Structural characterization of four prochlorosins - a novel class of lantipeptides produced by planktonic marine cyanobacteria

SUPPORTING INFORMATION

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Materials

All oligonucleotides were purchased from Integrated DNA Technologies and used as received. Restriction endonucleases, DNA polymerases and T4 DNA ligase were from New England Biolabs. Media components were obtained from Difco laboratories. *E. coli* DH5α and *E. coli* BL21 (DE3) cells were used as host for cloning and plasmid propagation and host for expression, respectively. Endoproteinases were ordered from Roche Biosciences. Unless specified otherwise, chemicals were purchased from Sigma Aldrich, Fisher Scientific or CalBiochem.

Table S1. Primer sequences for cloning of ProcAs.

Primer Name	Primer Sequence (5'-3')
ProcA1.1_EcoRI_FP_Duet	AAG AAT TCG ATG AAA AAG CGA CTC AAC
ProcA1.1_NotI_RP_Duet	AAG CGG CCG CTC AGC ACA CAT TGA TAG
ProcA4.3_EcoRI_FP_Duet	GGT GAG TGG AAT TCG ATG TCA GAA GAA CAA CTG AAG GC
ProcA4.3_NotI_RP_Duet	ATG ACC TAG CGG CCG CCT AGT AAC AAA ACA TAC

Figure S1. MALDI-TOF MS spectra of the core peptides of four selected prochlorosins modified by ProcM in *E. coli*. For peptide sequences, see Figure 4 of the main text. A. Pcn1.1. B. Pcn1.7 C. Pcn3.3 D. Pcn4.3.

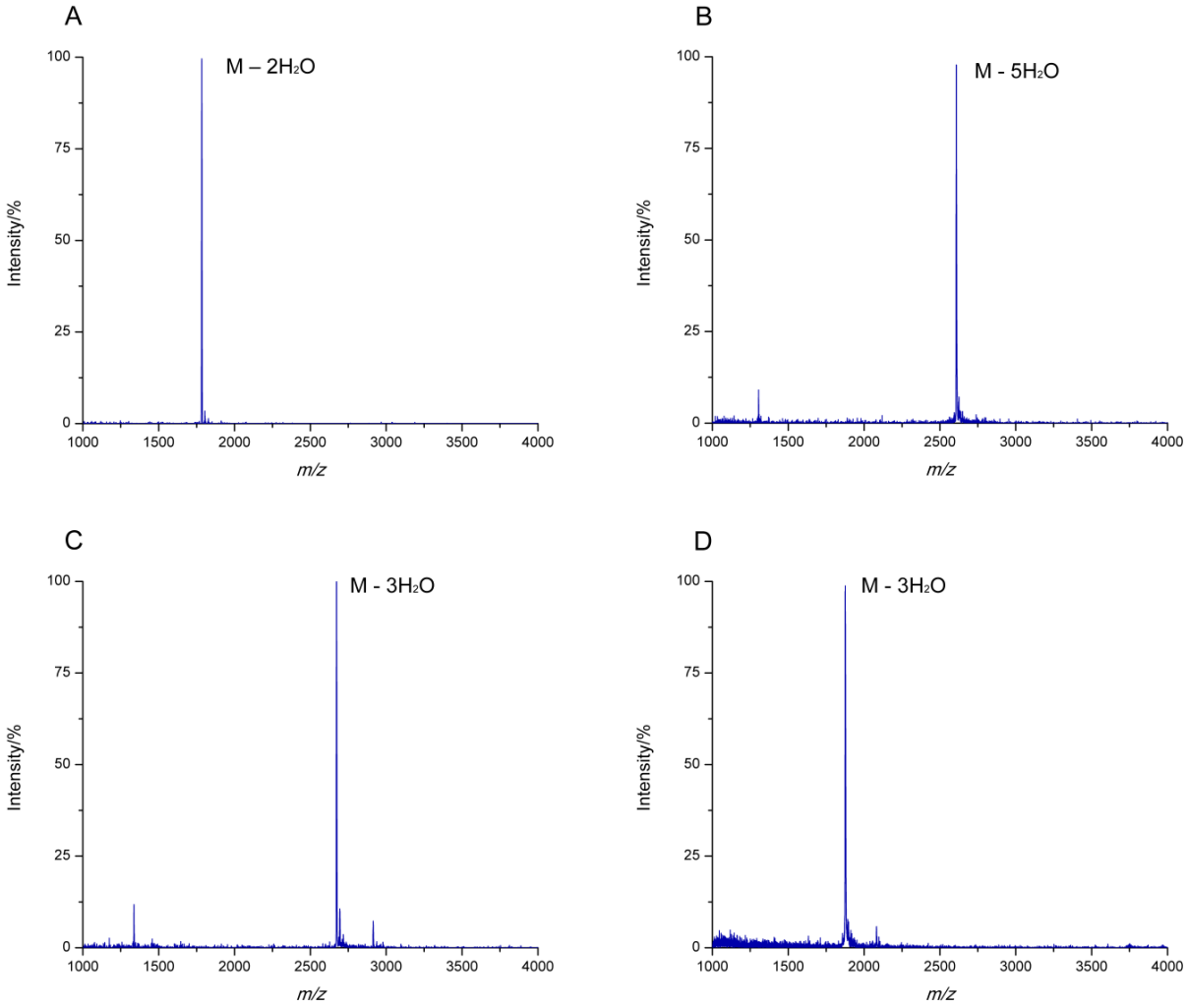

Figure S2. Water suppressed NOESY spectrum (mixing time = 0.40 s) for resonance amide assignments to amino acids from Pcn1.7.

