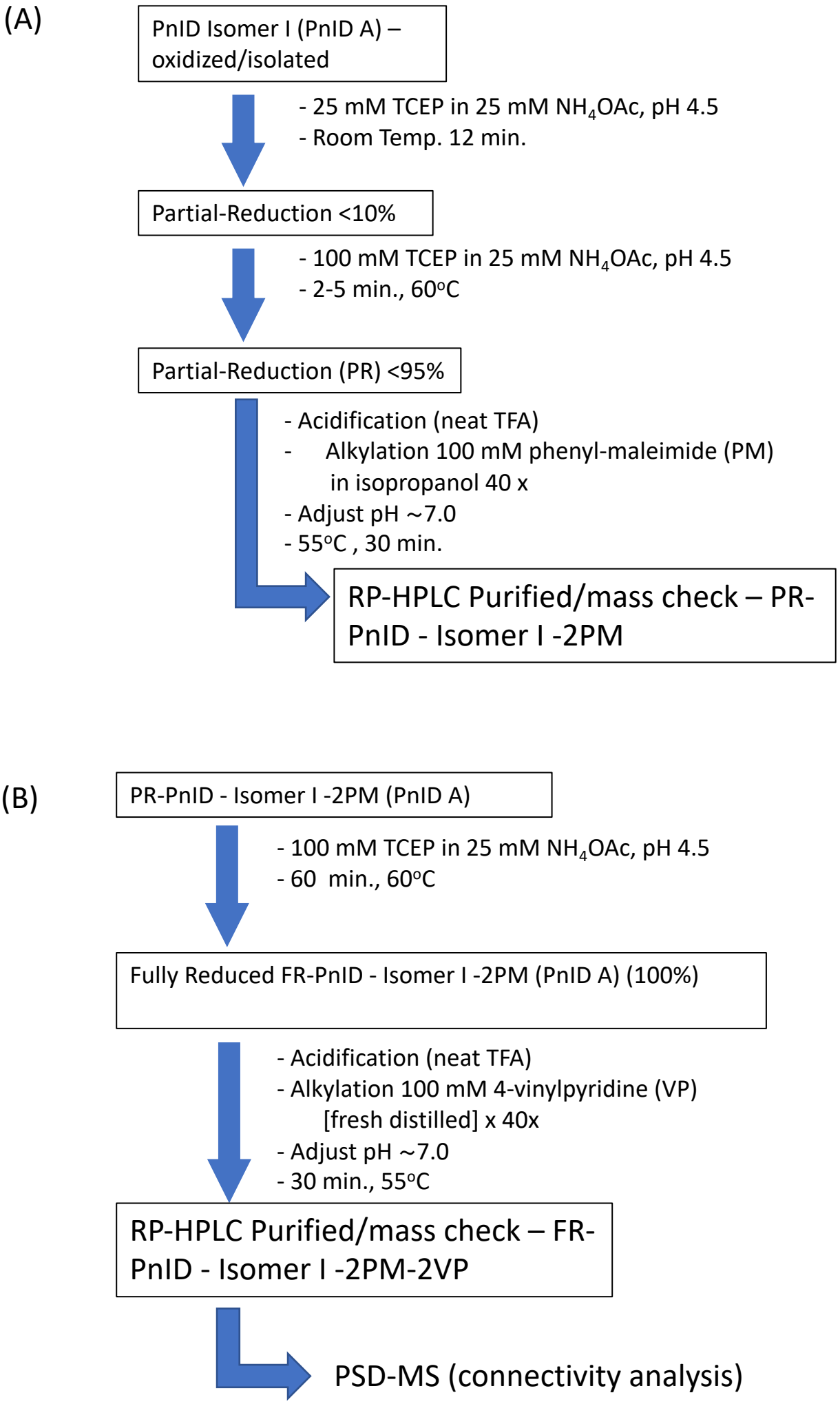
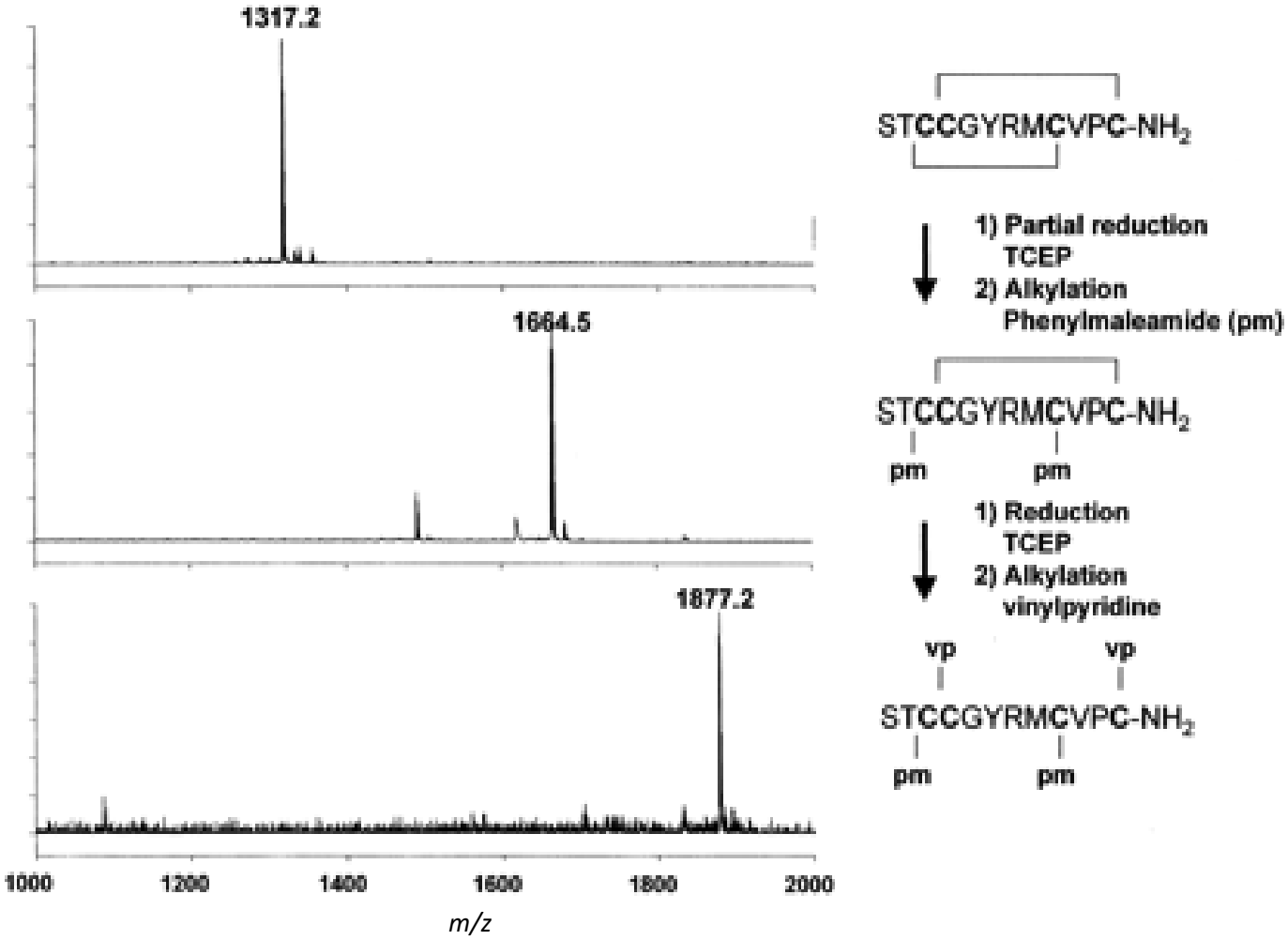
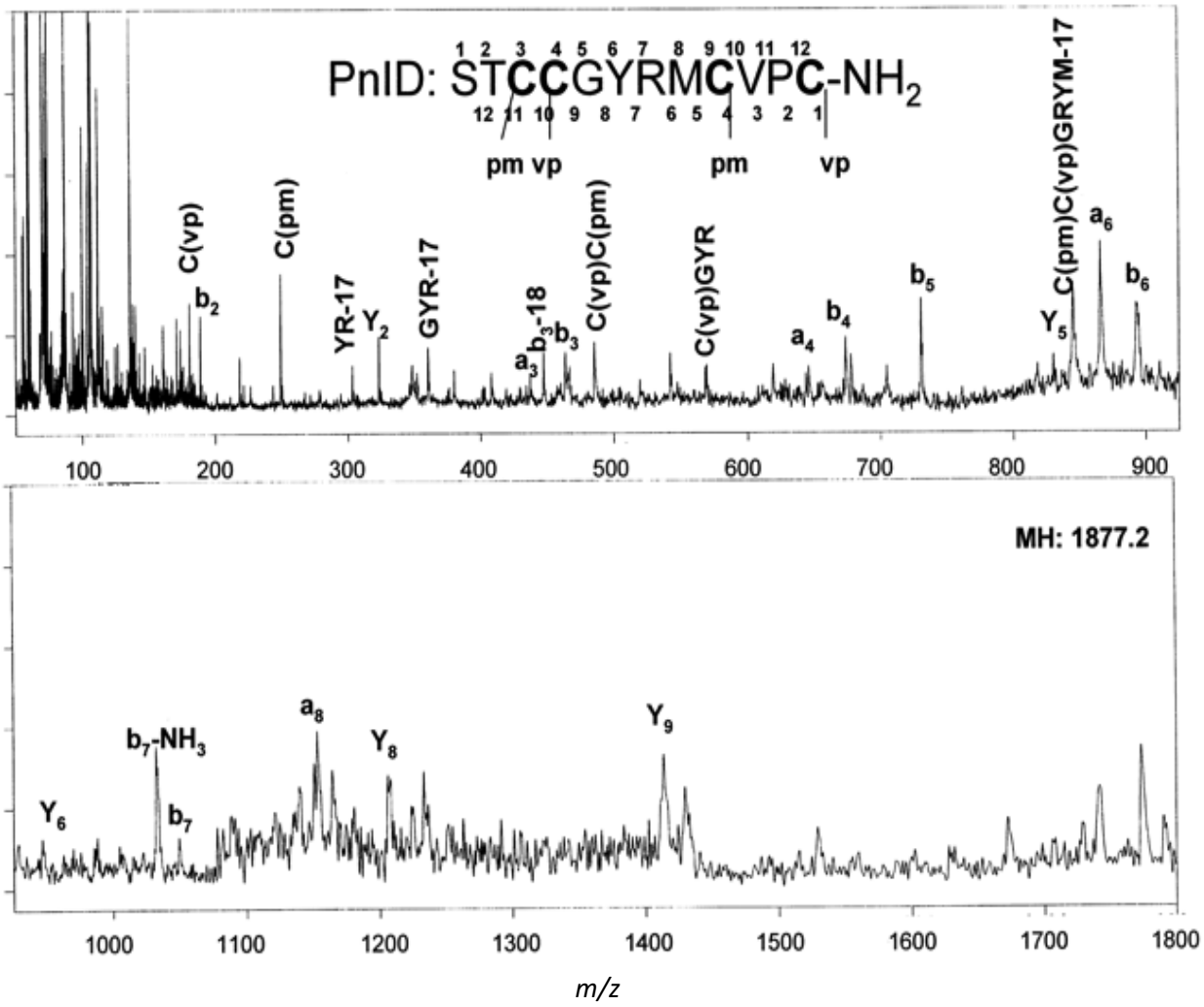


Supplemental Figure S1. Protocol for disulfide connectivity analysis for PnID A (PnID Isomer I; Figure 2A); (**A**) First disulfide bond selectively opened, followed by alkylation with Phenyl maleimide; (**B**) Second disulfide bond reduced and differentially alkylated with 4-vinylpyridine. Product then sequenced by MALD-PSD-MS, (see Supplemental Figure S3).

