SUPPORTING INFORMATION

Structure and substrate sequestration in the pyoluteorin peptidyl carrier protein PltL

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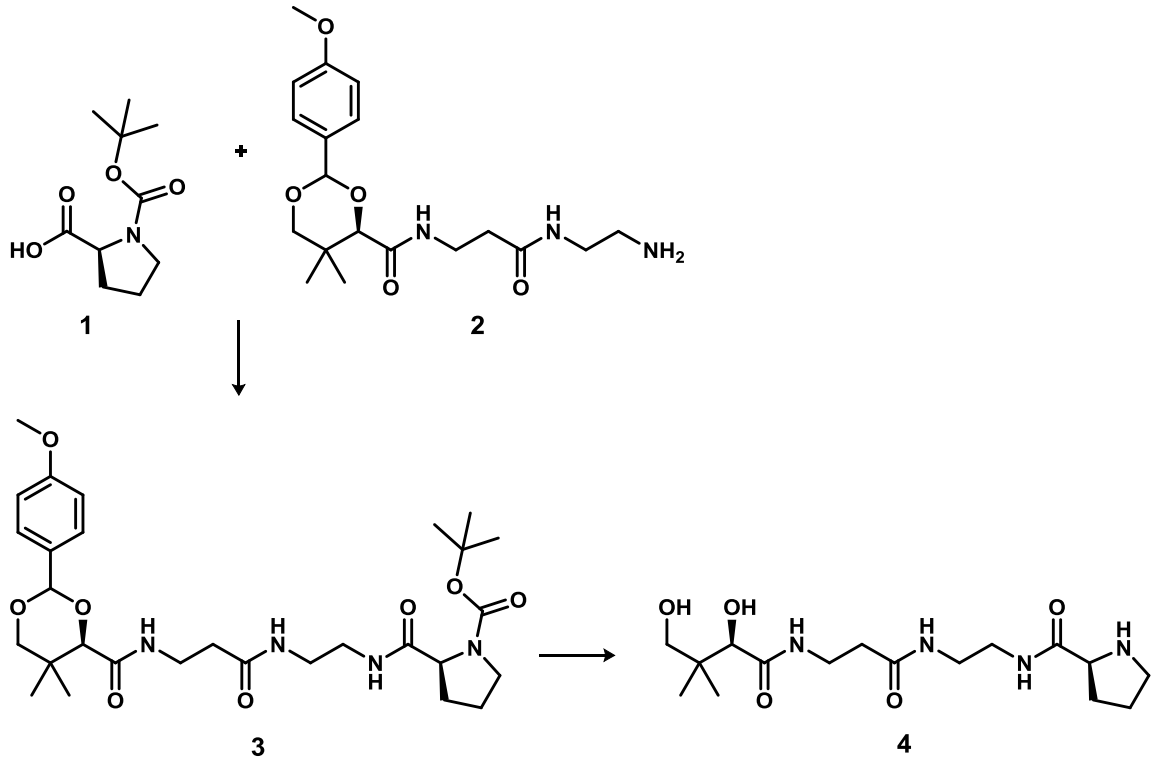
A. Synthesis of prolyl and pyrrolyl pantetheine probes

Unless otherwise noted, all reagents and chemical compounds were purchased from Alfa Aesar, Strem Chemicals, Sigma–Aldrich or TCI and used without further purification. Amine **2** was prepared according to [Meier, J. L.; Mercer, A. C.; Rivera, H. Jr.; Burkart, M. D. *J. Am. Chem. Soc.* **2006**, 128, 12174]. Flash chromatography was carried out on 40–63 mesh Geduran Silica Gel 60 (EMD Millipore). Thin layer chromatography (TLC) was conducted on 250 µm Silica Gel 60 F254 glass plates (EMD Millipore). NMR spectra were recorded on a Mercury Plus 400 MHz (Varian) or a VX 500 MHz equipped with XSens cold probe (Varian) spectrometer. FID files were processed using MestRenova version 8.1 (MestreLab Research). NMR spectra were referenced to residual solvent peaks according to S. Budavari, M.J. O'Neil, A. Smith, P.E. Heckelman, The Merck Index, an Encyclopedia of Chemicals, Drugs, and Biologicals, Eleventh Edition, Merck Co., Inc. Rahway, NJ, 1989. Mass spectrometric analyses were conducted on the following instruments: a LCQ Deca (ThermoFinnigan), MAT900XL (ThermoFinnigan), LTQ Orbitrap XL (ThermoScientific), or a LCT Premier (Waters) mass spectrometer. Reversed-phase HPLC separation was performed using a semi-preparative C18 Luna column (250 x 10 mm) at a flow rate of 2.5 mL/min using 600E pump (Waters) and Lambda-Max model 480 UV detector (Waters). Unless stated otherwise, anhydrous solvents were used for all chemical reactions. Reactions were conducted under Ar atmosphere in a round bottom flask or vial capped with a rubber septa and were stirred using a Teflon coated stir bar. All mixtures are provided as v:v ratios. All protein NMR experiments were conducted on a 600 MHz Avance system equipped with a cryoprobe (Bruker) at the UC San Diego Biomolecular NMR Facility. Proteins were purified by FPLC gel filtration over a HiPrep 26/60 Sephacryl S-100 HR column (GE Healthcare Life Sciences). Isotopes for NMR experiments were sourced from Cambridge Isotope Laboratories.

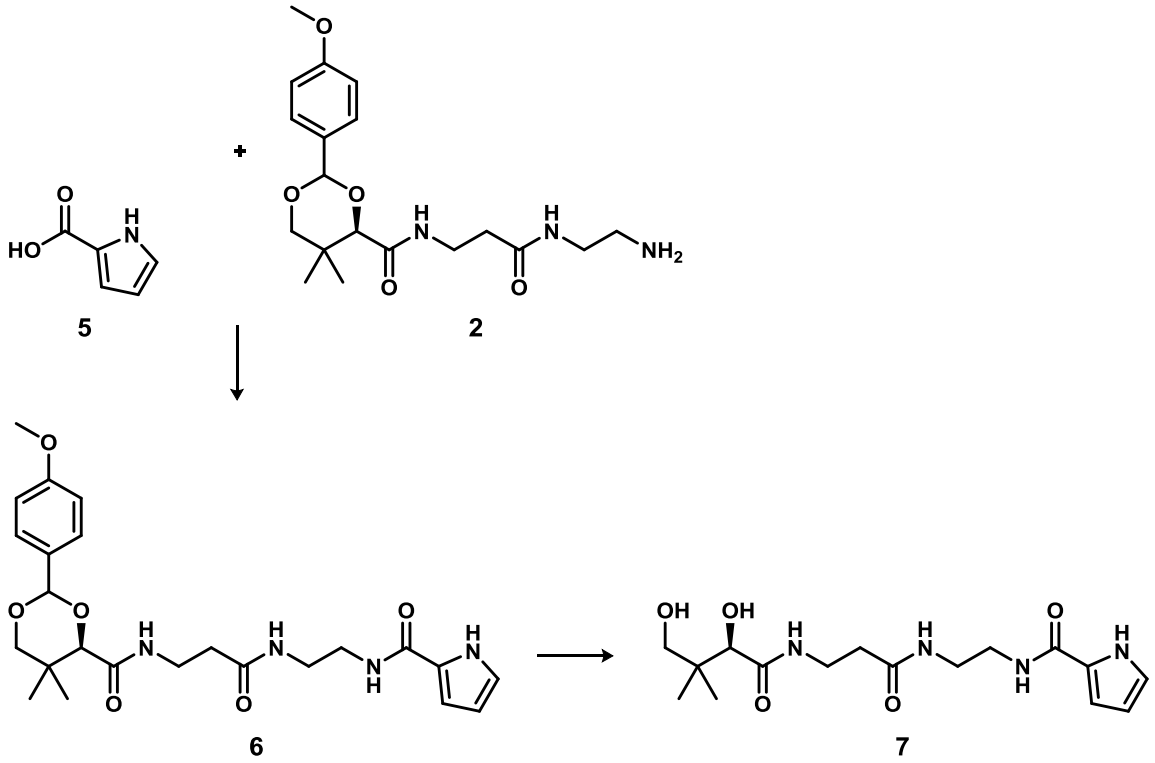
A.1. Synthesis of the prolyl-N-pantetheine probe.



tert-butyl(2R)-2-((2-(3-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carbox-amido)propanamido)ethyl)carbamoyl)pyrrolidine-1-carboxylate (3): 1-Ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDC) (107.7 mg, 0.694 mmol) and hydroxybenotriazole (HOBt) (93.7 mg, 0.694 mmol) were added to a mixture of N-Boc-L-proline (94.5 mg, 0.379 mmol) and amine 2 (120.0 mg, 0.316 mmol) dissolved in dry DMF (5 mL). Within 15 min, N,N-diisopropylethylamine (DIPEA) (121 uL) was added and the mixture was stirred at rt. After 12 h, the solvent was removed under reduced pressure, and the resultant oil was purified by column chromatography (EtOAc to 10% MeOH/EtOAc) to yield amide 3 as a white solid (91%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.44 (d), 7.04 (bs), 6.93 (d), 6.84 (bs), 5.47 (s), 4.16 (m), 4.09 (s), 3.83 (s), 3.68 (q), 3.70 (bs), 3.46 (m), 3.42 (m), 3.35 (m), 2.42 (m), 2.18 (bs), 1.97 (bs), 1.85 (m), 1.76 (quin), 1.45 (s), 0.93 (s), 0.92 (s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.41, 171.45, 169.42, 160.20, 155.54, 130.15, 127.55, 113.71, 101.34, 83.92, 80.45, 78.50, 60.48, 55.34 47.32, 39.27, 38.88, 36.27, 35.14 33.09, 30.84, 28.42, 24.65, 21.86, 19.14.

(R)-N-(2-(3-((R)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl)pyrrolidine-2-carboxamide (4): Amide 3 (167.1 mg, 0.290 mmol) was dissolved in 2 mL DCM and 400 uL TFA was slowly added. After 0.5 hr at rt the solvent was removed under reduced pressure. The solid was purified by column chromatography (EtOAc to 40% MeOH/EtOAc) to yield amide 4 as a white solid (50%). ^{1}H-NMR (400 MHz, CD_3OD) δ 8.14 (t), 8.06 (t), 4.24 (q), 3.91 (s), 3.49-3.30 (m), 2.42 (t), 2.41 (bs), 2.06 (m), 0.93 (s). ^{13}C-NMR (100 MHz, CD_3OD) δ 174.69, 172.90, 168.50, 75.80, 68.81, 59.79, 45.90, 39.19, 38.95, 38.34, 35.42, 35.03, 29.44, 23.68, 19.95, 19.45. HR-MS [M+H]+ calcd. 359.2289, found 359.2290.

A.2. Synthesis of the pyrrolyl-N-pantetheine probe.



N-(2-(3-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamido)-propan-amido)ethyl)-1H-pyrrole-2-carboxamide (6): EDC (68.6 mg, 0.442 mmol) and HOBt (59.7 mg, 0.442 mmol) were added to a mixture of 2-carboxylic acid pyrrole (27.0 mg, 0.243 mmol) and amine 2 (83.7 mg, 0.221 mmol) dissolved in dry DMF (5 mL). Within 15 min, N,N-diisopropylethyl-amine (DIPEA) (77 uL) was added and the mixture was stirred at rt. After 12 h, the solvent was

removed under reduced pressure, and the resultant oil was purified by column chromatography (EtOAc to 10% MeOH/EtOAc) to yield amide 6 as a white solid (55%).^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.32 (s), 7.39 (d), 7.38 (t), 7.25 (t), 7.05 (m), 6.88 (t), 6.87 (d), 6.68 (t), 6.16 (t), 5.38 (s), 4.02 (s), 3.75 (s), 3.62 (q), 3.48 (q), 3.44-3.26 (m), 2.39 (t), 1.07 (s), 1.02 (s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.49, 170.11, 162.30, 160.43, 130.31, 127.77, 125.86, 122.20, 113.93, 110.59, 109.97, 101.56, 84.06, 78.59, 55.52, 39.87, 36.29 35.60, 33.27, 22.05, 19.33. Contains residual ethyl acetate.

(R)-N-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl)-1H-pyrrole-2-carboxamide (7): Amide 6 (55.7 mg, 0.157 mmol) was dissolved in 80% aq. AcOH (10 mL). After 16 h at rt, the solvent was removed under reduced pressure, and the solid was purified by column chromatography (EtOAc to 20% MeOH/EtOAc) to yield amide 7 as a white solid (69%). ^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ 11.43 (s), 8.06 (t), 8.02 (t), 7.70 (t), 6.81 (s), 6.72 (s), 6.04 (s), 5.39 (bs), 4.47 (23), 3.68 (s), 3.26-3.13 (m), 2.25 (t), 0.79 (s), 0.76 (s). ^{13}C-NMR (125 MHz, $(CD_3)_2SO$) δ 173.29, 171.15, 161.24, 126.66, 121.69, 110.31, 108.95, 75.34, 68.40, 39.48, 39.02, 38.73, 35.64, 35.25, 21.41, 20.71. Contains residual ethyl acetate. HR-MS [M+H]+ calcd. 355.1976, found 259.1975.

B. Protein NMR Studies.

B.1. Site directed mutagenesis of PltL C87S

The pet22b-PltL plasmid was obtained from the laboratory of Christopher Walsh at Harvard University.[S1] A PltL C87S mutation was introduced via quickchange PCR to avoid inter-protein disulfide bridge formation through the thiol of the cysteine. The quickchange PCR was performed with the following primers:

C87S_F: 5'-GGCCGAGTCCGCCCTCGAGCACCACCACC-3'

C87S_R: 5'-CTCGAGGGCGGACTCGGCCTTTAGTTGCTCG-3'

B.2. Expression and purification of ^{15}N/^{13}C *apo*-PltL

pET22b-PltL (C87S) plasmid was transformed into BL21 (DE3) cells. Uniformly labeled ^{15}N-PltL was expressed by culturing cells in M9 minimal media (1 L) supplemented with 1 g ^{15}N-NH_4Cl, 4 g D-glucose, and 50 mg carbenicillin. Uniformly labeled ^{13}C,^{15}N-PltL was prepared by supplementing M9 minimal media (1 L) with 4 g ^{13}C-D-glucose, 1 g ^{15}N-NH_4Cl, and 50 mg carbenicillin. Expression was induced with 0.5 mM IPTG at an OD_{600} of 0.9, and the cells were incubated an additional 16 h at 16 °C. The cells were harvested by centrifugation (1000 RCF). The pelleted cells were re-suspended in 30 mL of lysis buffer (150 mM NaCl, 50 mM Tris pH 7.5, 10% glycerol) and lysed in a French pressure cell. The lysate was then centrifuged (12000 RCF) for 1 h to remove insoluble debris. The His_6-tagged proteins were purified using Ni-NTA resin (Novagen) to yield *apo*-PltL.

B.3. Expression and purification of ^{15}N/^{13}C *holo*-PltL

pET22b-PltL (C87S) and pRep4-Sfp (obtained from the laboratory of Christopher Walsh at Harvard University) plasmids were co-transformed into BL21 (DE3) cells. Isotopically labelled *holo*-PltL was expressed and purified as mentioned in section **B.2**. *holo*-PltL was isolated from Sfp (no his-tag) during Ni-NTA purification.

B.4. Preparation of prolyl-PltL, pyrrolyl-PltL

Loading of the prolyl and pyrrolyl mimetic probes were achieved by a previously described one pot chemoenzymatic reaction converting pantetheinamides to their corresponding CoA analog *in situ* using ATP and three of the *E. coli* CoA biosynthetic enzymes (CoaA, CoaD, CoaE), followed by loading onto *apo*-PltL by Sfp.[S2] In this study, this was achieved by treating 1.5 ml of

400 μM *apo*-PltL with 12.5 mM $MgCl_2$, 8 mM ATP, 2 mM mimetic **4** and **7**, 1.5 μM Sfp, 0.5 μM *E. coli* CoaA, 0.7 μM *E. coli* CoaD, 0.6 μM *E. coli* CoaE, 0.02% Triton X, 0.1% NaN_3, and 5 mM TCEP in a 150 mM NaCl and 50 mM Tris pH 7.4 buffer containing 10% glycerol. After incubation at 37°C for 12 h, the reactions were purified by FPLC into a 50 mM KPi 7.4 buffer. *Crypto*-PltL species eluted between 155-180 mL.

B.5. Preparation of *holo*-PltL

To prepare pure *holo*-PltL, *apo*-PltL from section C.3 was incubated with 50 mM Tris pH 7.5, 150 mM NaCl, 12.5 mM $MgCl_2$, 2 mM coenzyme A, 3 mM TCEP, 0.1% NaN_3 and 1.5 μM Sfp at 37 °C overnight. The solution was purified by FPLC.

B.6. HPLC analysis of ACP proteins

Loading of probes in sections C.4 was monitored by HPLC analysis. The *crypto*-PltL proteins were passed over a C18 column (Burdick & Jackson) using 10% solvent B for 5 min, then increasing 10–50% B over 5 min, and finally 50-63.3% over 10 min (solvent A = H_2O, 0.05% trifluoroacetic acid; solvent B = CH_3CN 0.05% trifluoroacetic acid). Analyses were conducted on HP 1100 series HPLC (Agilent) equipped with a G1315A DAD detector (Agilent). The elution of the protein was monitored by absorbance at 210 nm.