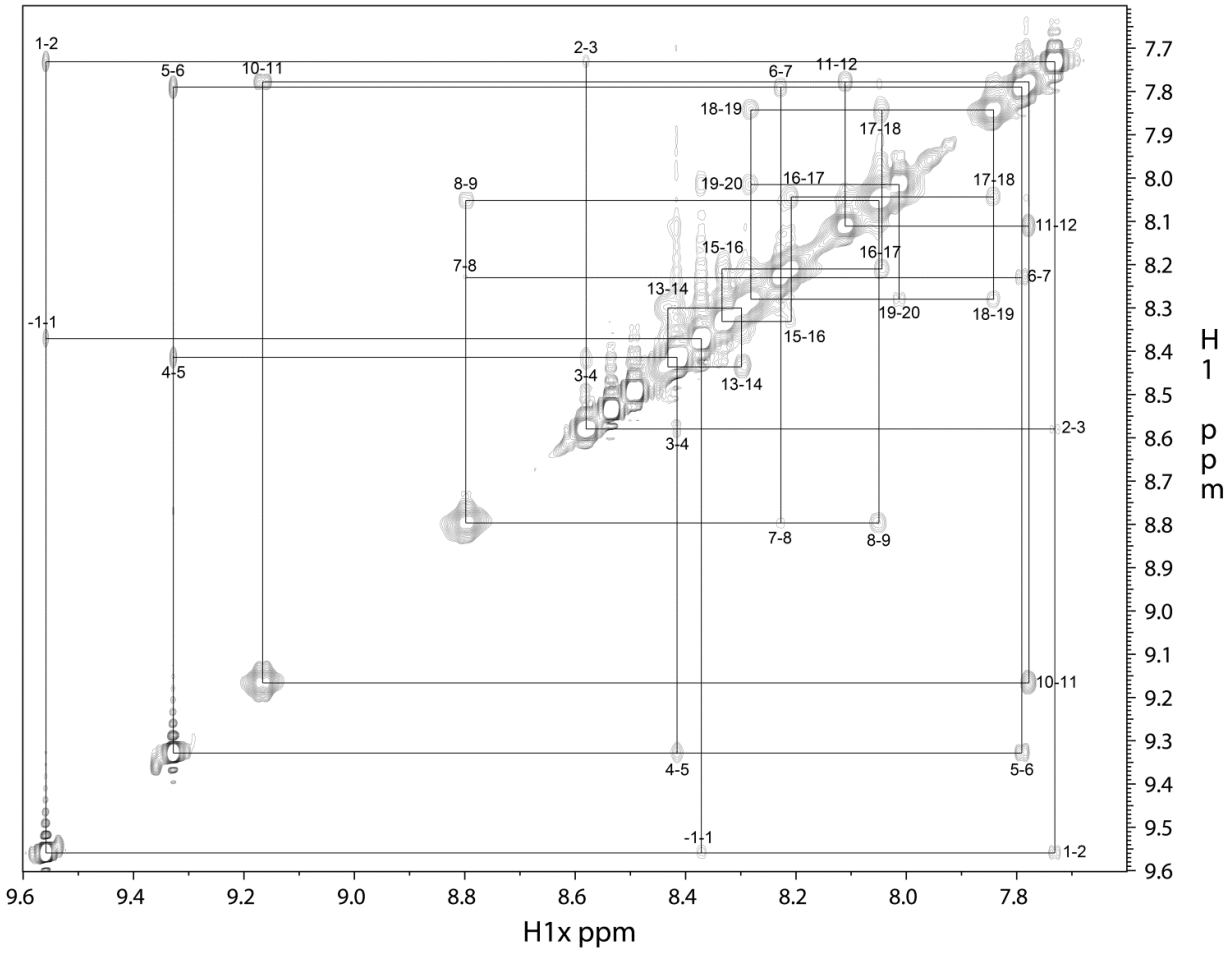

Figure S3. Water suppressed TOCSY spectrum identifying all residues of Pcn1.1.

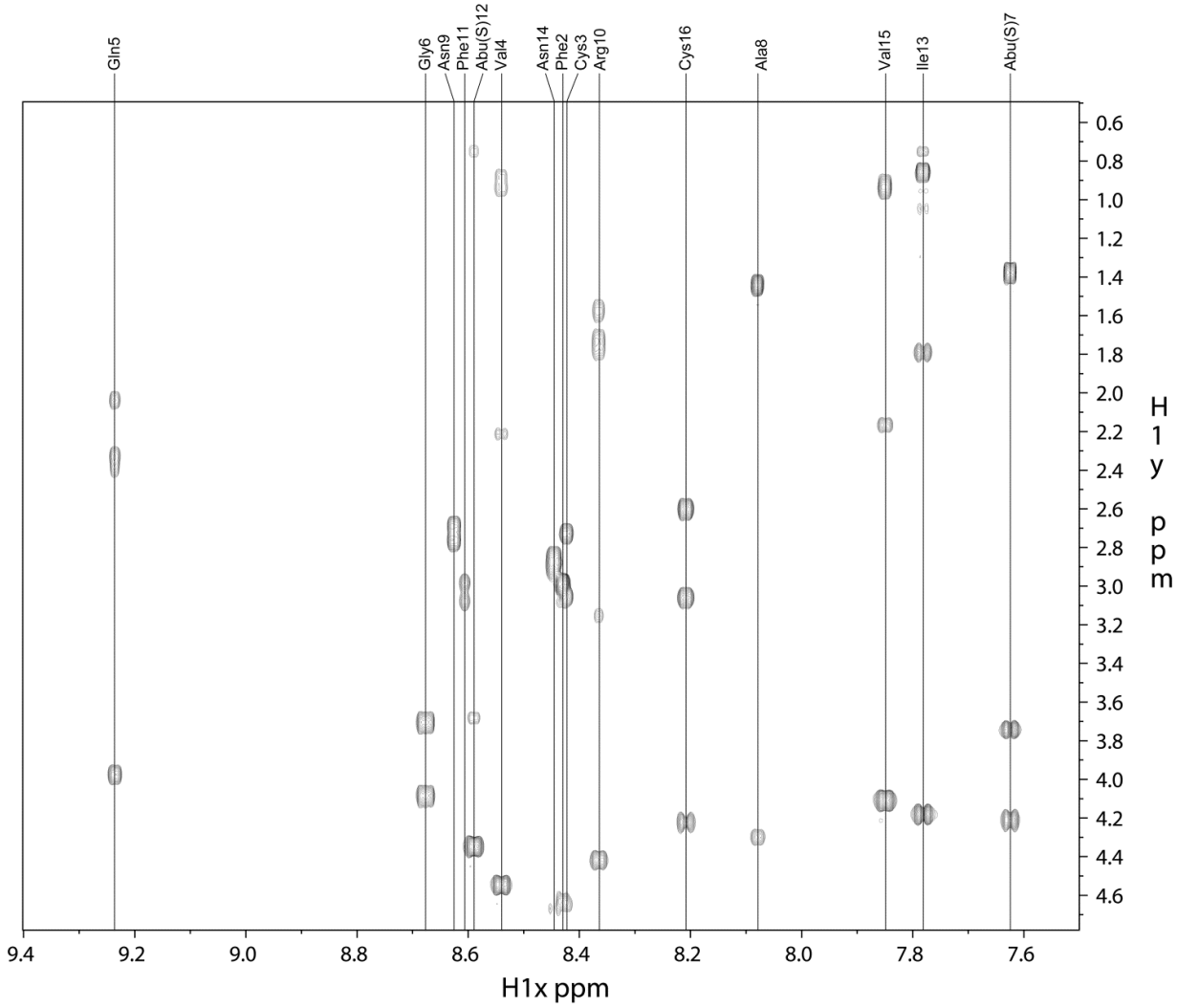

Figure S4. Water suppressed NOESY spectrum (mixing time = 0.20 s) for assignments of amide resonances to specific amino acids of Pcn1.1. In turn this information was used to assign the spin systems in Figure S3 to specific amino acids.

