Supporting information

Site-specific incorporation of selenocysteine by genetic encoding as a photocaged unnatural amino acid

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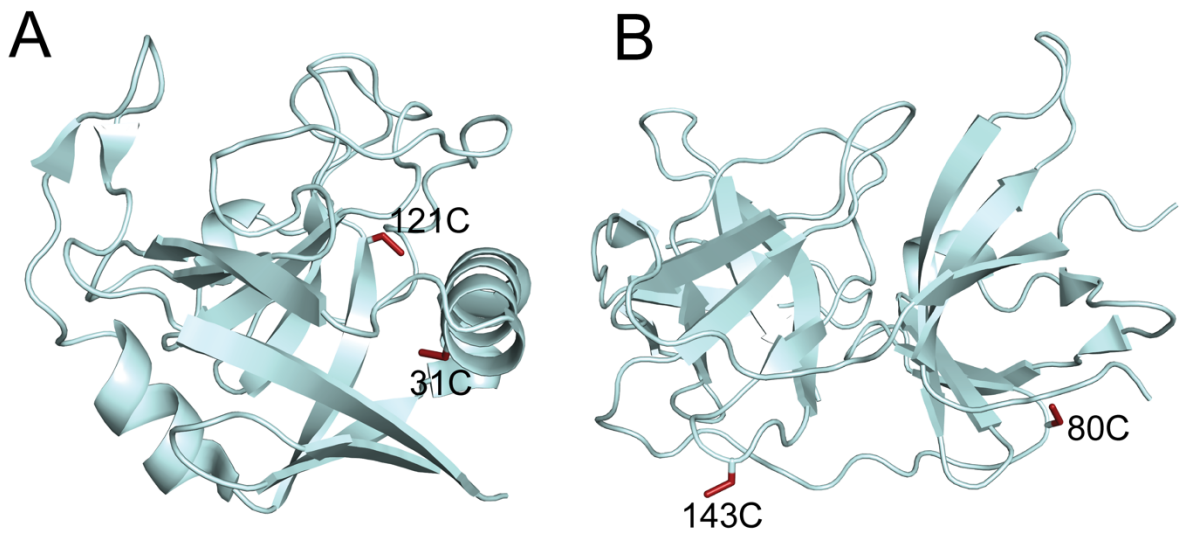


Figure S1. Cysteine residues in PpiB are buried inside the protein, while cysteine residues in ZiPro are fully solvent exposed. (A) Ribbon representation of the crystal structure of PpiB (PDB ID 2NUL[1]), highlighting the side chains of cysteine residues in red. (B) Same as (A), but for ZiPro (PDB ID 5LC0[2]).