B.7. Protein NMR experiments

NMR samples were prepared by first concentrating the FPLC pure proteins in 50 mM KPi pH 7.4. A 450 μL aliquot of the appropriate PltL form was then prepared for NMR by adding 50 μL of D_2O, 5 μL of 10% (w/v) NaN_3 and 5 μL of 0.5 M TCEP adjusted to pH 7.4. The final concentration of the samples evaluated was as follows: 1 mM *holo*-PltL; 1 mM prolyl-PltL; 1 mM pyrollyl-PltL. After adding the sample, each NMR tube was flushed with argon, capped, and sealed with Teflon tape. All NMR spectra were acquired at 25 °C with a 1.2 s recycle delay. HSQC spectra were collected for each sample prepared in the same buffer for CSP analysis, collecting 2048 points (R+I) in the ^{1}H direct dimension and 256 points (R+I) in the ^{15}N indirect dimension. Chemical shifts were measured by peak maxima, and chemical shift perturbation was calculated using the formula $CSP = ([(0.2\delta N)^2 + (\delta H)^2]/2)^{0.5}$.

Samples were prepared and subjected to an HNCACB experiment with 2048 points (R+I) in the direct ^{1}H dimension and 96 points (R+I) in both the ^{13}C and ^{15}N indirect dimensions. Standard backbone-assignment techniques correlating the backbone amides to their CA and CB, and those for the neighboring residue were employed to confirm our peak assignments. NMR spectra were processed using nmrPipe (NIH),[S3] and analyzed using the Sparky (UC San Francisco) and CARA software suites.

B.8. PltL Protein NMR Data Collection and Structure Calculations

NMR spectra were collected at 25 °C using NMR spectrometers at the UC San Diego Biomolecular NMR Facility operating at ^{1}H frequencies of 500, 600, and 800 MHz. Backbone and sidechain assignments were made automatically assigned and manually inspected in CARA and Sparky. Initial backbone assignments were obtained through a 3D HNCACB experiment by standard backbone-assignment techniques correlating the backbone amides to their CA and CB atoms. The side chain ^{1}H and ^{13}C assignments were obtained from ^{13}C-edited NOESY-HSQC and TOCSY-HSQC experiment (mixing time 60 ms). NOE data was obtained from 3D ^{15}N-edited and ^{13}C-edited NOESY-HSQC experiments (mixing time 150 μs). NOE peaks were picked through restricted peak picking in Sparky and inspected manually. Torsion angle restraints (φ and ψ) were generated from backbone chemical shift data using TALOS-N.[S4] NOE peaks were subject to automated assignment and structure calculations with CYANA[S5] in accordance with

the chemical shift list and TALOS-N restraints. After CYANA calculations, the family of structures were refined in discrete solvent using the Crystallography and NMR System (CNS) 1.3,[S6,S7] and scripted by Roberto Tejero's WaterRefCNS[3]. Output files were analyzed and prepared for submission by PdbStat.[S8] Topology, parameter, and linkage files for phosphopantetheinylated serine (PNS) and pyrrole phosphopantetheinylated serine (PYA) were constructed using a combination of known atom types and additional information from the PRODRG server.[S9]

Table S1. Solution NMR Structure Statistics

Assignment Statistics (%) (residues 1-90)	*holo*	*pyrrolyl*
backbone H	97.8	97.8
backbone non-H	97.4	64.4
side chain H	98.6	98.4
side chain non-H	78.5	75.7
Structure Calculations		
Distance restraints	3023	3275
short-range, | *i-j* | <= 1	2217	2584
medium range, 1<| *i-j* |<5	476	381
long-range, | *i-j* |>=5	330	310
Dihedral Angle Restraints	139	130
Structure Statistics		
Average final CYANA target function ($Å^2$)	1.32	1.2
Average cycle1 CYANA target function ($Å^2$)	40.10	64.27
CNS energies (kcal/mol)	-2949.35	-2932.7
Restraint Violations		
Max. distance restraint violation (Å)	0	0.07
Violated distance restraints > 0.1 Å	0	0
Violated dihedral angles	0	0
CYANA Ramachandran Plot		
Residues in most favored regions	82.1%	83.3%
Residues in additionally allowed regions	17.9%	15.7%
Residues in generously allowed regions	0.0%	0.9%
Residues in disallowed regions	0.0%	0.1%
CYANA RMSD (residues 4-86)		
Average backbone RMSD to mean	0.40 ± 0.09 Å	0.55 ± 0.11 Å
Average heavy atom RMSD to mean	0.84 ± 0.08 Å	1.06 ± 0.12 Å

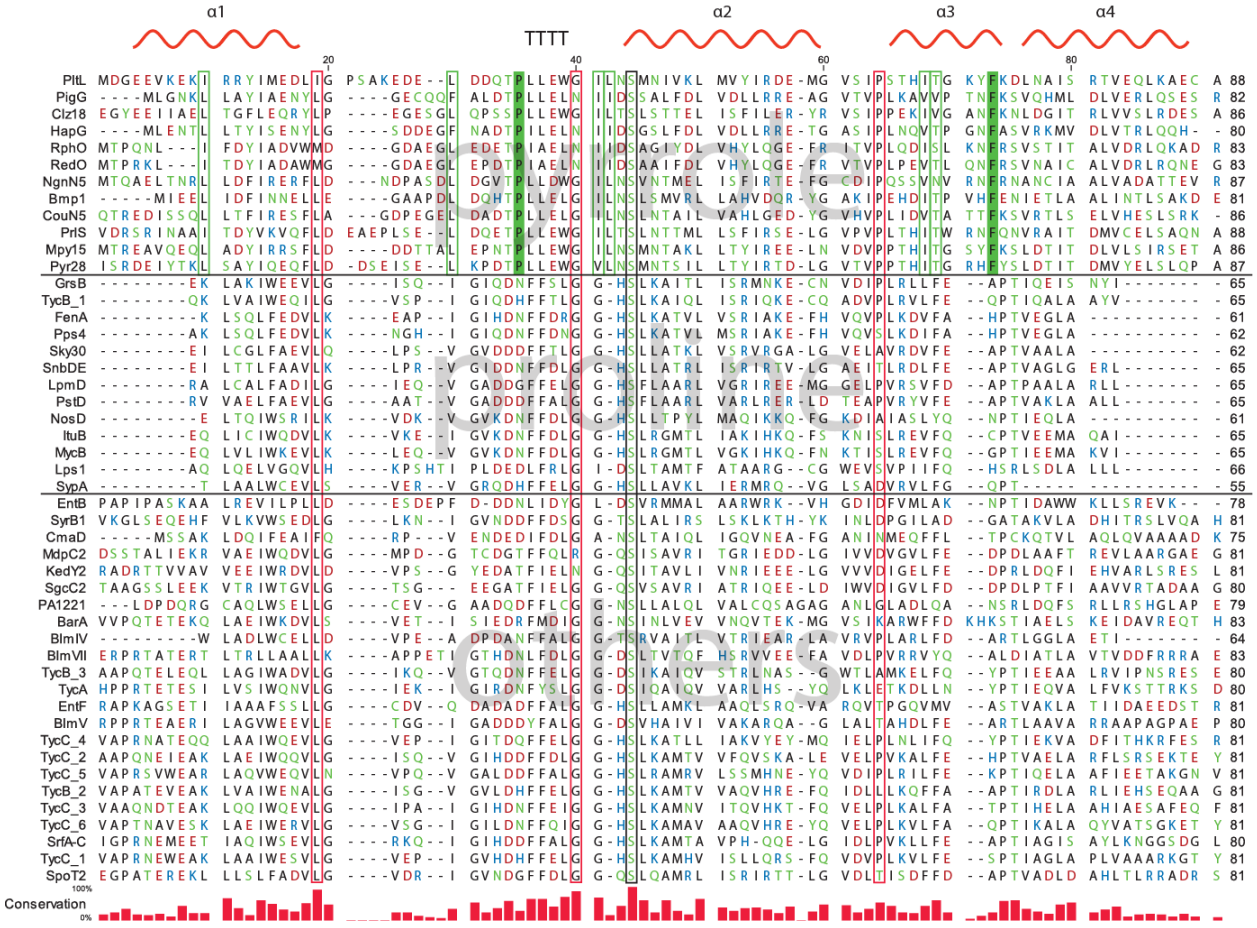


Figure S1 - Sequence alignment of PCPs generated with MUSCLE.[S11] The sequences are separated by the natural final product that tethers to the PCP, in this case, pyrrole, proline, and other amino acids. The three groups are separated by horizontal black lines and the final peptidyl product is listed in the background in large font. The secondary structure of PltL is depicted above the sequences. Red, blue, green, and black font indicate negative, positive, polar, and nonpolar amino acids, respectively. We included many proline CPs to compare with the pyrrole CPs, since the pyrrole CPs load a proline also. We added various other PCPs in which the structure is known (SrfA-C, PA1221, EntF, etc…) and which are standalone type II PCPs (KedY2, SgcC2, CmaD, etc…).

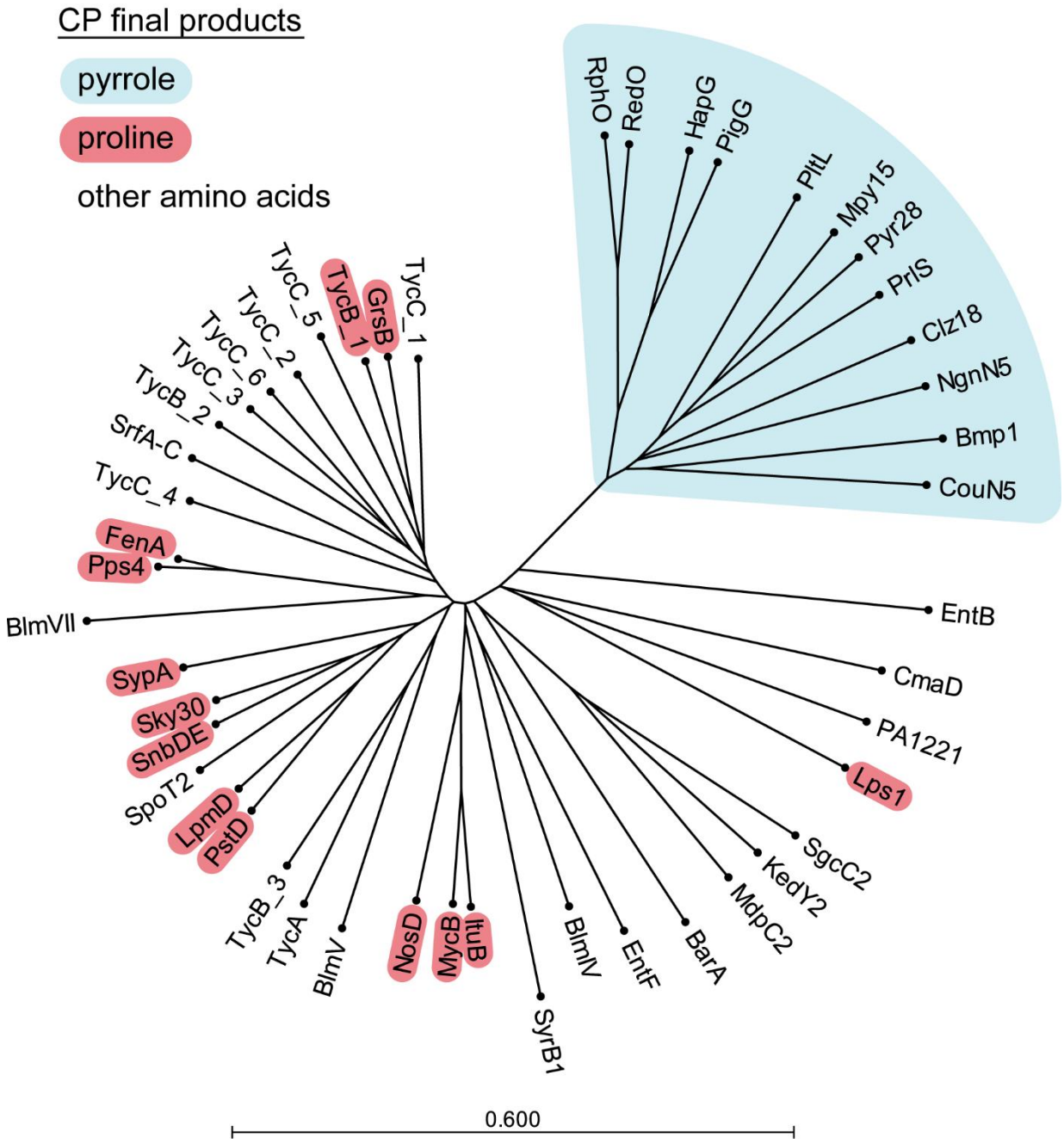


Figure S2 - Phylogenetic tree of PCPs generated with ClustalW.[S12]

Figure S3 - 20% UREA Page Gel of apo, holo, prolyl, and pyrrolyl PltL. The PltL Species were stained with coomassie dye. To note, *apo* PltL runs slower in the gel possible due to having a more positive charge (does not contain the Ppant) and/or a more relaxed conformation, which is common for carrier proteins (a wider species will travel slower in the gel).

Figure S4A – HPLCMS data for prolyl-PltL.

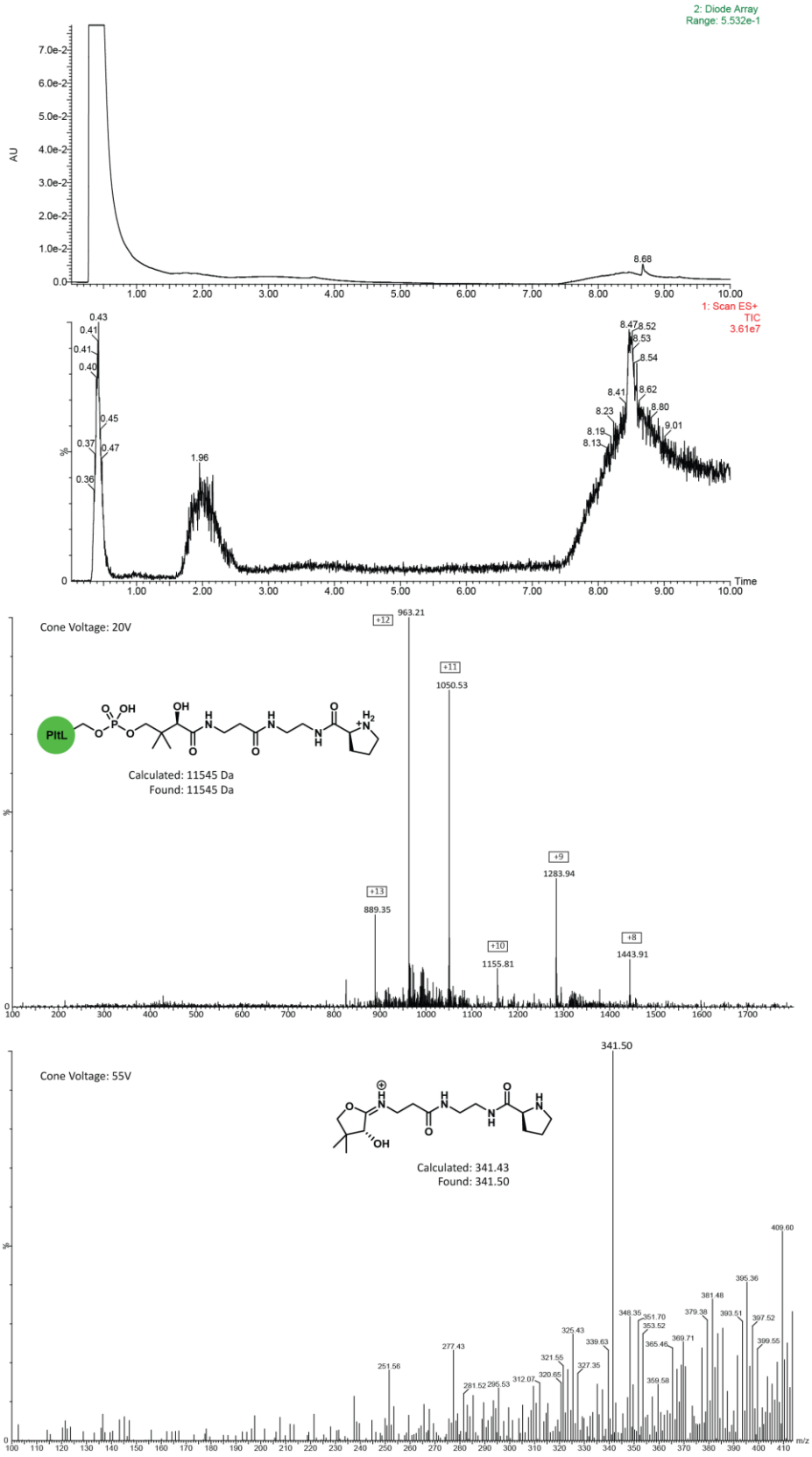


Figure S4B – HPLCMS data for pyrrolyl-PltL.