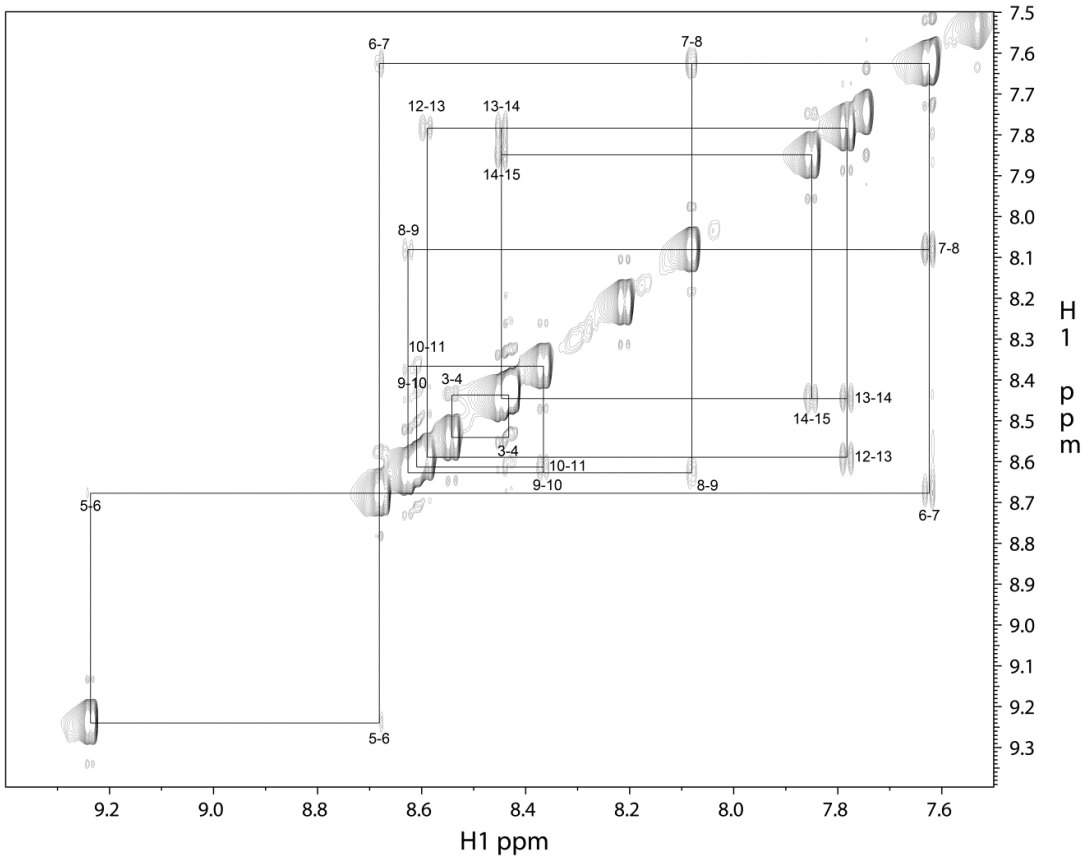

Figure S5. NOESY spectrum of Pcn1.1 in D_2O (mixing time = 0.20 s) for ring topology assignment. The assignments are indicated on the spectrum. Note that only the nOe signals pertaining to the assignment of ring topology are labeled. No efforts were made to utilize the additional nOe signals to determine a three-dimensional structure since the pH of the sample was non-physiological (pH 3.5).

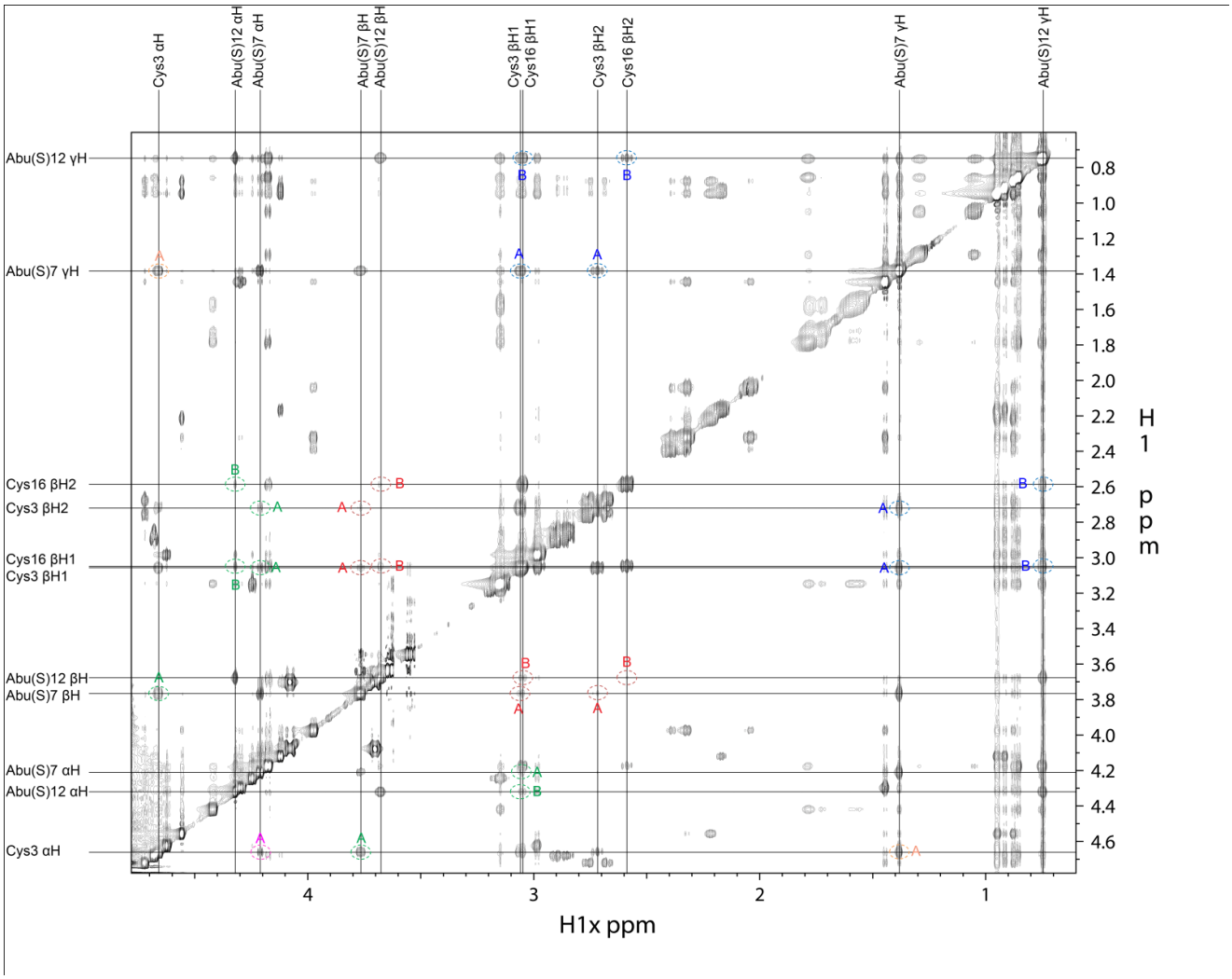

Figure S6. Water suppressed TOCSY spectrum identifying all amide resonances of Pcn3.3.

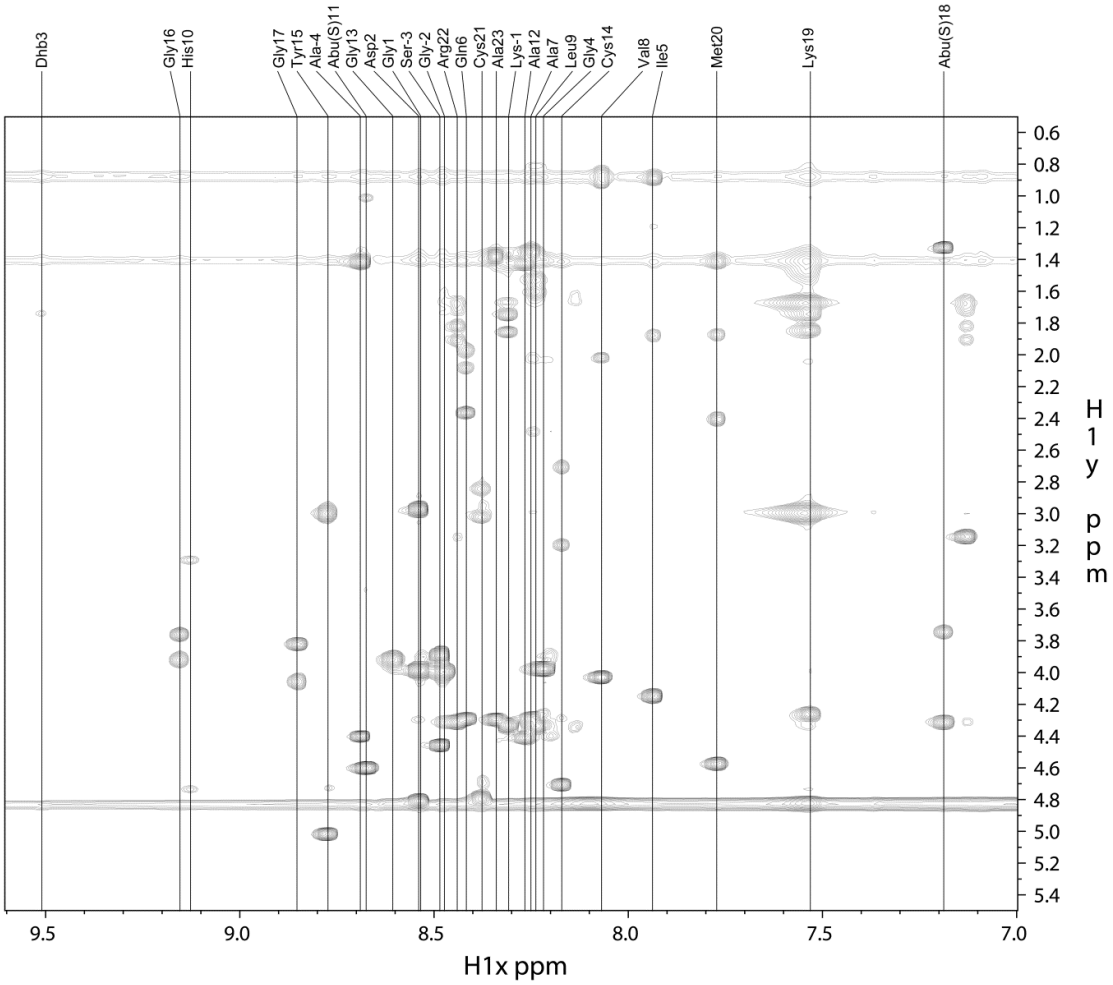

Figure S7. Water suppressed NOESY spectrum (mixing time = 0.20 s) for assignment of the amide resonances to specific amino acids of Pcn3.3. In turn this information was used to assign the spin systems in Figure S6 to specific amino acids.