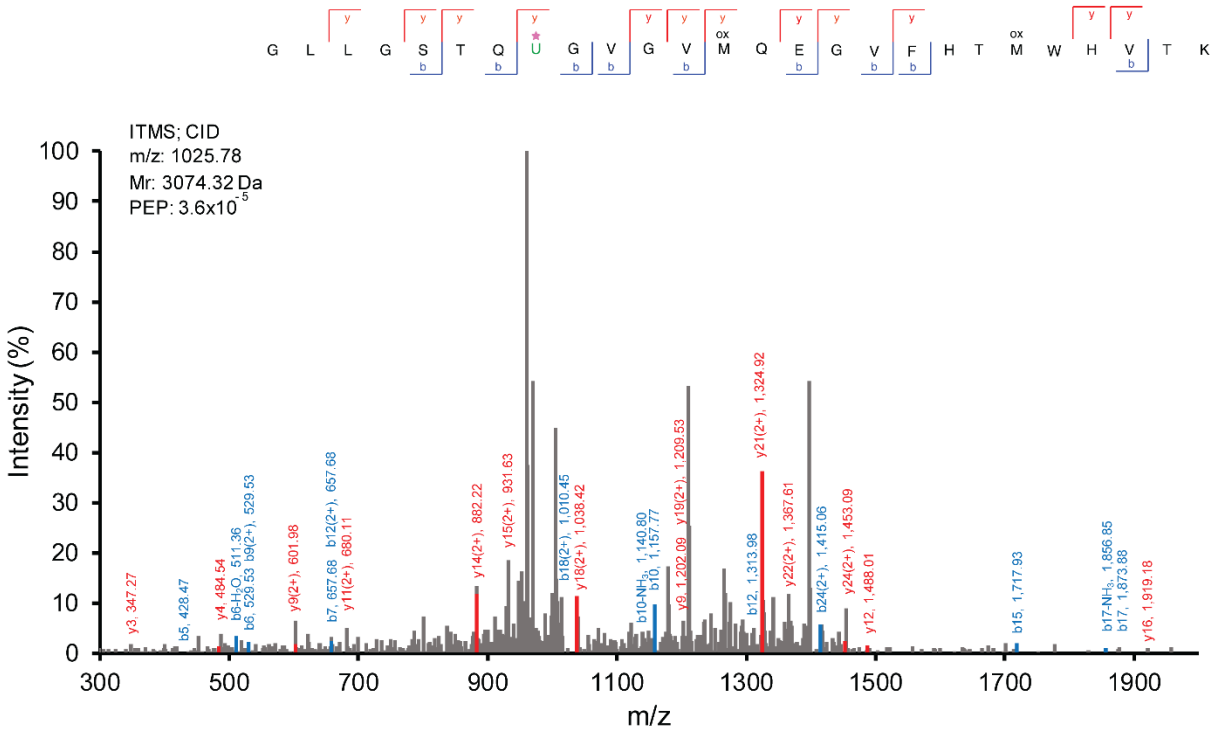


Figure S2. LC-MS/MS analysis of ZiPro V36PSc showing PSc incorporation at the amber codon. The triple-charged (+3) ion with m/z of 1025.78 was fragmented to produce the MS/MS spectrum. Annotated peaks in the MS/MS spectrum correspond to the series of b ions (blue) and y ions (red) that resulted from fragmentation of the peptide from ZiPro V36PSc shown at the top. b- and y-ion fragmentation is also indicated in the amino acid sequence, where the position of the PSc residue is marked by a star.