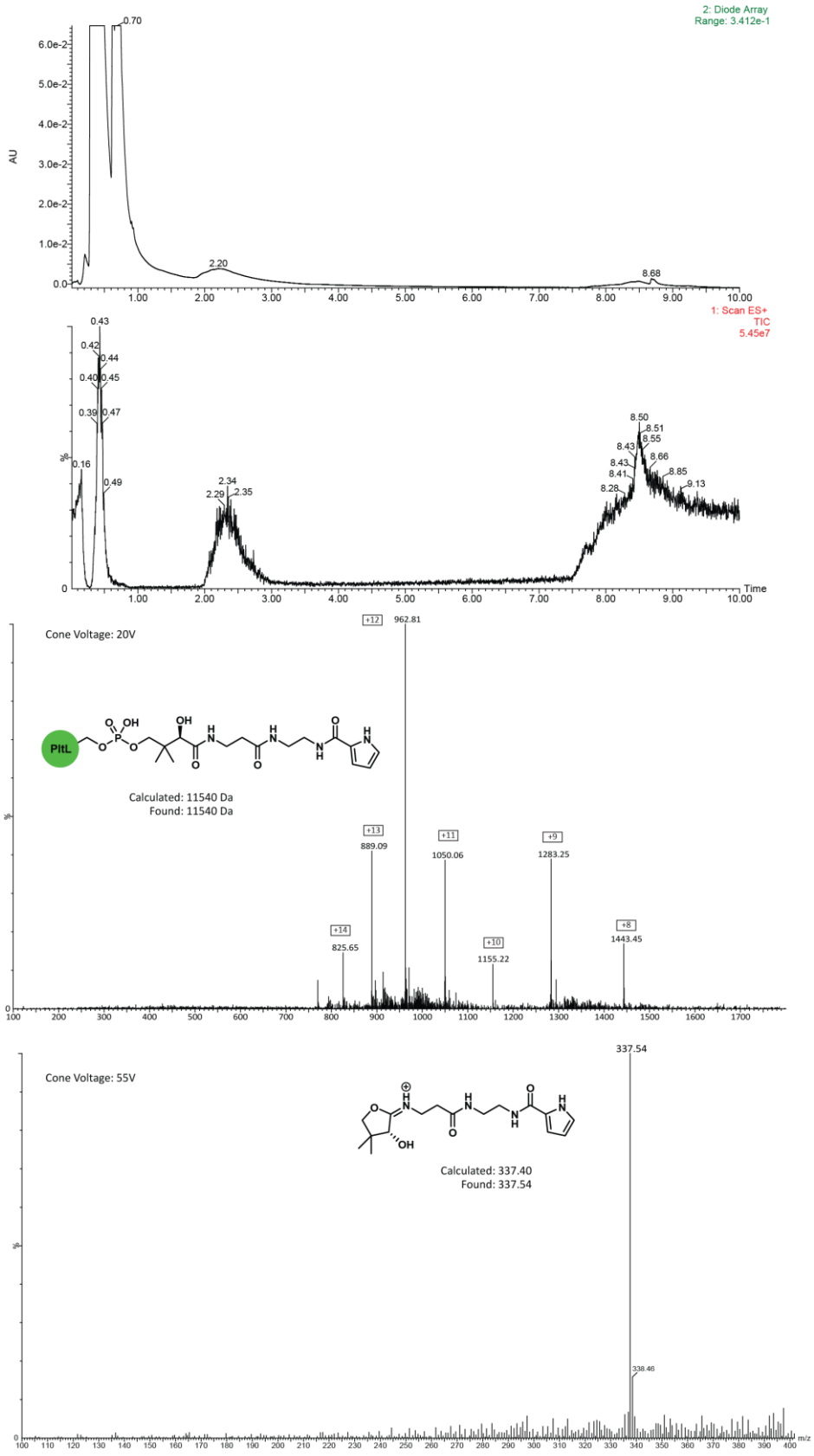


Figure S4 – HPLC-MS spectra of proylyl- and pyrrolyl-PltL species. PltL species from in vitro loading (see section C.4) were analyzed by HPLC-MS. HPLC-MS analysis was performed on a Waters Acquity system using an Acquity binary solvent manager, an Acquity column manager, a 2777c robotic autosampler, an Acquity TUV detector and an SQ detector. Samples in buffer (2 µl) were separated on a Waters C18 Xbridge (3.0 x 50mm, 2.5µ) at 0.8 ml/min using a linear gradient of 95% H_2O containing 0.1% formic acid to 30% acetonitrile, containing 0.1% formic acid, in 30 seconds, followed by a linear gradient to 50% acetonitrile (containing 0.1% formic acid) in 7 minutes, followed by cleaning and re-equilibrating the column (total run time of 10 minutes). The column was maintained at 55 °C. Protein molecular weights were calculated using Expasy (http://web.expasy.org/protparam/) and ESI-MS (operated in positive mode) data deconvoluted using Spectraphile (http://home.iprimus.com.au/pakholt/lcms/spectraphile.html). A) UV absorbance chromatogram at 273 nm and total ion count (TIC) MS chromatogram of prolyl-PltL. The mass specta corresponds to the peak at 1.96 minutes. The top and bottom spectra corresponds to the PltL fragmentation pattern at a cone voltage of 20V and the MS-ejection[S13] of the phosphopantetheine probe at a cone voltage of 55V, respectively. B) UV absorbance chromatogram at 273 nm and total ion count (TIC) MS chromatogram of pyrrolyl-PltL. The mass spectrum corresponds to the peak at 2.34 minutes. The top and bottom spectra corresponds to the PltL fragmentation pattern at a cone voltage of 20V and the MS-ejection[S7] of the phosphopantetheine probe at a cone voltage of 55V, respectively.

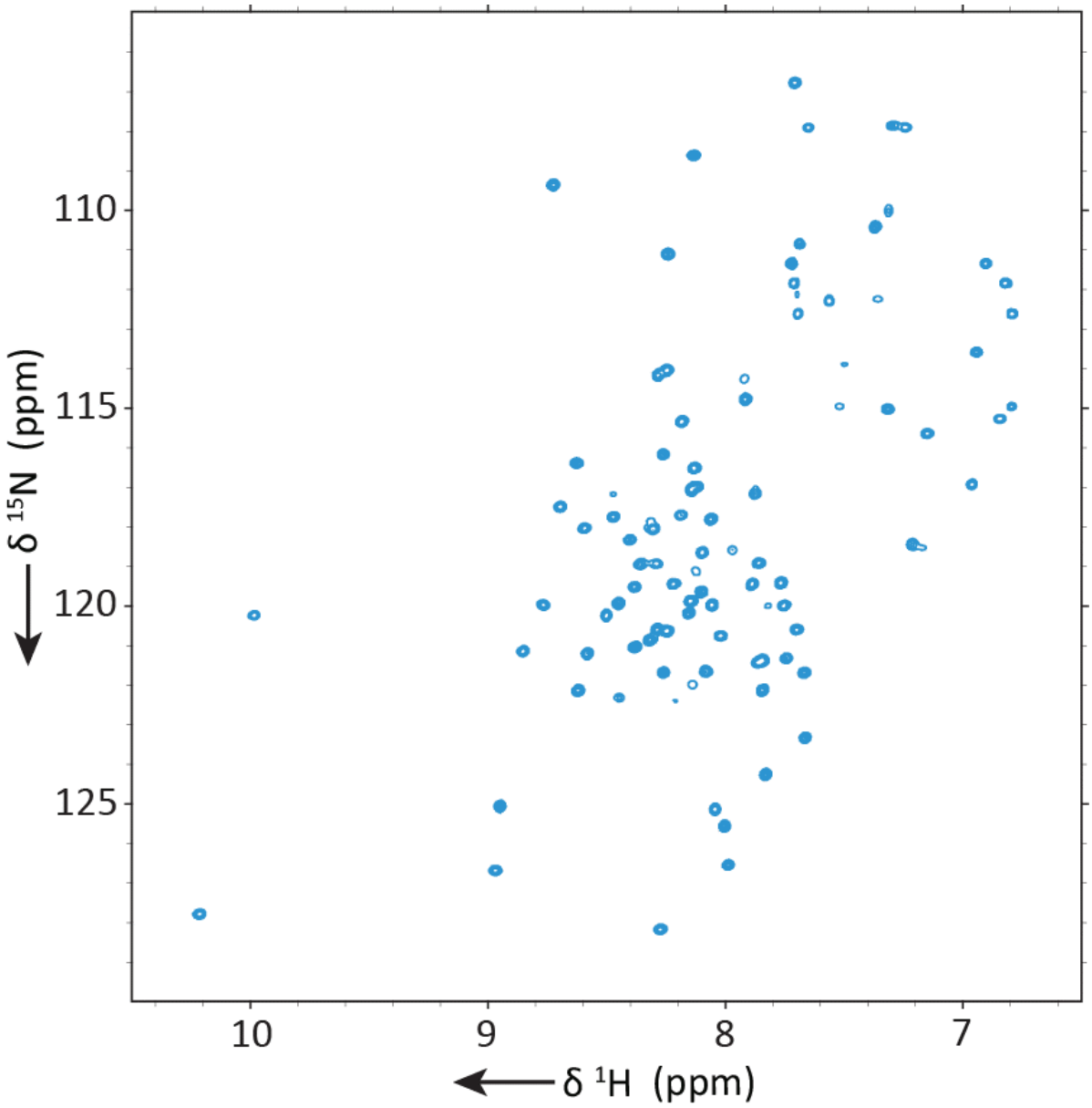


Figure S5 - ^{1}H-^{15}N HSQC of *holo*-PltL.

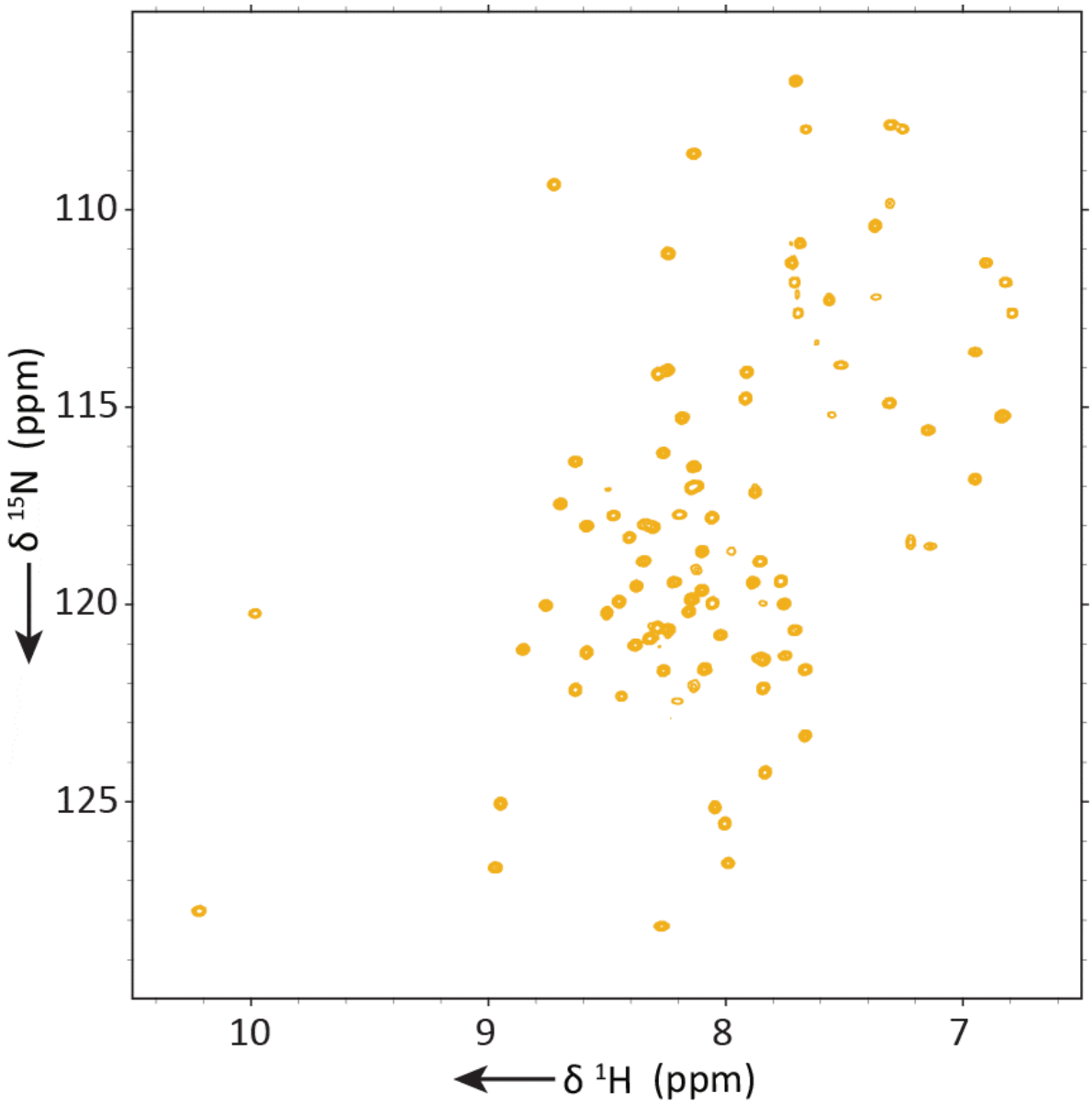


Figure S6 - ^{1}H-^{15}N HSQC of prolyl-N-PltL.

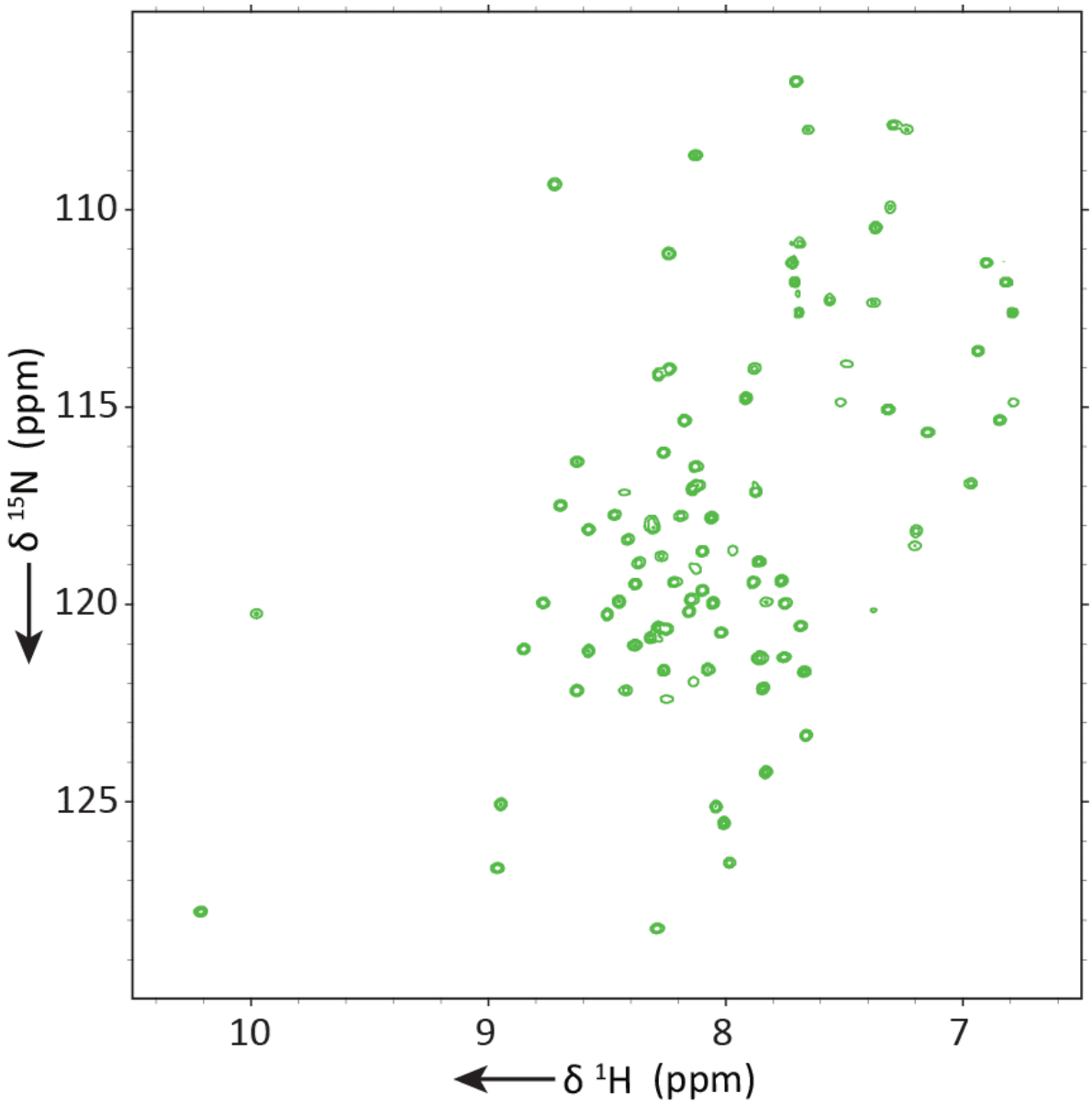


Figure S7 - ^{1}H-^{15}N HSQC of pyrrolyl-N-PltL.