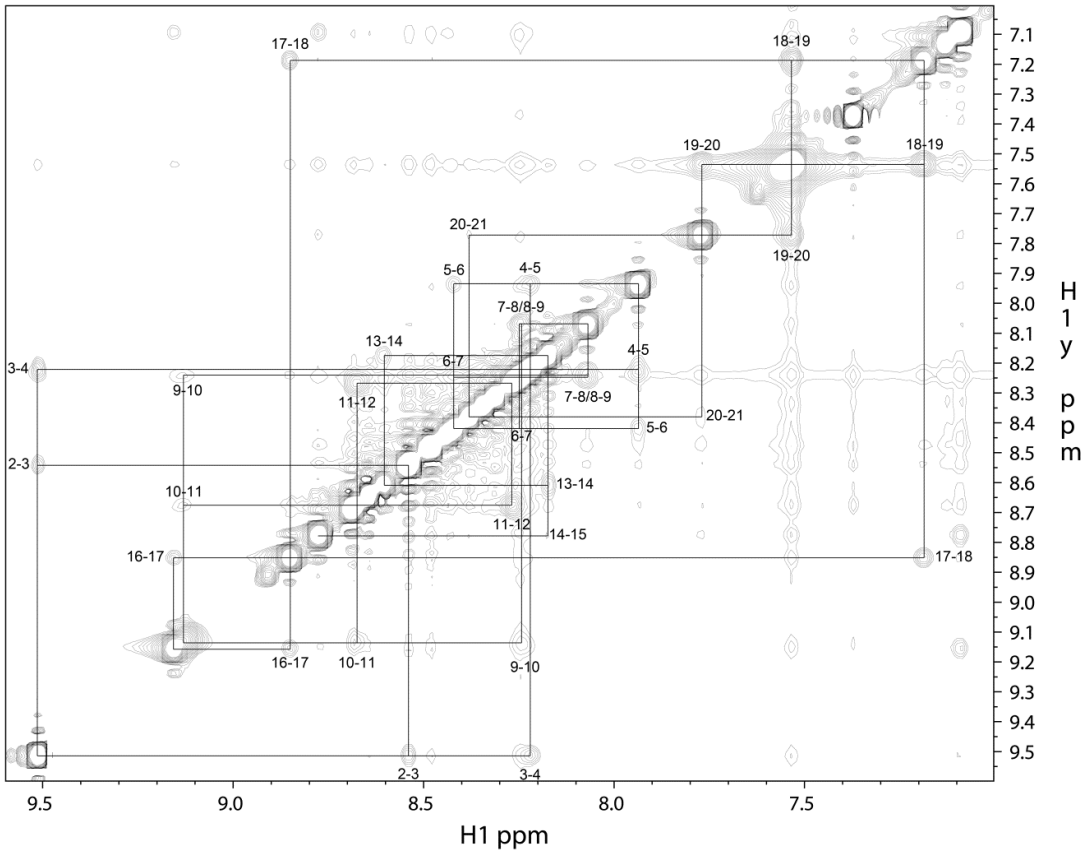

Figure S8. NOESY spectrum of Pcn3.3 in D_2O (mixing time = 0.20 s) for ring topology assignment. The assignments are indicated on the spectrum.

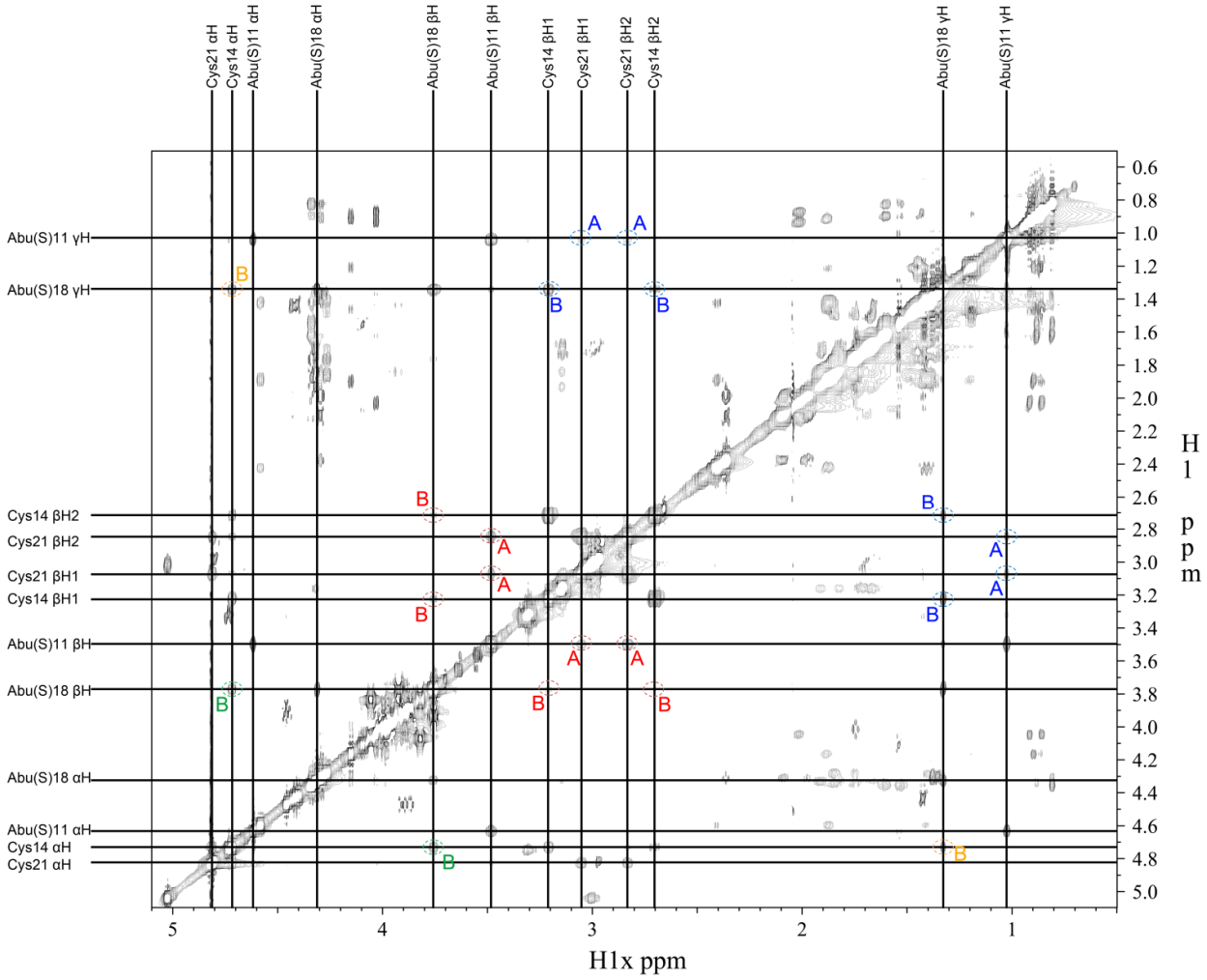

Figure S9. Water suppressed TOCSY spectrum identifying all amide resonances of Pcn4.3.