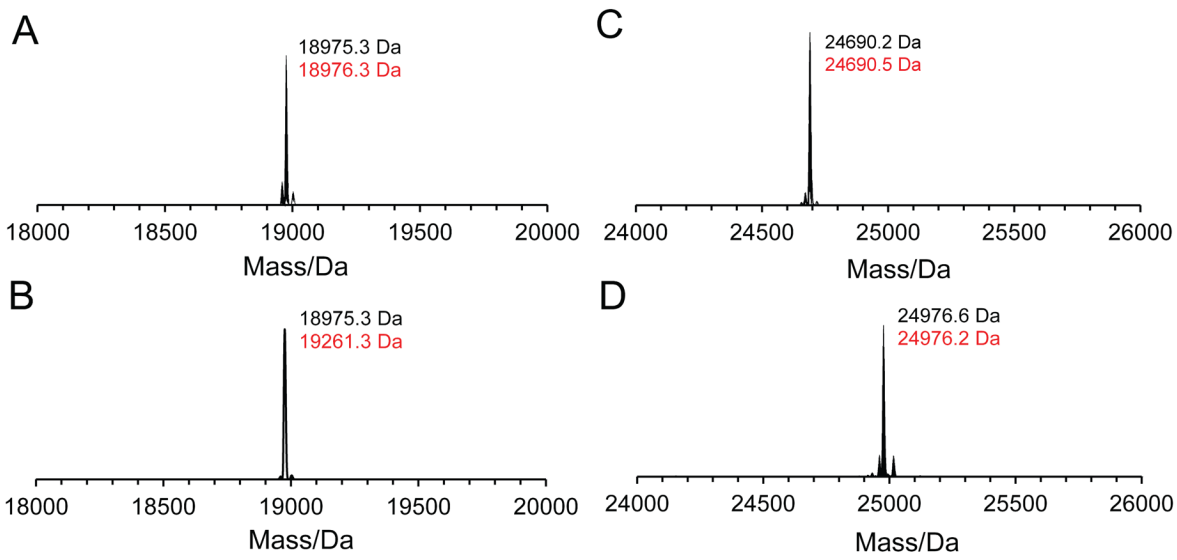


Figure S3. Mass spectra of wild-type PpiB and ZiPro before and after overnight incubation with TMS-1 at pH 7.5 and room temperature. Observed and expected masses are indicated in black and red, respectively. (A) Wild-type PpiB. (B) Wild-type PpiB after incubation with TMS-1. The unchanged mass highlights the inaccessibility of the cysteine residues in the interior of the three-dimensional structure of PpiB. (C) Wild-type ZiPro. The observed mass corresponds to the protein without N-terminal methionine. (D) Wild-type ZiPro tagged with TMS-1. The protein contains two solvent-exposed cysteine residues. Ligation with two TMS-1 tags leads to an expected increase in mass of 286 Daltons.

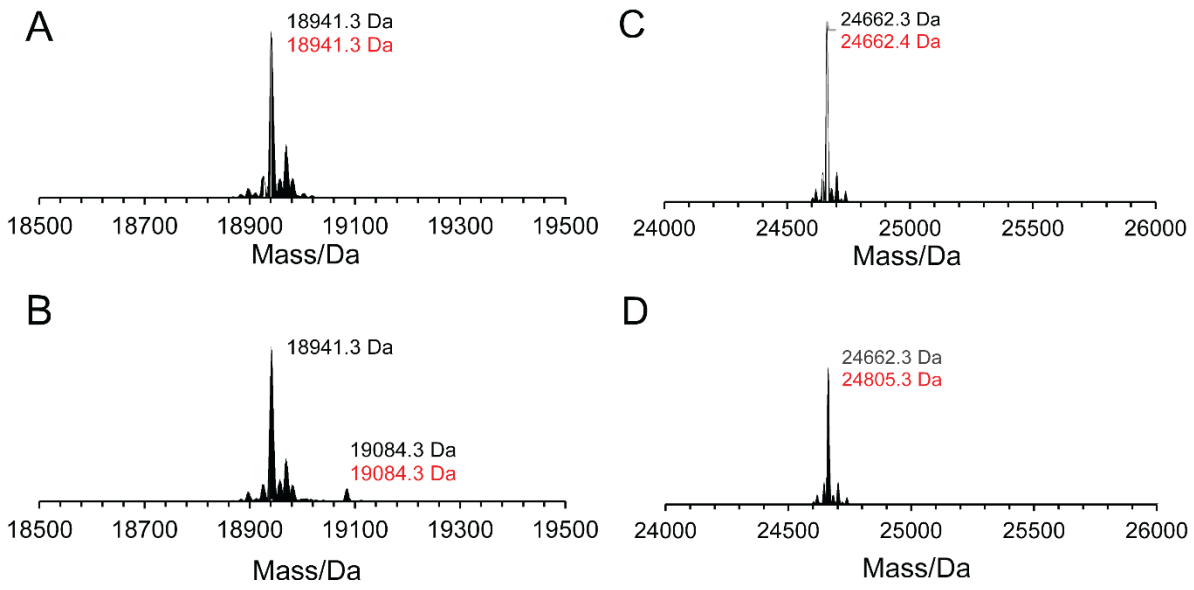


Figure S4. Mass spectra illustrating the poor reactivity of solvent-exposed cysteine residues of PpiB H147C and ZiPro V36C/C80S/C143S at pH 5.1 and room temperature towards overnight reaction with TMS-1. Observed and expected masses are indicated in black and red, respectively. (A) PpiB H147C mutant. (B) PpiB H147C after reaction with TMS-1. The small peak at 19084.3 Da indicates a tagging yield of less than 10%. (C) ZiPro V36C/C80S/C143S mutant. (D) Mass spectrum of ZiPro V36C/C80S/C143S after reaction with TMS-1. No evidence for reaction product was observed.