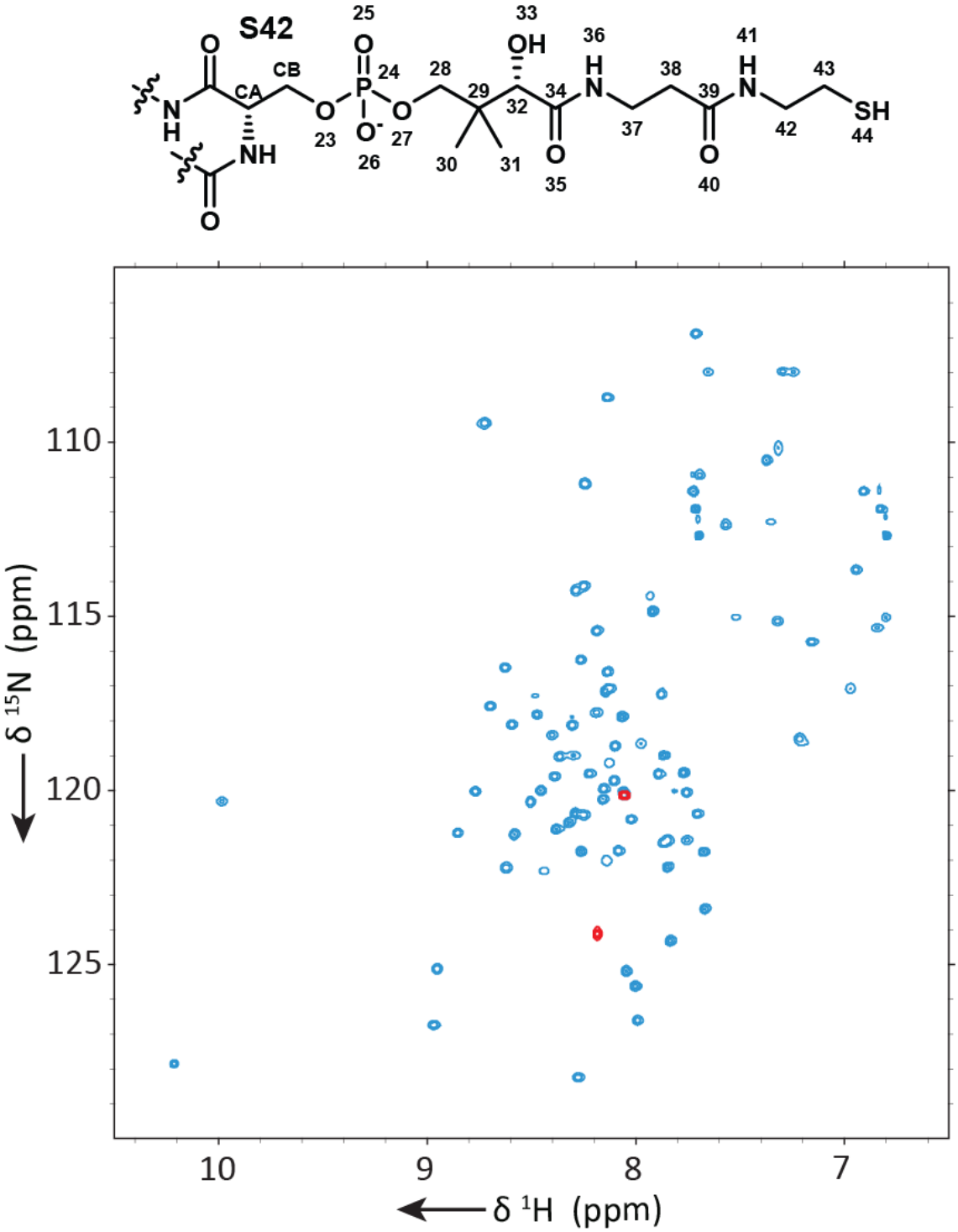


Figure S8 - ^{1}H-^{15}N HSQC of ubiquitous ^{15}N-labelled *holo*-PltL. The red peaks correspond to the phosphopantetheine amides (H-N36: δ 8.05, 120.1; H-N41: δ 8.19, 123.9). The phosphopantetheine arm was ^{15}N-labelled by co-expressing PltL and Sfp in BL21 cultured in ^{15}N minimal media.

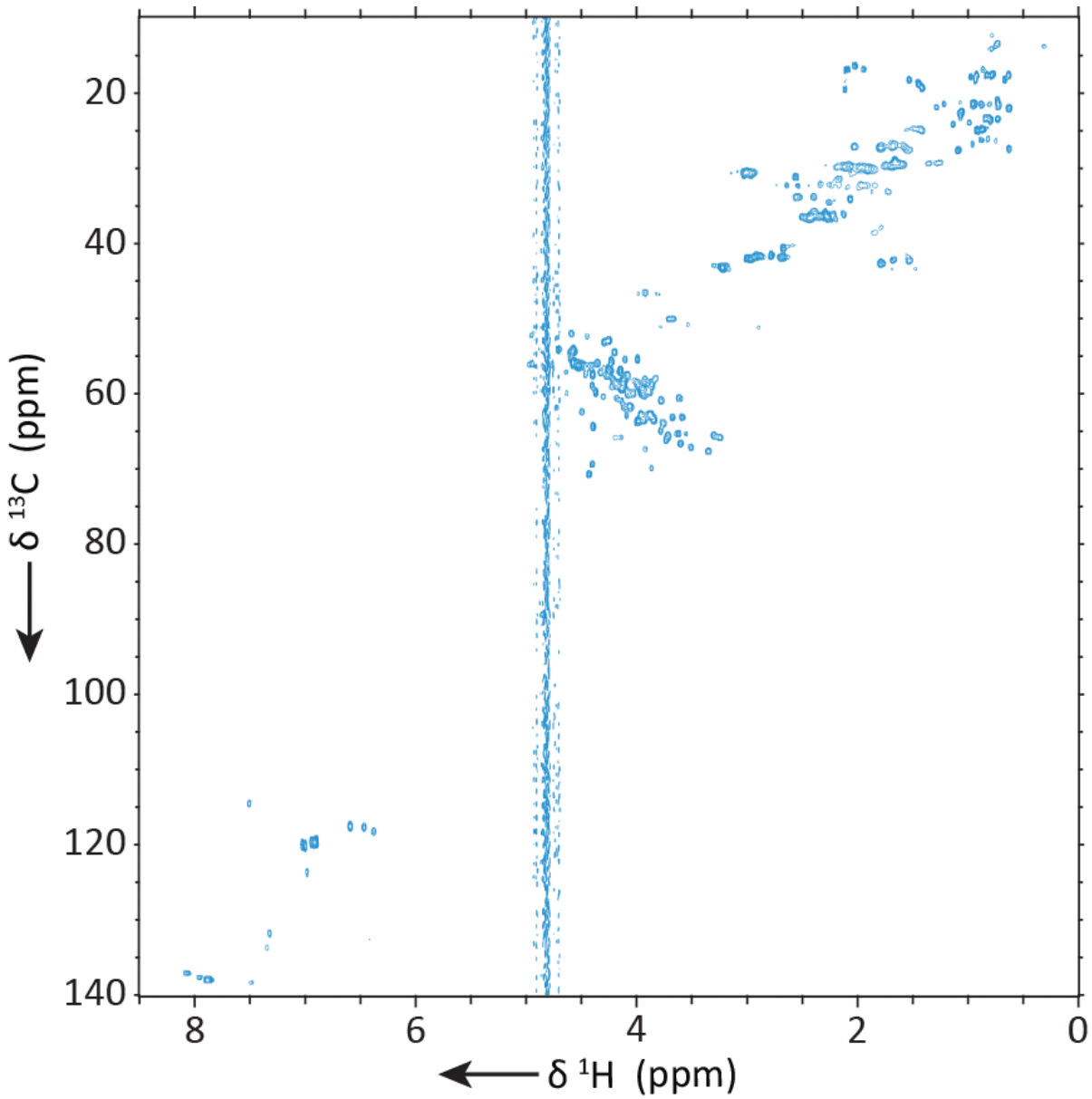


Figure S9 - ^{1}H-^{13}C HSQC of *holo*-PltL.

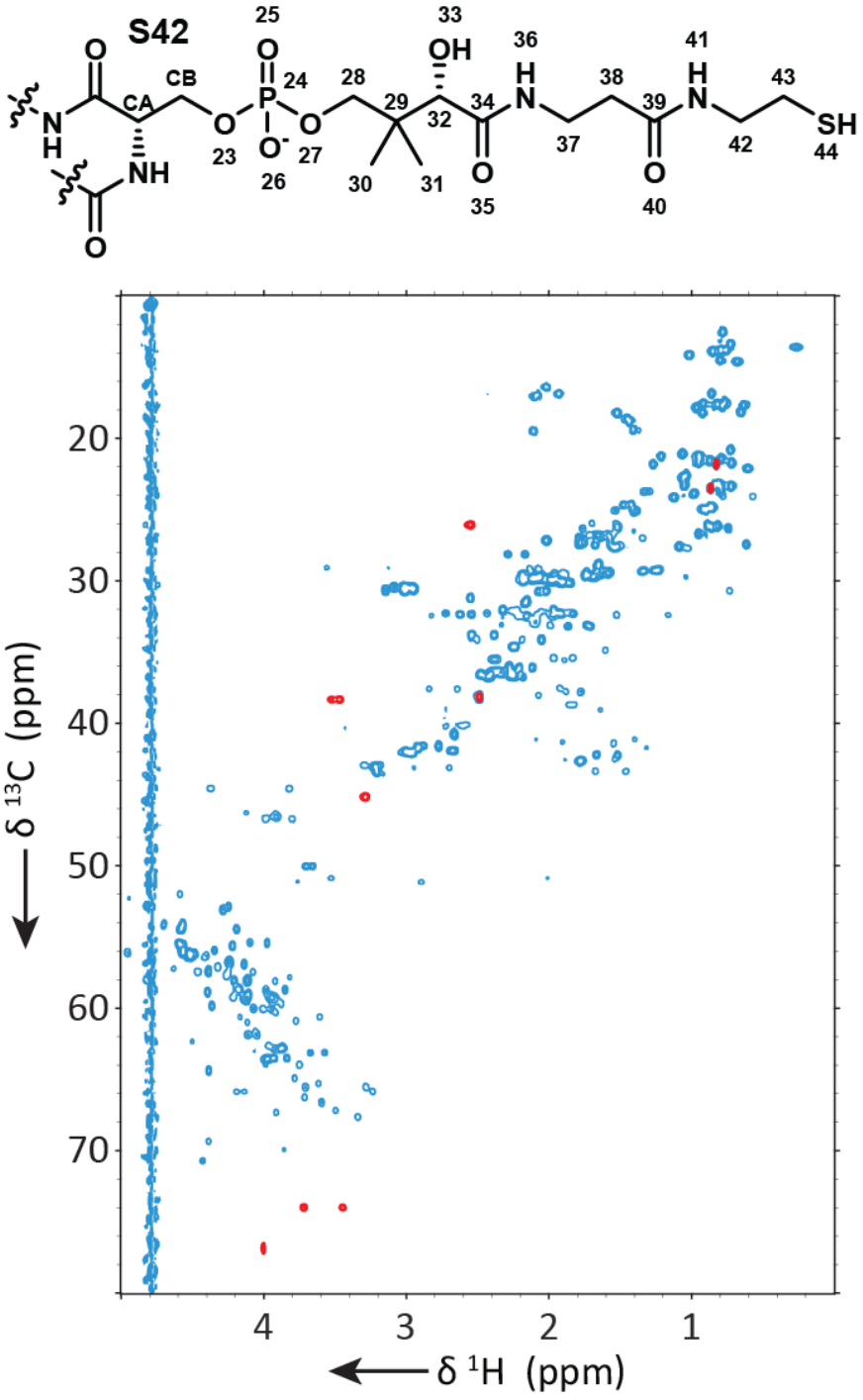


Figure S10 - ^{1}H-^{13}C HSQC of ubiquitous ^{15}N,^{13}C-labelled *holo*-PltL. The red peaks correspond to the phosphopantetheine aliphatic proton-carbon pairs (H-H-C28: δ 8.45 , 3.72 , 73.9; H-C30: δ 0.83, 21.8; H-C31: δ 23.53, 0.87; H-C32: δ 4.00, 76.9; H-H-C37: δ 3.47, 3.53, 38.3; H*-C38: δ 2.49, 38.1; H*-C42: δ 3.29, 45.2; H*-C43: δ 2.55, 26.1). The phosphopantetheine arm was ^{13}C-labelled by co-expressing PltL and Sfp in BL21 cultured in ^{13}C minimal media. * denotes pseudoatoms.

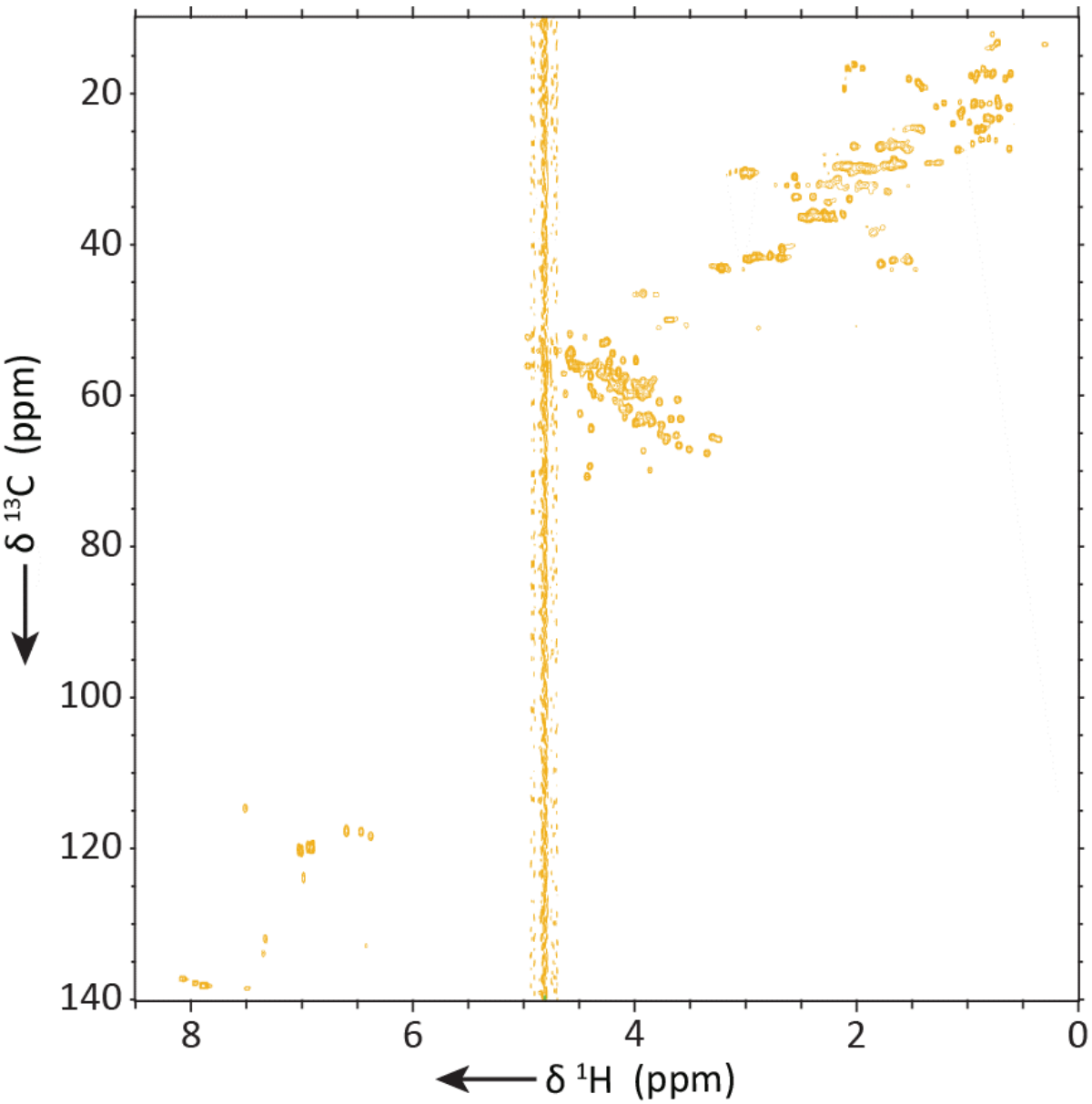


Figure S11 - ^{1}H-^{13}C HSQC of prolyl-PltL.

