MS of VGAGIGFPAYRG macrocyclization.

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