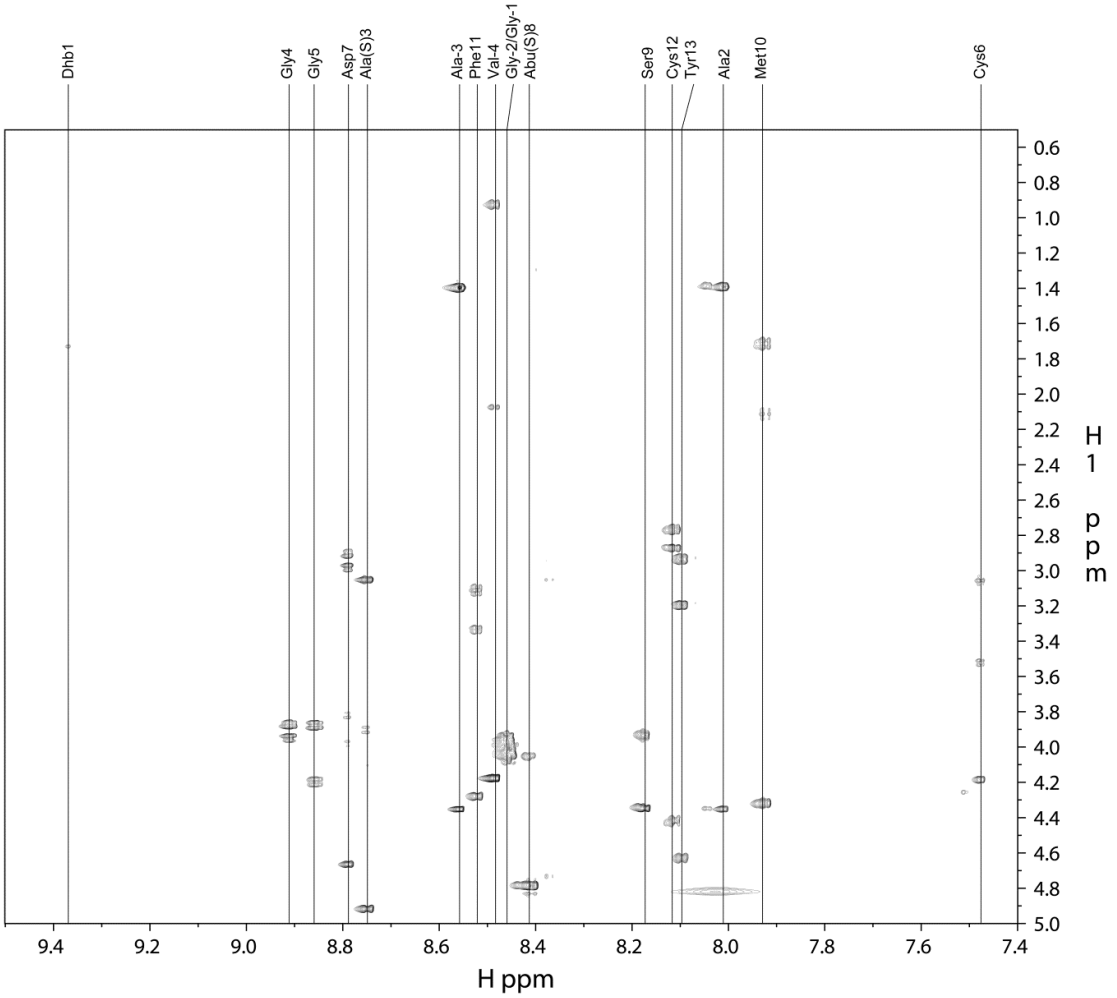

Figure S10. Water suppressed NOESY spectrum (mixing time = 0.20 s) for assignments of the amide resonances to specific amino acids in Pcn4.3. In turn this information was used to assign the spin systems in Figure S9 to specific amino acids.

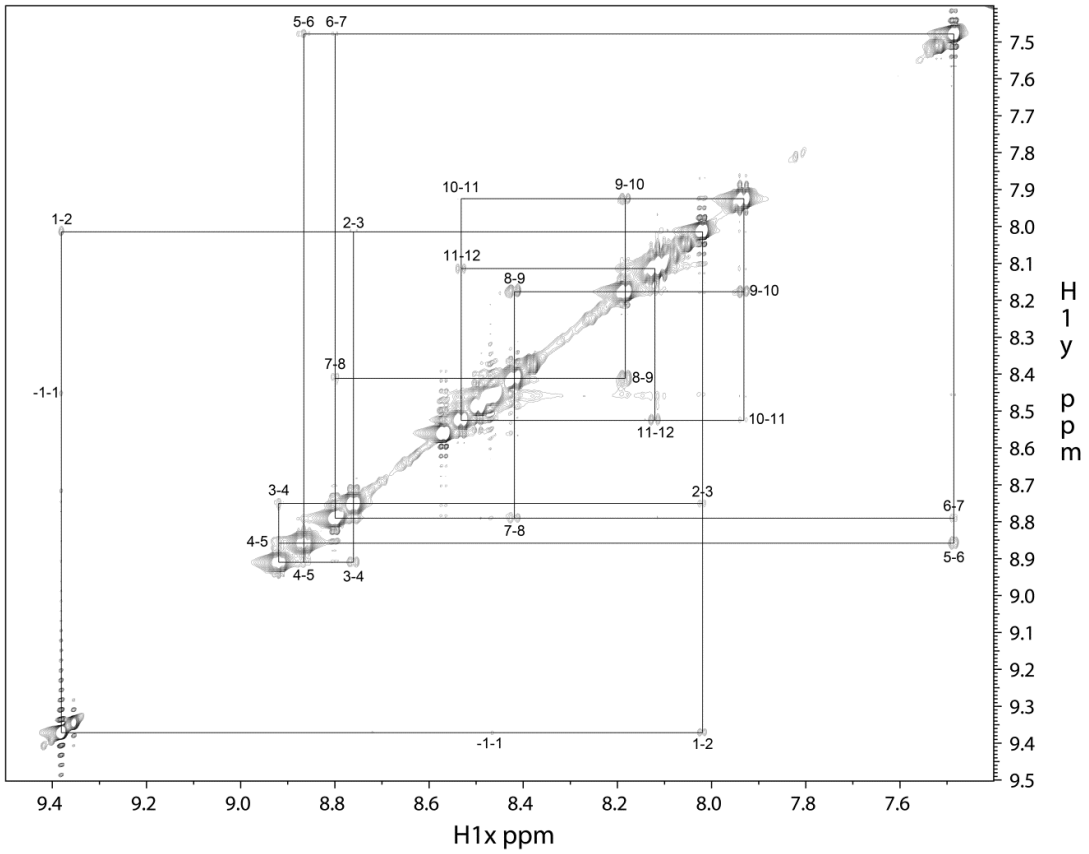

Figure S11. NOESY spectrum of Pcn4.3 in D_2O (mixing time = 0.40 s) for ring topology assignment. The assignments are indicated on the spectrum.