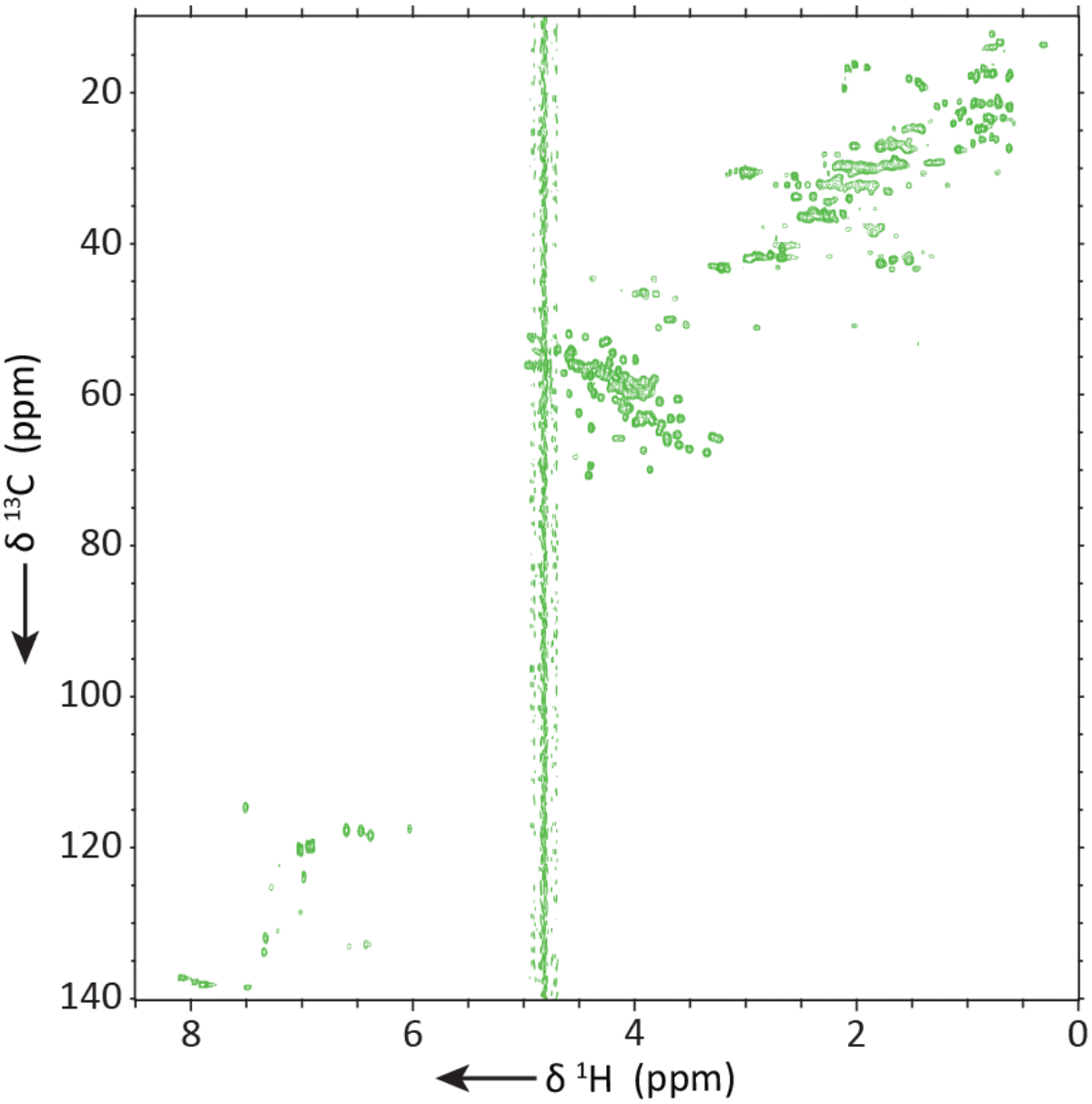


Figure S12 - ^{1}H-^{13}C HSQC of pyrrolyl-PltL.

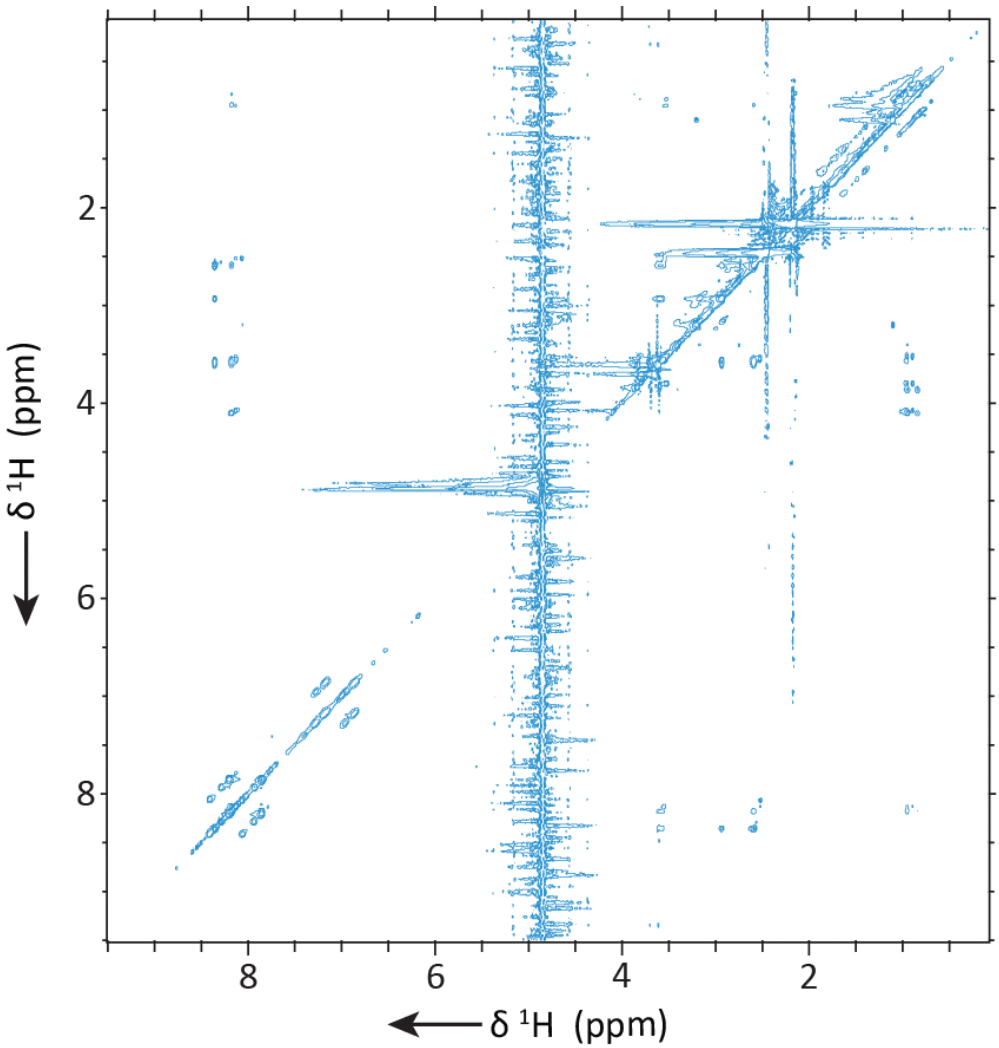


Figure S13 - $^{13}C/^{15}N$ *F*1F2-filtered NOE spectrum of *holo*-PltL. Only intramolecular NOEs of the unlabeled phosphopantetheine are visible.

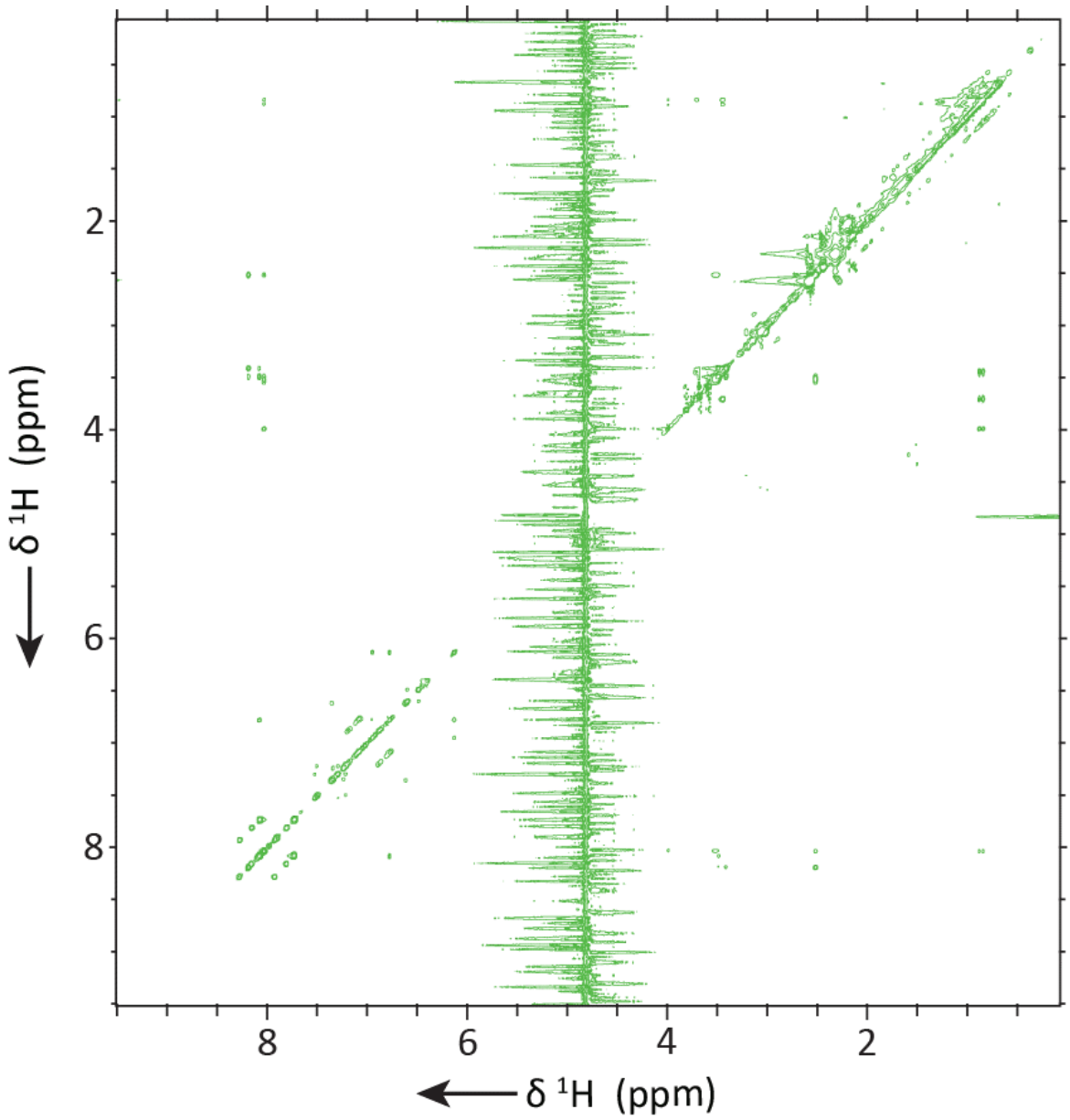


Figure S14 - $^{13}C/^{15}N$ *F*1F2-filtered NOE spectrum of pyrrolyl-N-PltL. Only intramolecular NOEs of the unlabeled phosphopantetheine-N-pyrrolyl probe are visible.

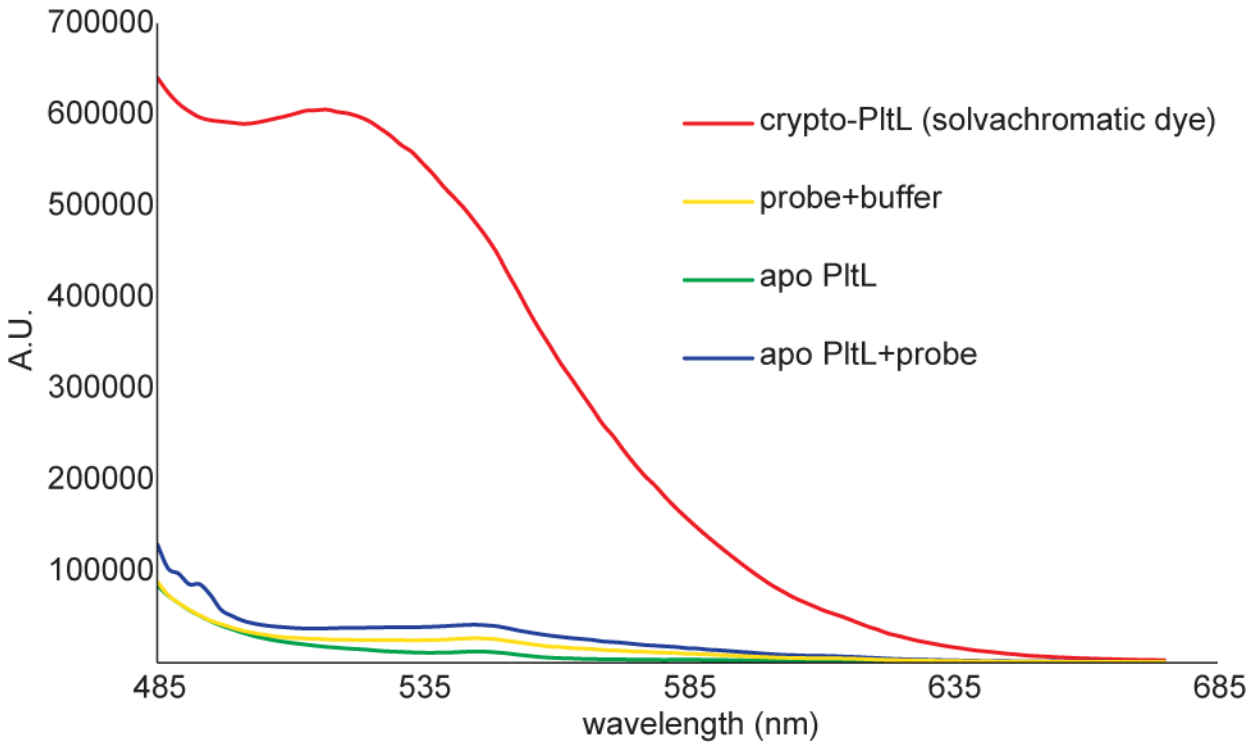


Figure S15 - The interaction of PltL and attached solvatochromic 4-DMN (4-N,N-dimethyl-amino-1,8-naphthalimide) pantetheine probe. We recently developed solvatochromatic pantetheine probes to observe interactions between attached substrates and carrier proteins. 4-DMN is fluorescent when exposed to a hydrophobic environment. Fluorsecence is only seen when the 4-DMN pantetheine probe is attached to PltL (red) and not fluorescence when the 4-DMN is alone in solution (yellow) or in a solution with apo PltL (blue). For methods, see Beld et. al 2014 publication.[S14]

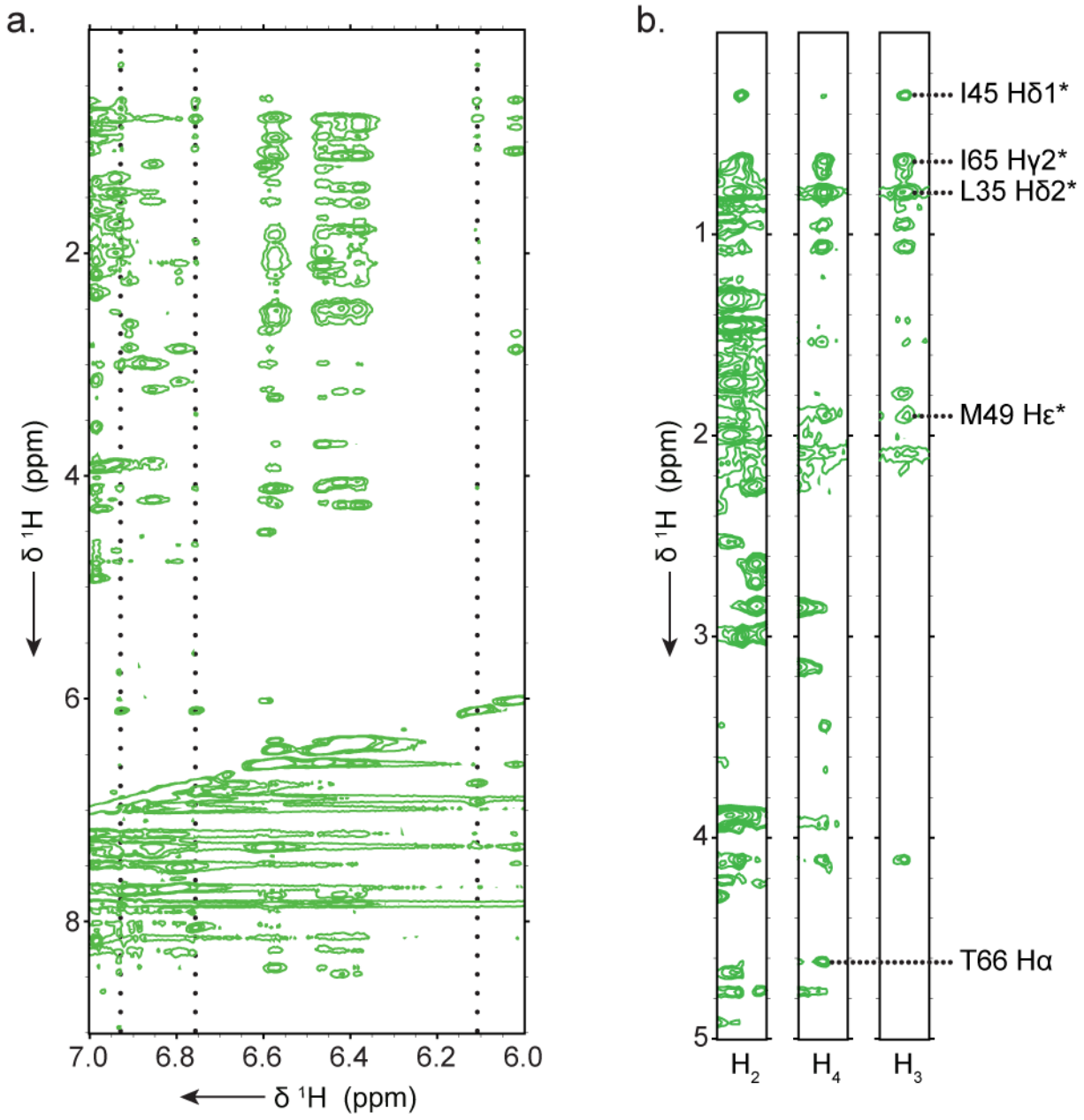


Figure S16 - ^{1}H-^{1}H NOE spectra of pyrrolyl-N-PltL focused on the protons of the pyrrole. a, Pyrrolyl-N-PltL dissolved in H_2O/KPi buffer. Dotted lines indicate the resonances of the pyrrole protons (δ 6.93, 6.76, 6.11). b, NOE strips of the pyrrole protons with peaks corresponding to atoms in PltL labelled.