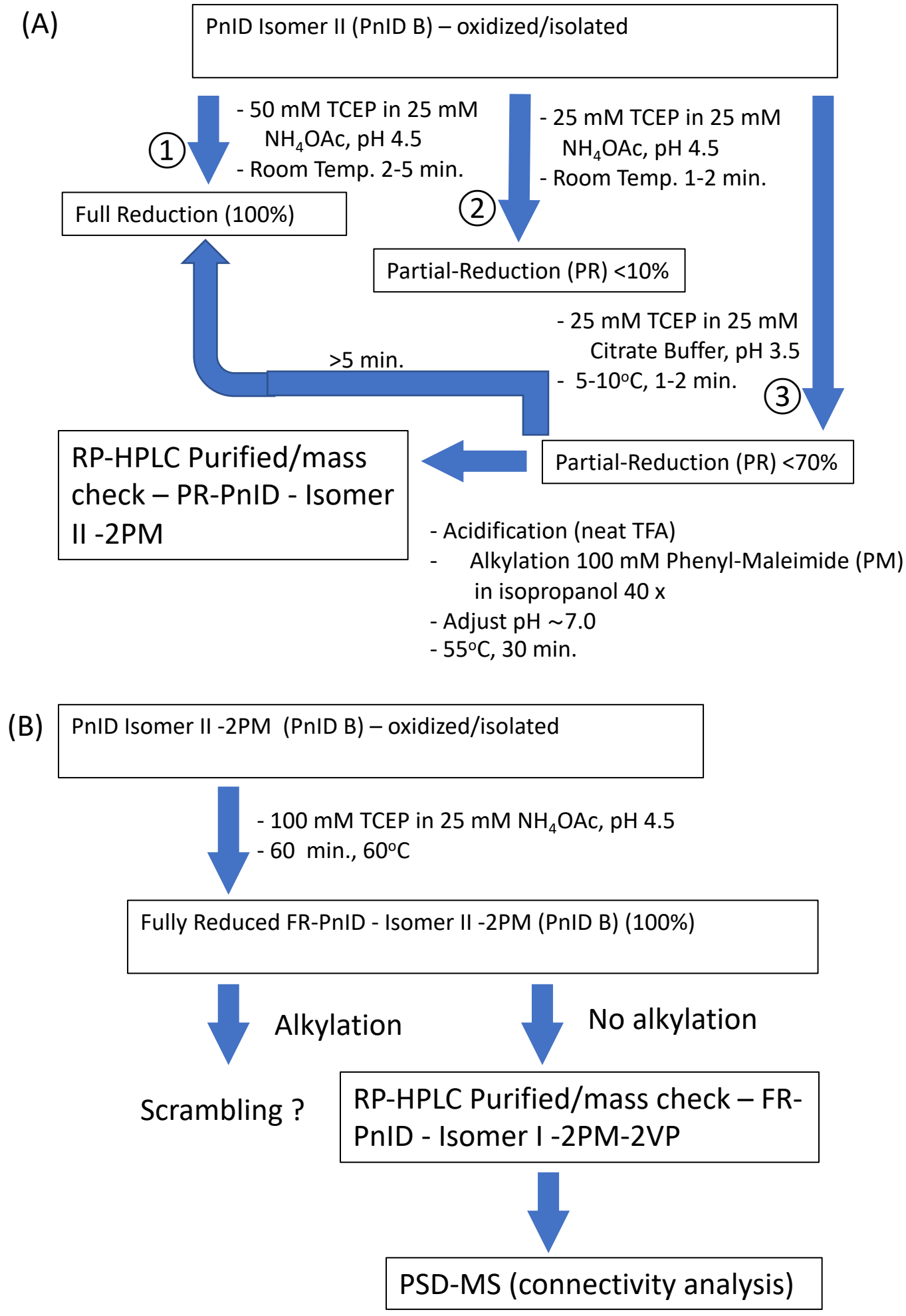


Supplemental Figure S2. MALD-MS of the resulting: (**A**) Oxidized material, PnID isomer I (PnID A) *m/z* 1317.5 Da; (**B**) the product from selectively opening the first disulfide bond and then alkylating with Phenyl-maleimide, *m/z* 1665.4 Da; (**C**) The production of the fully reduced and differentially alkylated PnID isomer I (**A**) alkylated with 4-vinylpyridine, *m/z* 1877.2 Da. This RP-HPLC purified material was then subjected to PSD-MS sequence analysis, as shown in Supplemental Figure S3.

Supplemental Figure S2



Supplemental Figure S3. MALDI-PSD sequence analysis of the differentially alkylated PnID Isomer I (PnID A) 2PM-2VP: Mass spectra, 0-1800 *m/z* indicating the differential alkylated positions within PnID A, indicating connectivity as: 3-9 (PM) + 4-12 (VP) – as determined by matching alkylation positions and their additional molecular mass to the peptide fragment(s).



Supplemental Figure S4. The multiple step protocol for disulfide connectivity analysis for PnID B (PnID Isomer II; Figure 2A); (**A**) First disulfide bond selectively opened, followed by alkylation with Phenyl maleimide; which demonstrates a higher level of susceptibility to reduction, and is then compounded by the possibility of disulfide scrambling upon final full reduction and alkylation of the (**B**) Second disulfide bond. Here only the first disulfide bond connectivity, 3-12 was assigned with certainty (not shown).