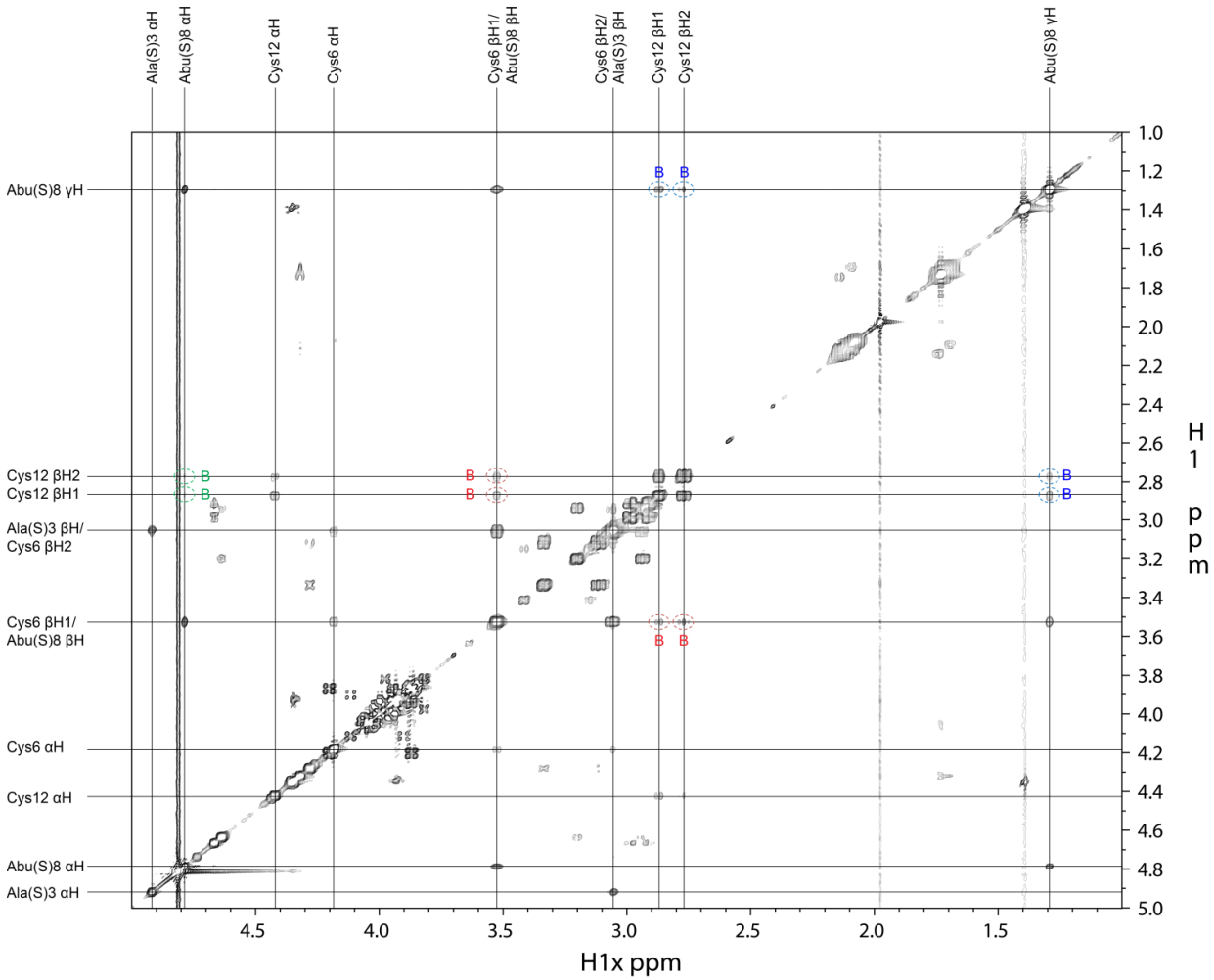

Figure S12. GC/MS traces (single ion monitoring, SIM, at 365 Da for Lan and 379 Da for MeLan) of derivatized (Me)Lan standards and co-injections with derivatized Lan/MeLan obtained from Pcn1.1. A. Hydrolyzed and derivatized amino acids from Pcn1.1 (red line) and coinjected with DL-Melan standard with Pcn1.1 (blue line). B. Hydrolyzed and derivatized amino acids from Pcn1.1 (red line) and coinjected with LL-Melan standard (blue line). Traces of derivatized MeLan from Pcn1.1 (red lines) used for overlay were adjusted to 70% intensity for clarity.

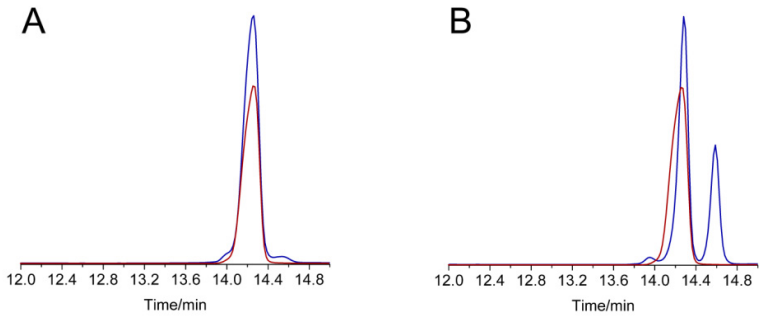

Figure S13. GC/MS traces (single ion monitoring, SIM, 379 Da for MeLan) for coinjections of derivatized MeLan standards with hydrolyzed and derivatized amino acids of Pcn3.3. A. Hydrolyzed and derivatized amino acids from Pcn3.3 (red line) and coinjected with DL-MeLan standard (blue line). B. Hydrolyzed and derivatized amino acids from Pcn3.3 (red line) and coinjected with LL-MeLan standard (blue line). Derivatized MeLan from Pcn3.3 used for overlays were adjusted to 70% intensity for clarity.

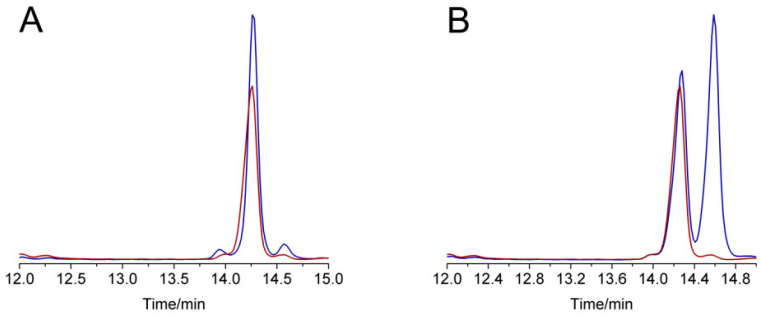

Figure S14. GC/MS traces (single ion monitoring, SIM, 365 Da for Lan and 379 Da for MeLan) for coinjection of derivatized (Me)Lan standards and hydrolyzed and derivatized amino acids from Pcn4.3. A. Hydrolyzed and derivatized amino acids from Pcn4.3 (red line) and coinjected with derivatized DL-MeLan standard (blue line). B. Hydrolyzed and derivatized amino acids from Pcn4.3 (red line) and coinjected with derivatized LL-MeLan standard (blue line). C. Hydrolyzed and derivatized amino acids from Pcn4.3 (red line) and coinjected with derivatized DD-Lan standard (blue line). D. Hydrolyzed and derivatized amino acids from Pcn4.3 (red line) and coinjected with derivatized DL-Lan standard (blue line). E. Hydrolyzed and derivatized amino acids from Pcn4.3 (red line) and coinjected with derivatized LL-Lan standard (blue line). Traces of derivatized Lan and MeLan from Pcn4.3 (red lines) used for overlay were adjusted to 70% intensity for clarity.