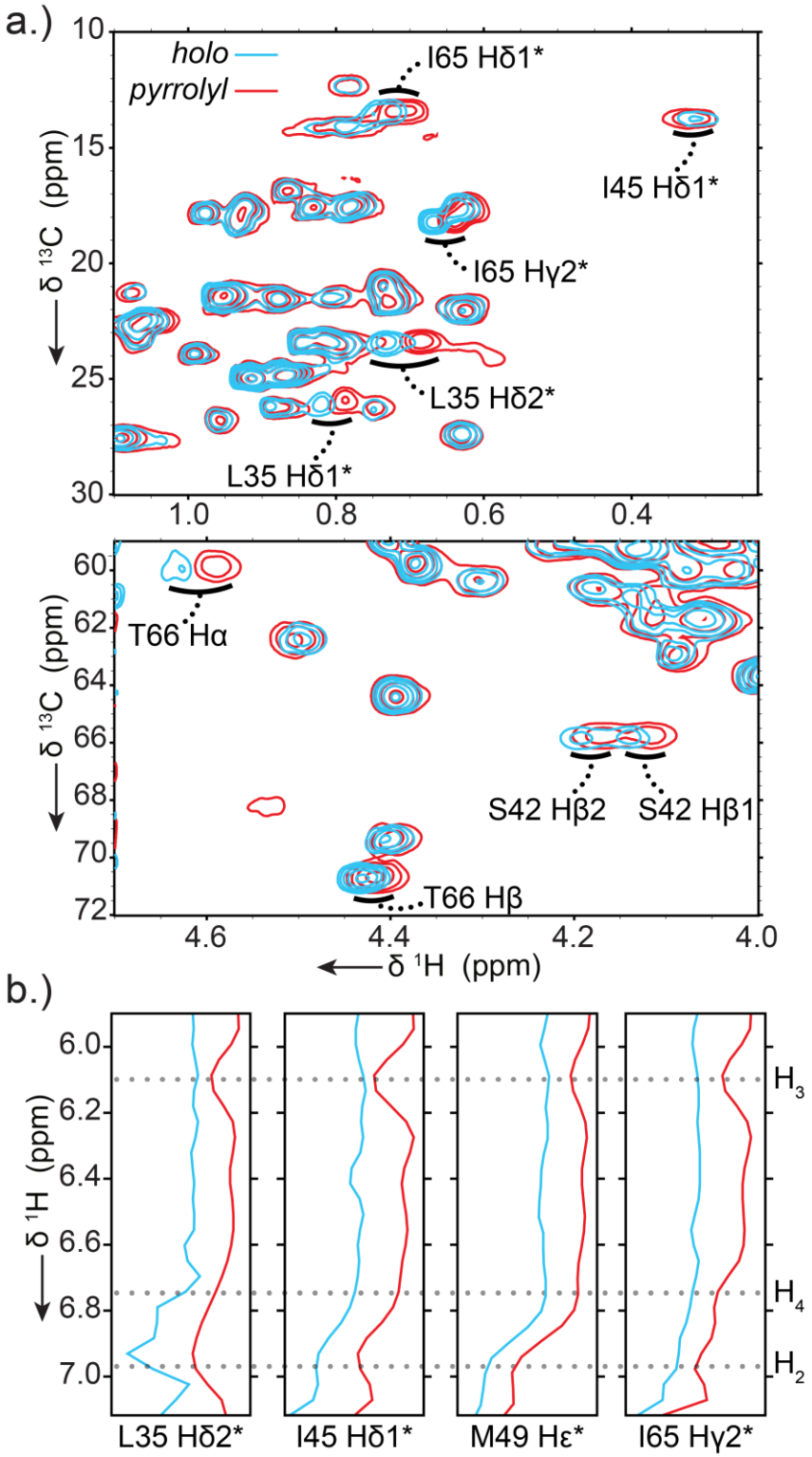


Figure S17 – Pertrubations and NOEs of PltL due to pyrrole. a, Chemical shift perturbations due to pyrrole attachment of key atoms in PltL. Shown are methyl (top) and alpha (bottom) regions of overlaid *holo* (blue) and pyrrolyl (red) ^{1}H-^{13}C HSQCs. b, 1D slices of L35 Hδ2*, I45 Hδ1*, M49 Hε*, and I65γ2* NOE strips. Dotted lines indicate the resonances of the pyrrole protons (δ 6.93, 6.76, 6.11).

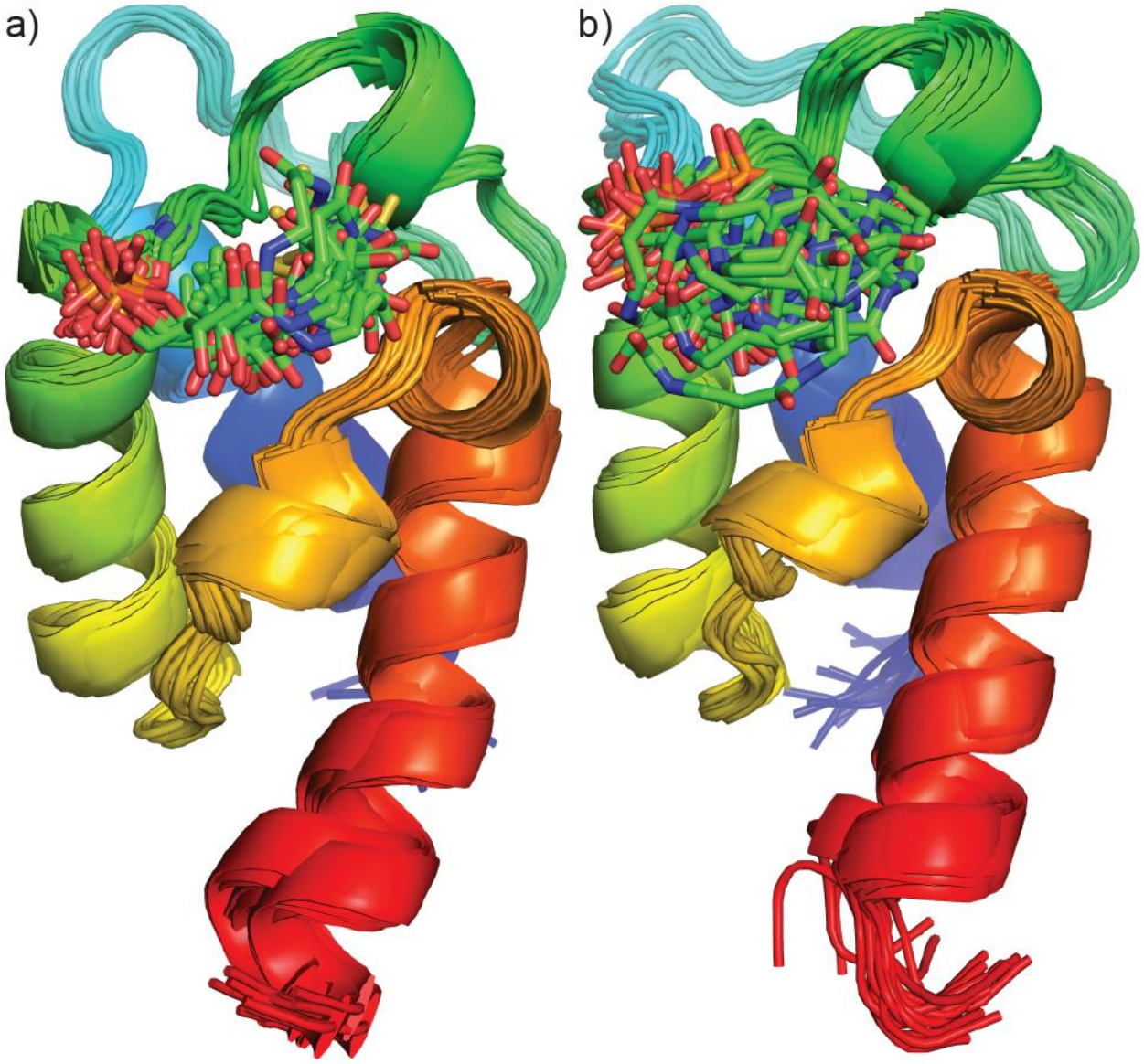


Figure S18 - Solution NMR structures of PltL species, represented as a family of structures with the 20 lowest CYANA target functions. a, *holo*-PltL. b, Pyrrolyl-PltL.

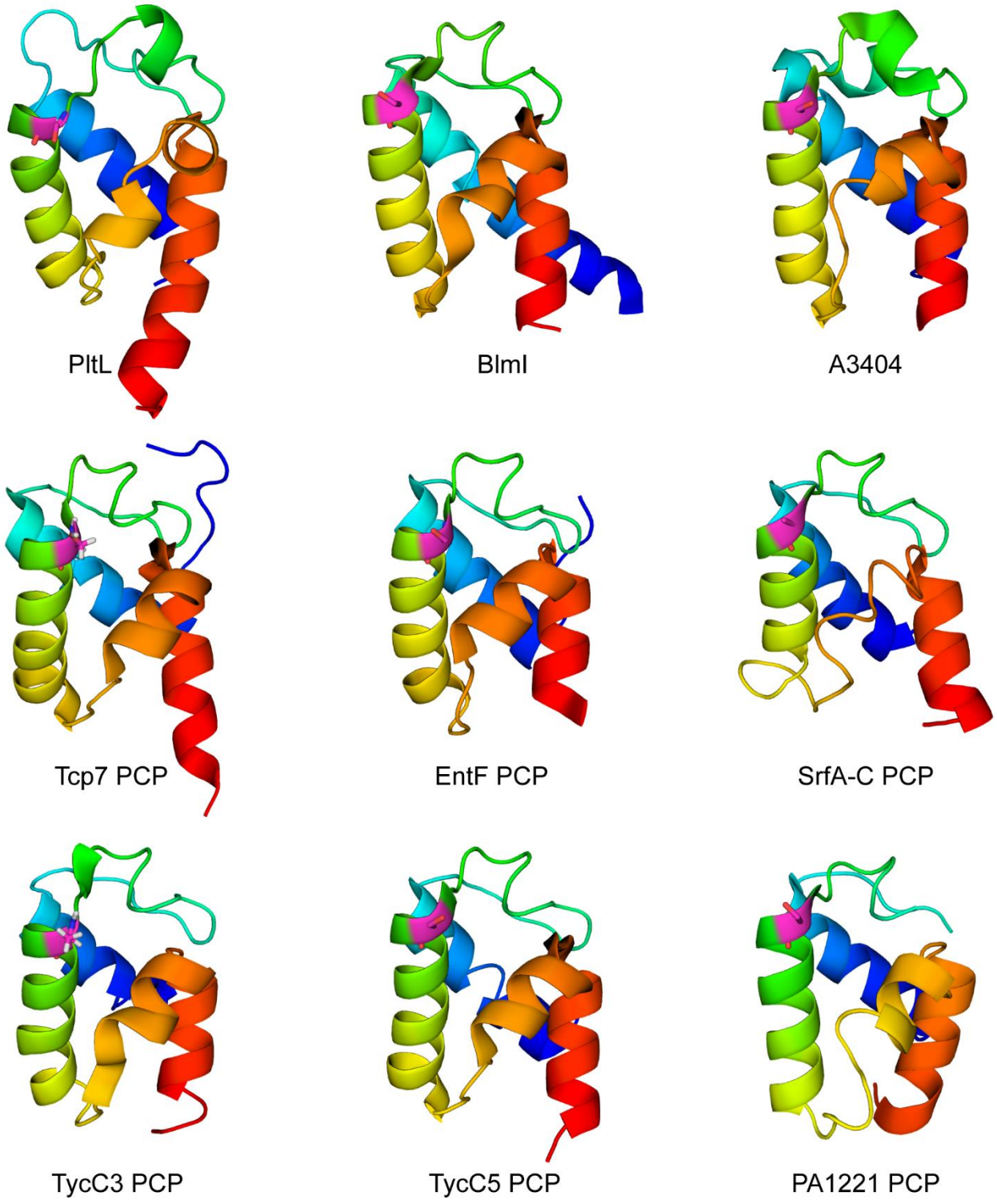


Figure S19 - Structure comparison of PltL to other peptidyl carrier proteins. The top row consists of all type II PCPs structure determined. PDB IDs: PltL (2n5h), 4neo (BlmI), 4hkg (A3404), 2mr8 (Tcp7 PCP), 3tej (EntF PCP), 2vsq (SrfA-C PCP), 4mrt (TycC3 PCP), 2jgp (TycC5 PCP), 4dg9 (PA1221). The conserved PPant serine is highlighted in magenta.

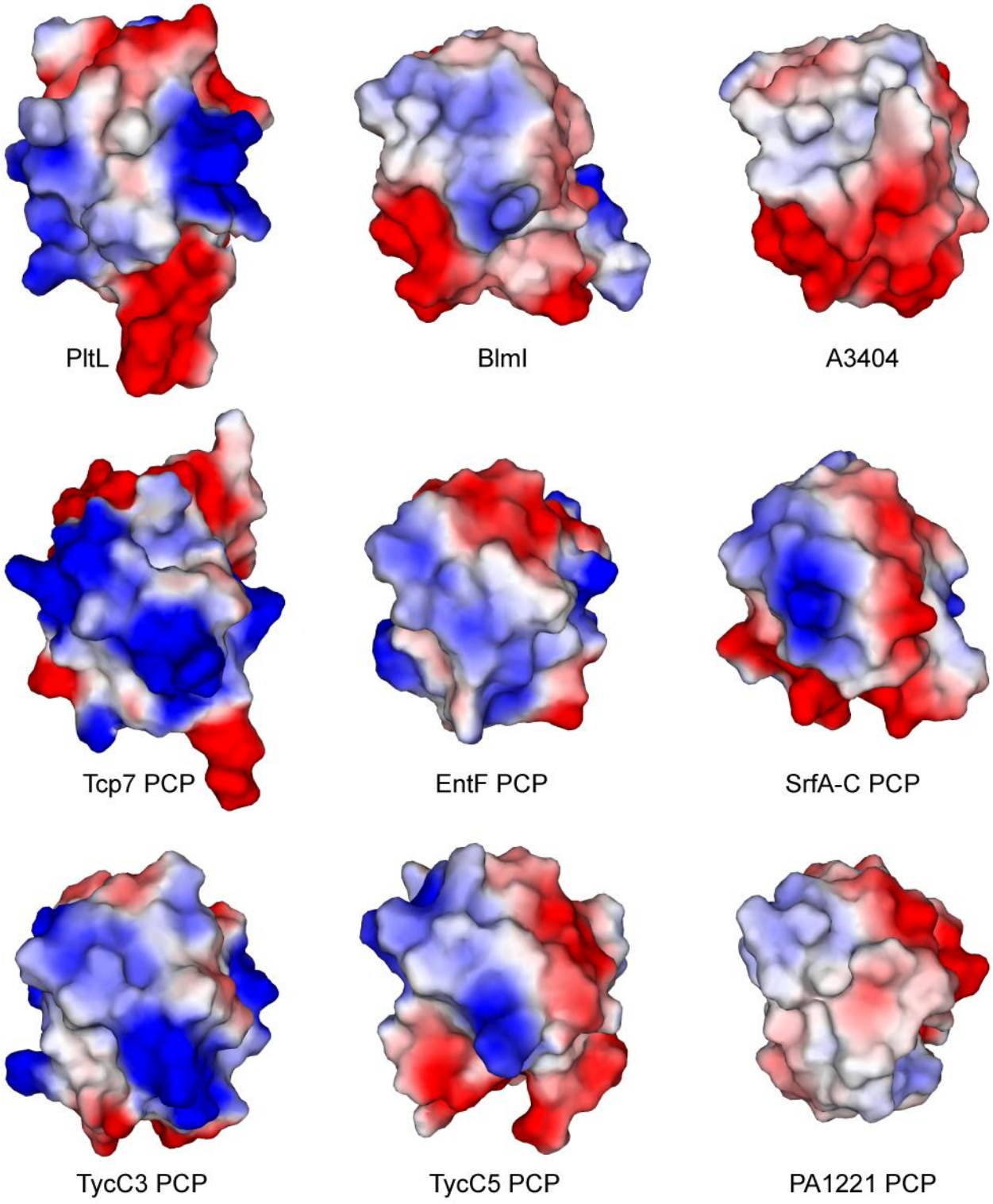


Figure S20 - Electrostatic comparison of PltL to other peptidyl carrier proteins. The top row consists of all type II PCPs structure determined. Carrier proteins are in the same orientation and order as Fig. S19. Red, white, and blue are negative, neutral, and positive charged, respectively.

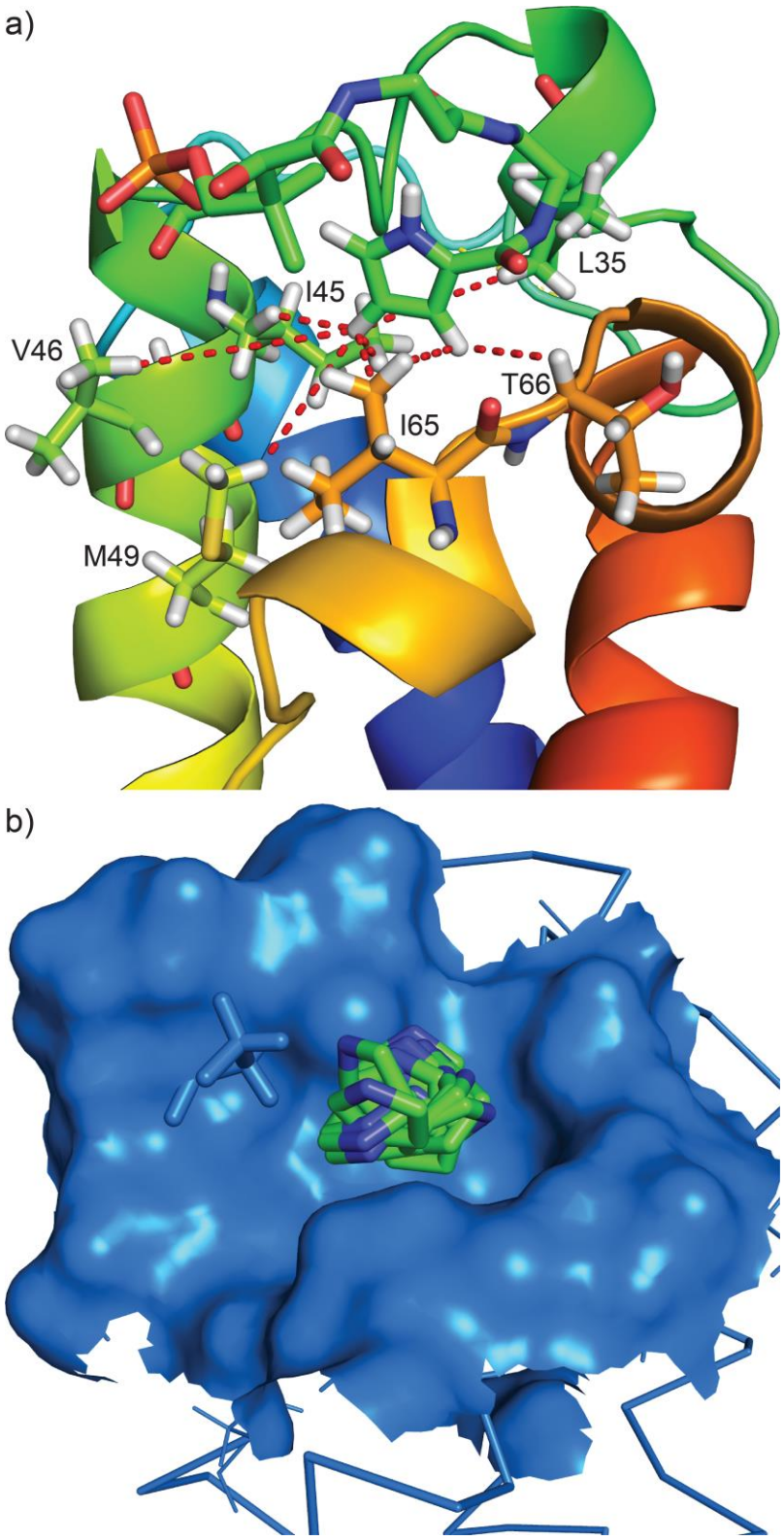
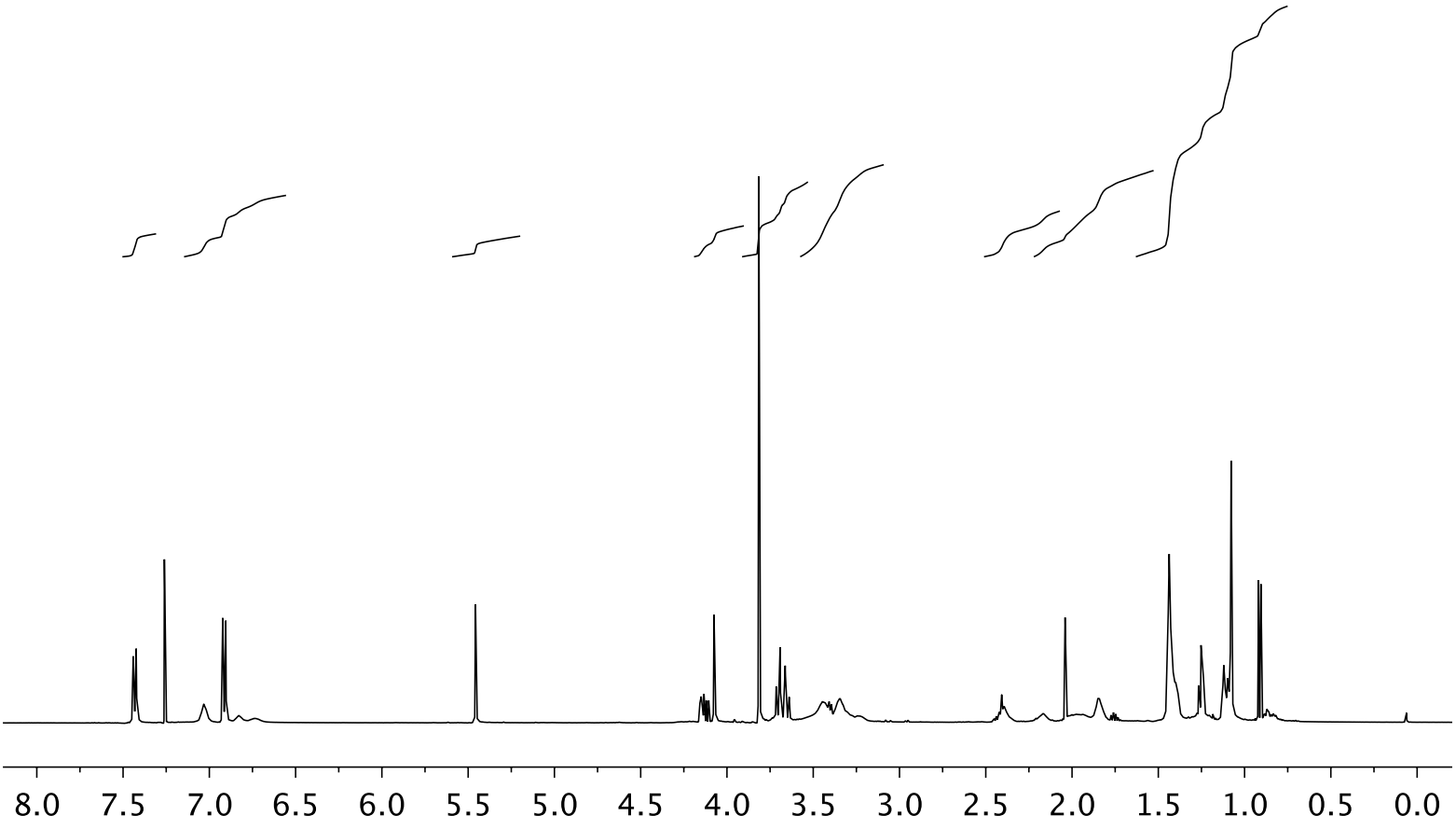


Figure S21 – NOEs between PltL and the pyrrole. a, Key NOEs are shown between the pyrrole and PltL. b, Pyrrole sequestration into the helix II/III hydrophobic pocket of PltL. 20 pyrrole rings are shown from the 20 lowest target function structures from CYANA.

Figure S22. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra of **3** in $CDCl_3$



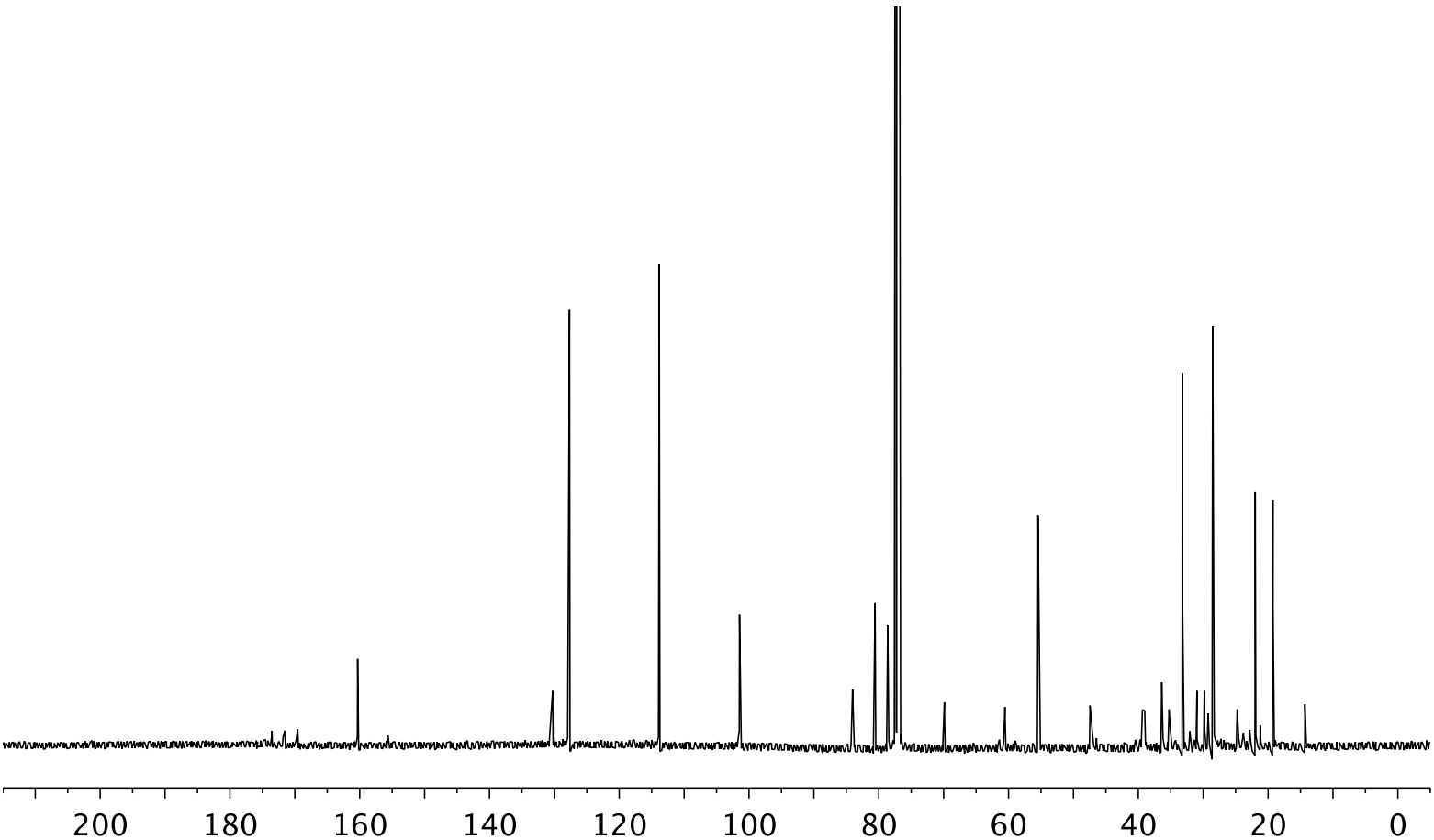
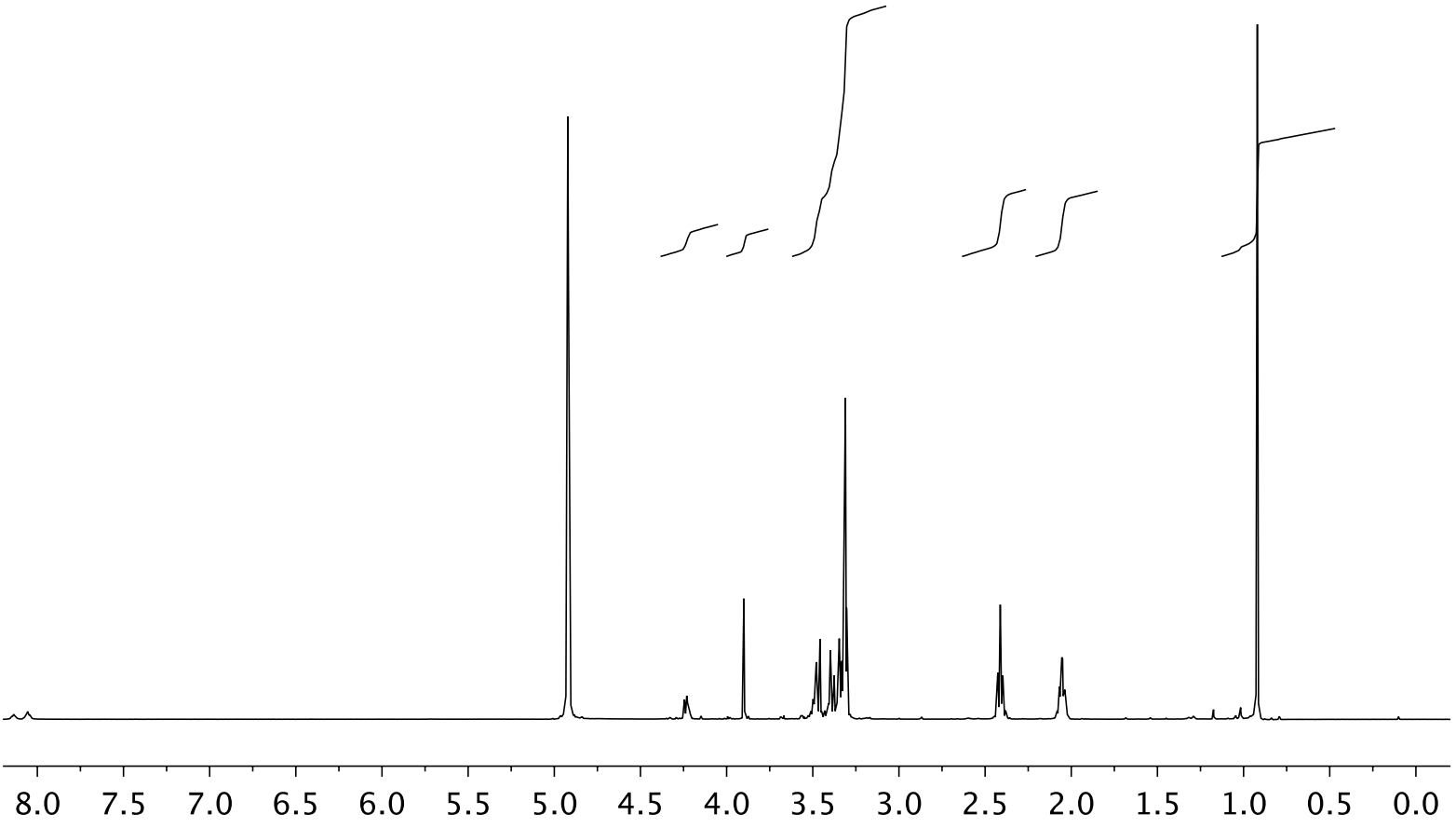


Figure S23. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra of **4** in CD_3OD.