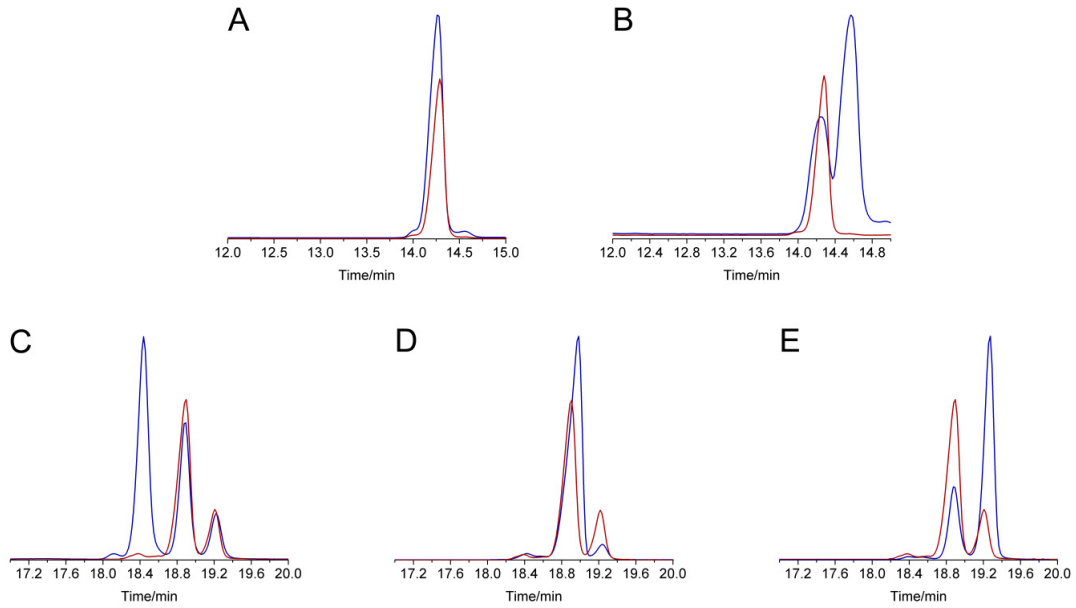

Figure S15. GC/MS traces (single ion monitoring, SIM, 365 Da for Lan and 379 Da for MeLan) for coinjection of derivatized (Me)Lan standards and hydrolyzed and derivatized amino acids from Pcn2.8. A. Hydrolyzed and derivatized amino acids from Pcn2.8 (red line) and coinjected with derivatized DD-Lan standard (blue line). B. Hydrolyzed and derivatized amino acids from Pcn2.8 (red line) and coinjected with derivatized DL-Lan standard (blue line). C. Hydrolyzed and derivatized amino acids from Pcn2.8 (red line) and coinjected with derivatized LL-Lan standard (blue line). Traces of derivatized Lan and MeLan from Pcn2.8 (red lines) used for overlay were adjusted to 70% intensity for clarity. As can be seen most clearly in panel B, slightly more DD and LL-Lan is observed in the sample of hydrolyzed Pcn2.8 than in the standard. The same slight increase in epimer content was also observed for hydrolyzed nisin, suggesting that the hydrolysis and/or derivatization procedure for the lantipeptides results in more epimerization than observed for the standards that are synthesized in their derivatized form.

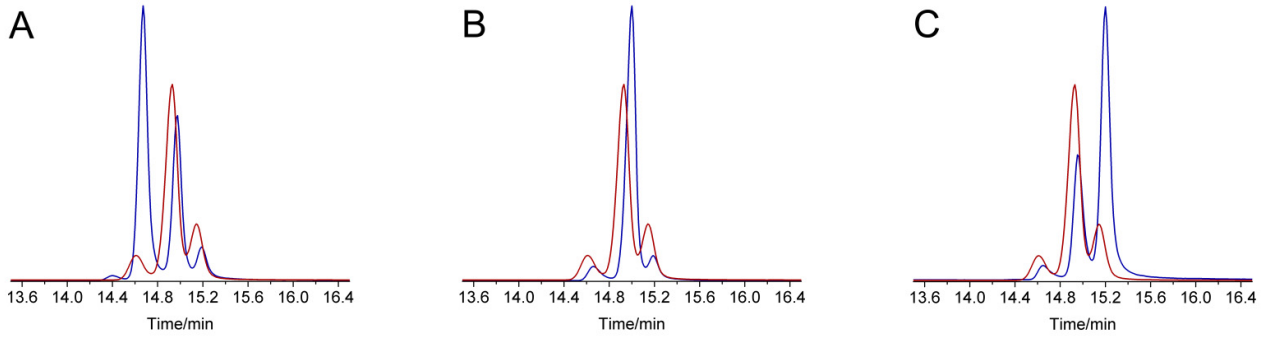

Figure S16. GC/MS traces (single ion monitoring, SIM, 365 Da for Lan and 379 Da for MeLan) for coinjection of derivatized (Me)Lan standards and hydrolyzed and derivatized amino acids from Pcn2.11. A. Hydrolyzed and derivatized amino acids from Pcn2.11 (red line) and coinjected with derivatized DL-MeLan standard (blue line). B. Hydrolyzed and derivatized amino acids from Pcn2.11 (red line) and coinjected with derivatized LL-MeLan standard (blue line). C. Hydrolyzed and derivatized amino acids from Pcn4.3 (red line) and coinjected with derivatized DD-Lan standard (blue line). D. Hydrolyzed and derivatized amino acids from Pcn2.11 (red line) and coinjected with derivatized DL-Lan standard (blue line). E. Hydrolyzed and derivatized amino acids from Pcn2.11 (red line) and coinjected with derivatized LL-Lan standard (blue line). Traces of derivatized Lan and MeLan from Pcn2.11 (red lines) used for overlay were adjusted to 70% intensity for clarity.