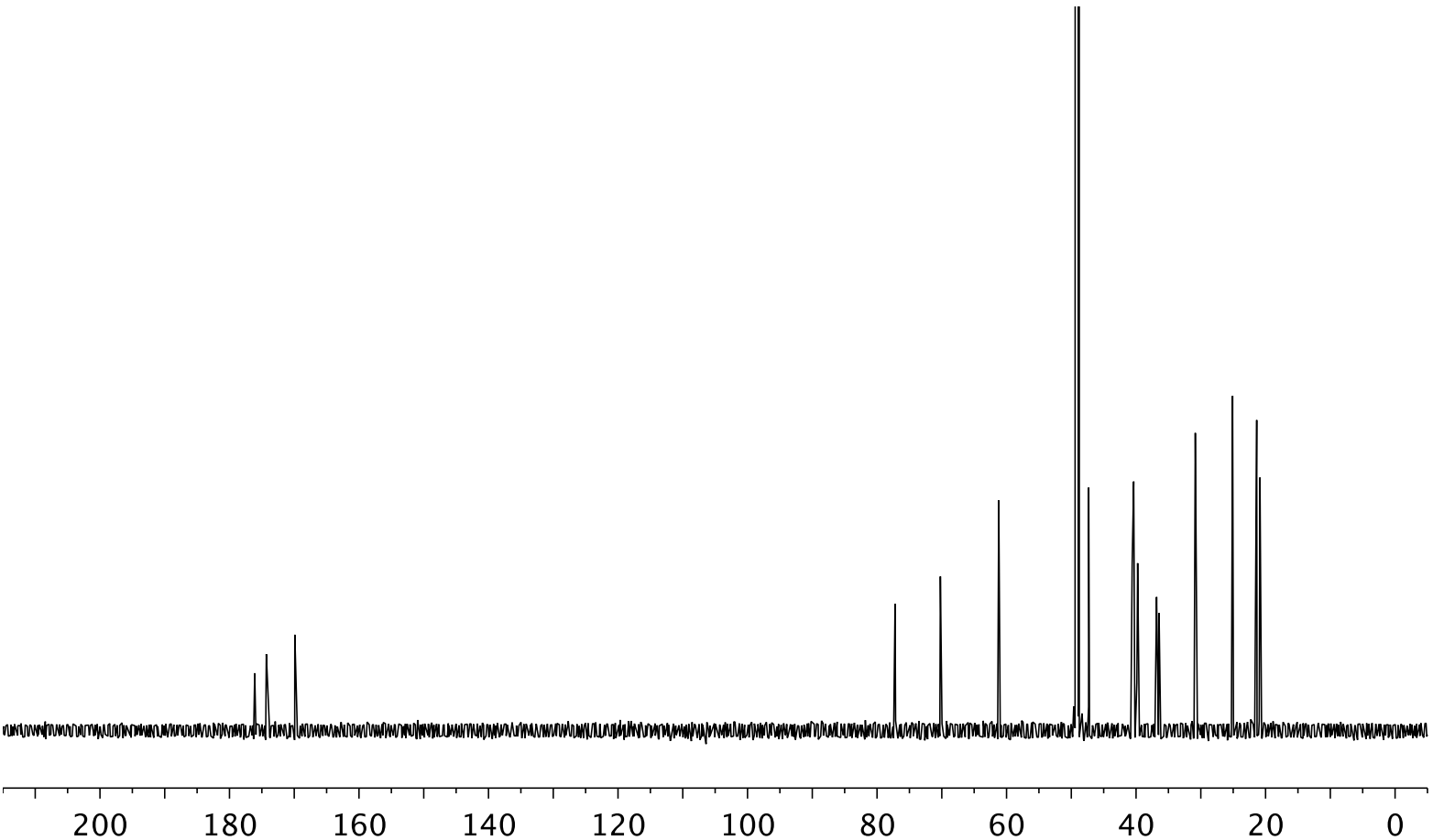
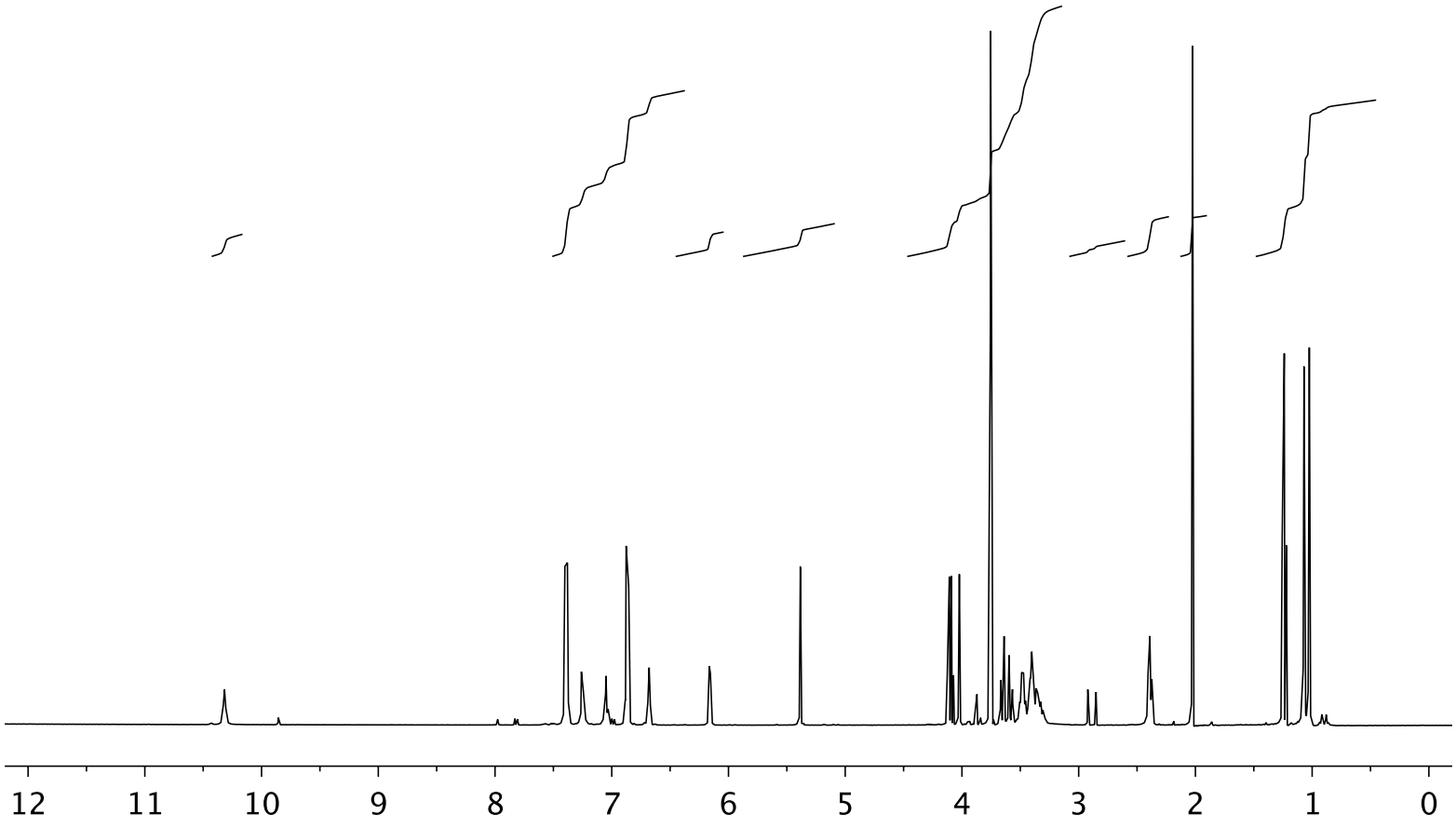


Figure S24. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra of **6** in $CDCl_3$



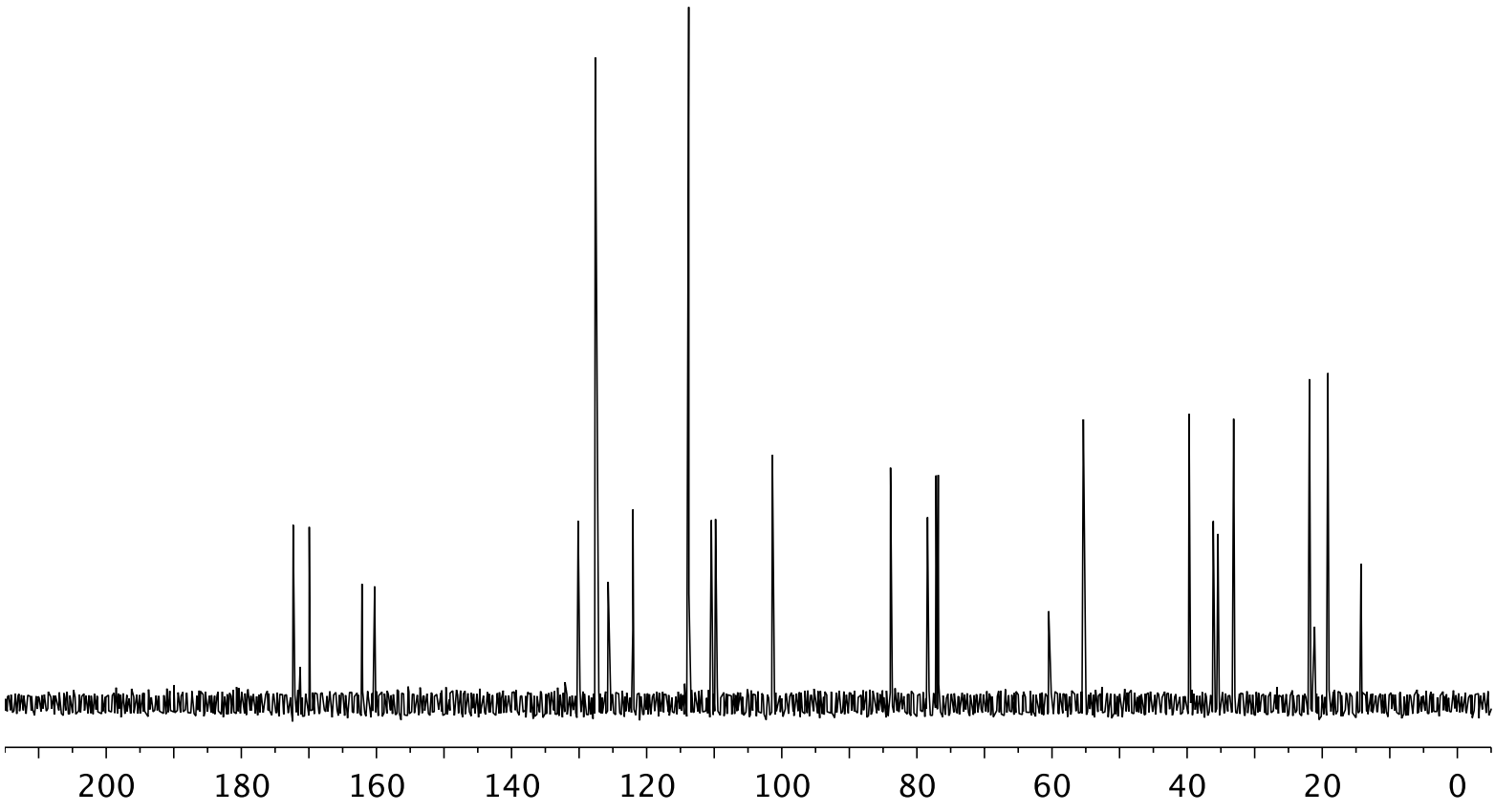
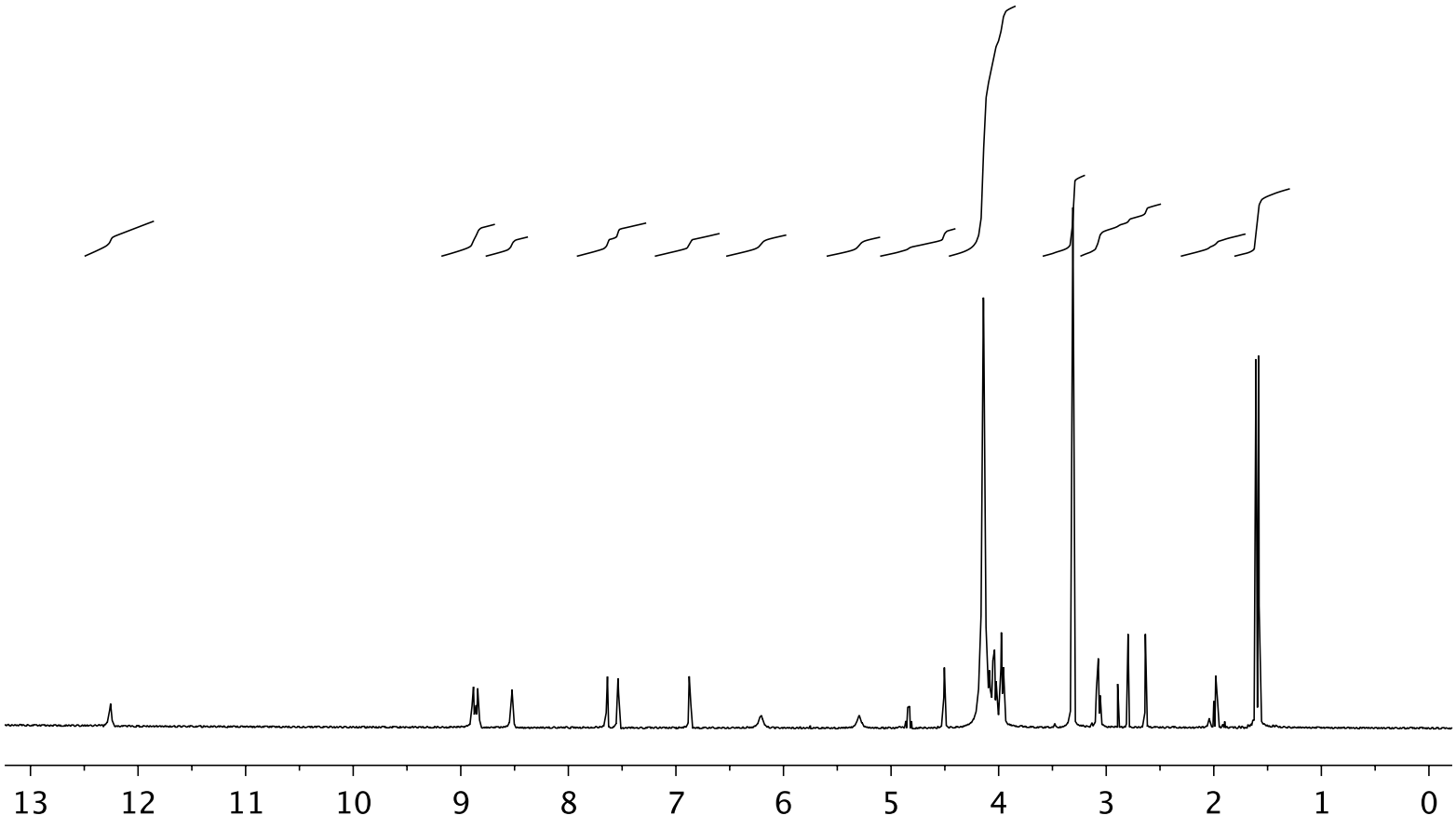
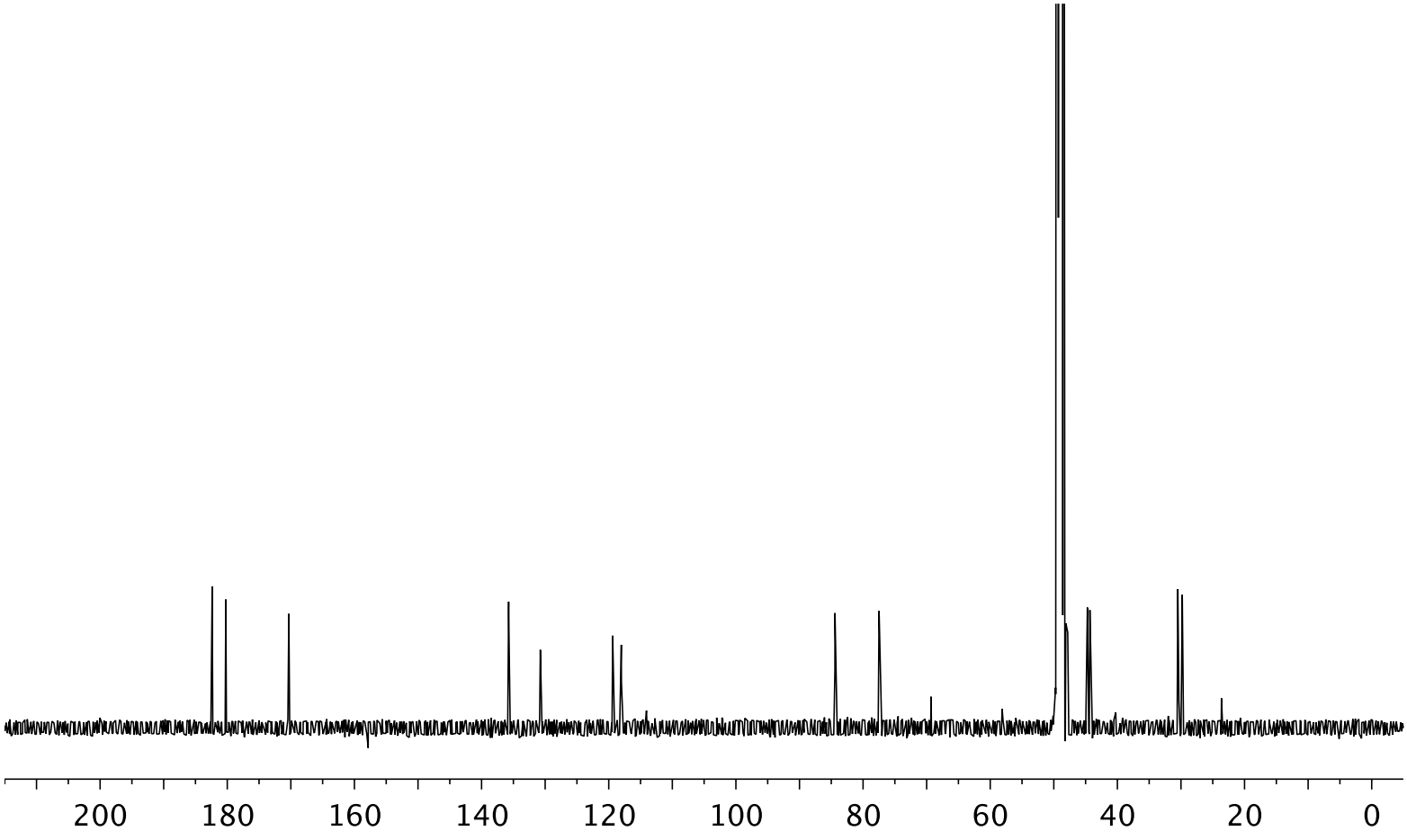


Figure S25. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra of **7** in $CDCl_3$





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