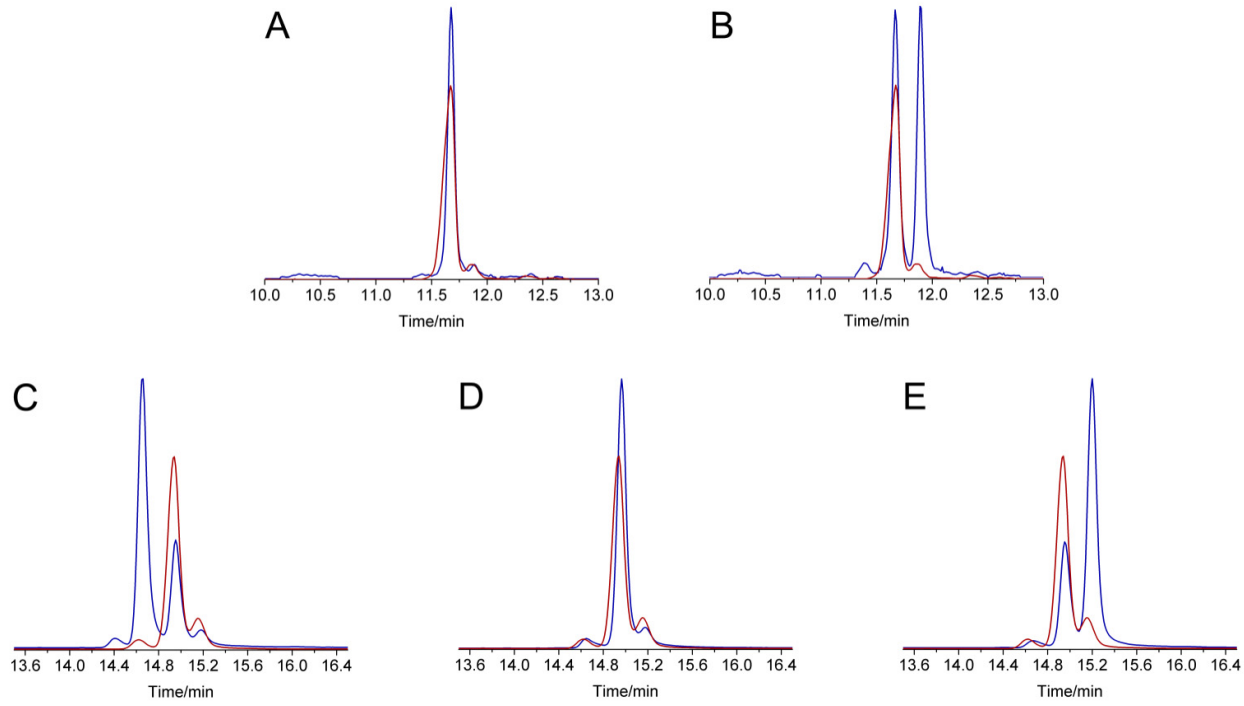

Figure S17. GC/MS traces (single ion monitoring, SIM, 365 Da for Lan and 379 Da for MeLan) for coinjection of derivatized (Me)Lan standards and hydrolyzed and derivatized amino acids from Pcn3.2. A. Hydrolyzed and derivatized amino acids from Pcn3.2 (red line) and coinjected with derivatized DL-MeLan standard (blue line). B. Hydrolyzed and derivatized amino acids from Pcn3.2 (red line) and coinjected with derivatized LL-MeLan standard (blue line). C. Hydrolyzed and derivatized amino acids from Pcn3.2 (red line) and coinjected with derivatized DD-Lan standard (blue line). D. Hydrolyzed and derivatized amino acids from Pcn3.2 (red line) and coinjected with derivatized DL-Lan standard (blue line). E. Hydrolyzed and derivatized amino acids from Pcn3.2 (red line) and coinjected with derivatized LL-Lan standard (blue line). Traces of derivatized Lan and MeLan from Pcn3.2 (red lines) used for overlay were adjusted to 70% intensity for clarity.

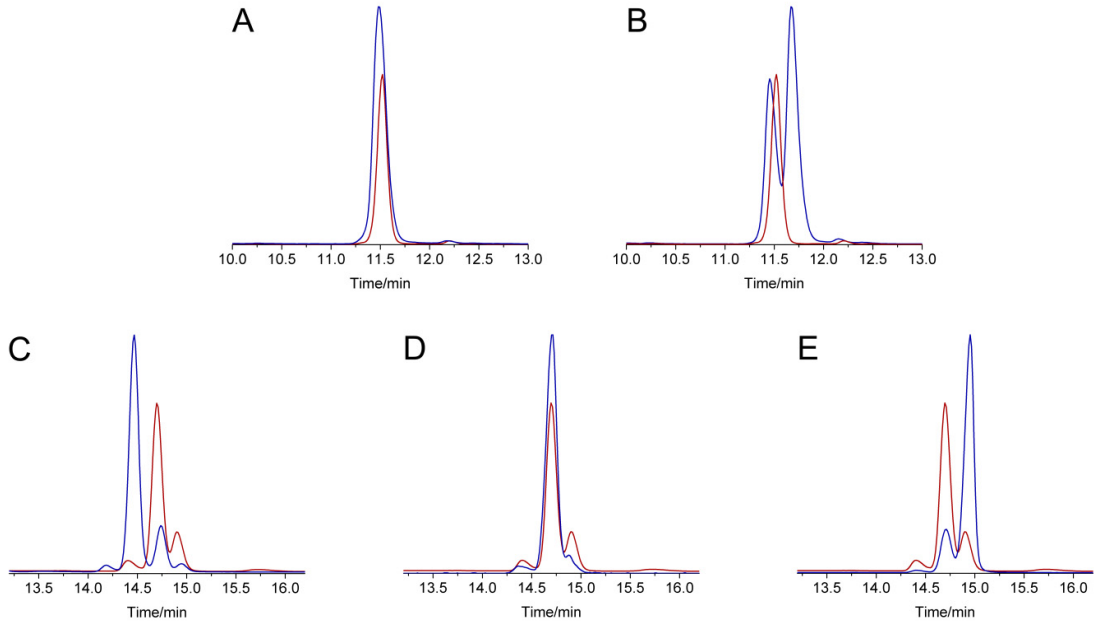

Figure S18. Enlargement of Figure 3B: Section of the NOESY spectrum (mixing time = 0.40 s) of Pcn1.7 in D_2O showing the correlations between β-protons.

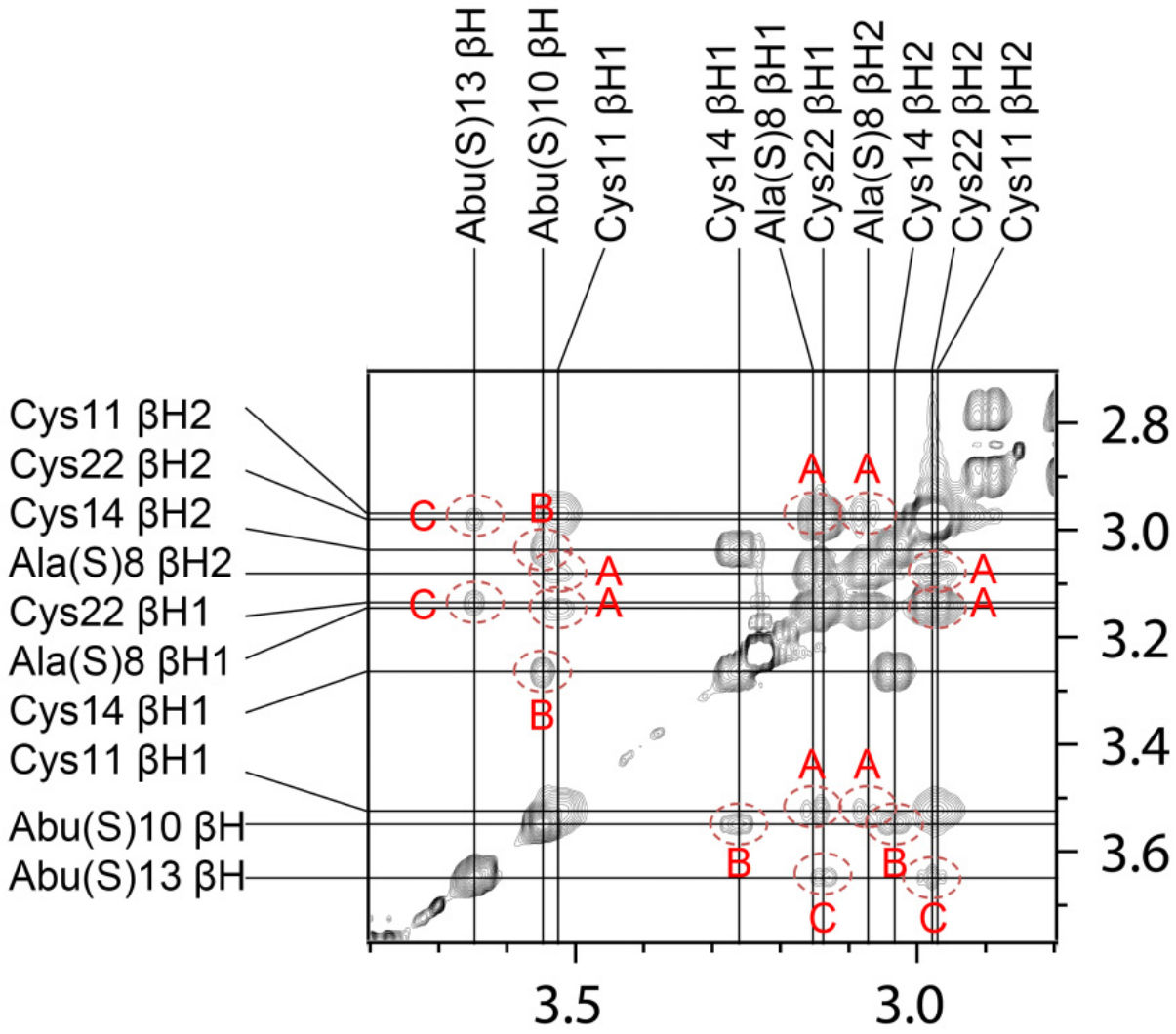

Figure S19. Enlargement of Figure 3C: Section of the NOESY spectrum showing the correlations of the α protons with α and β protons across the thioether bridge.