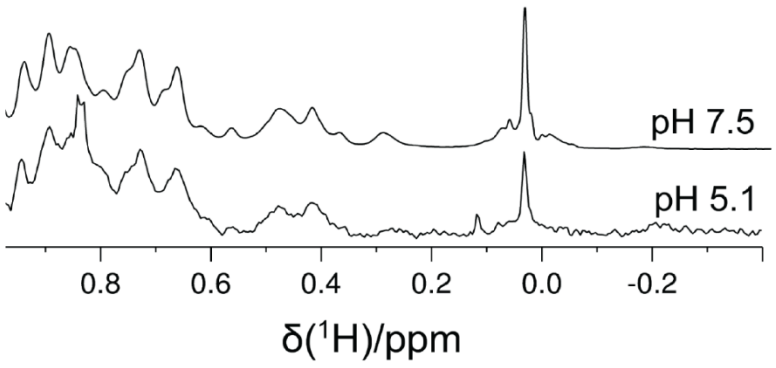
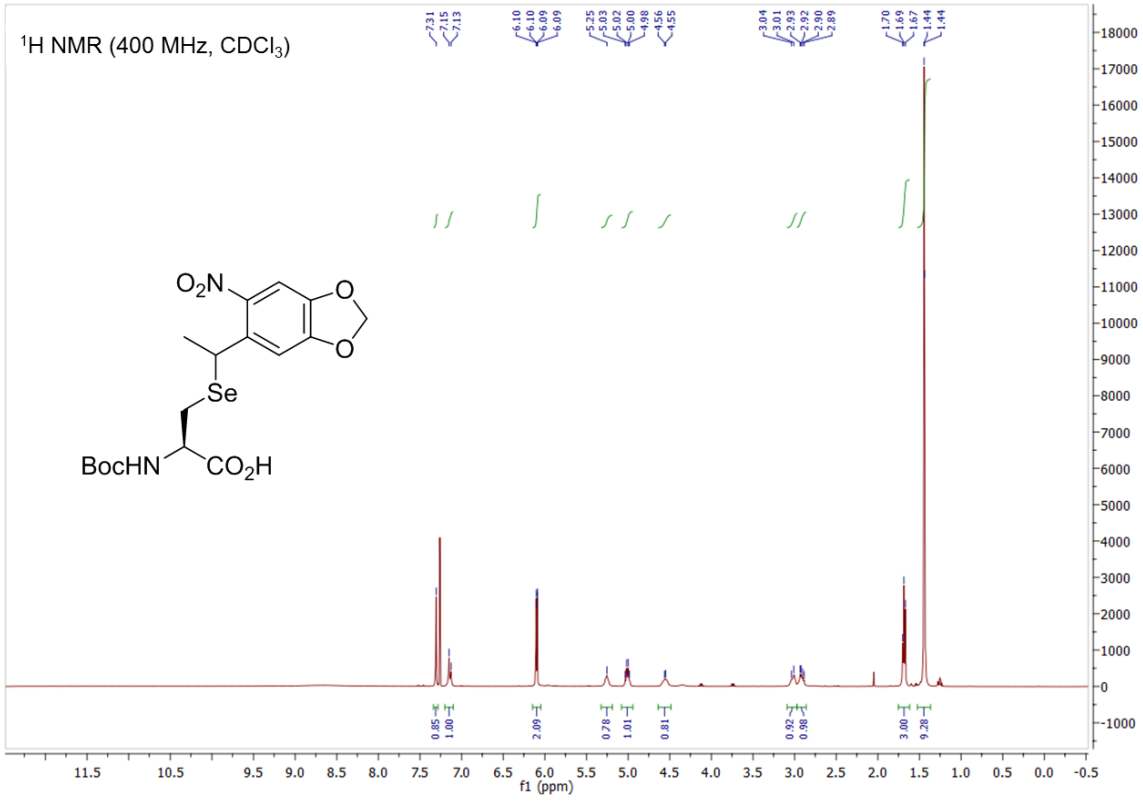
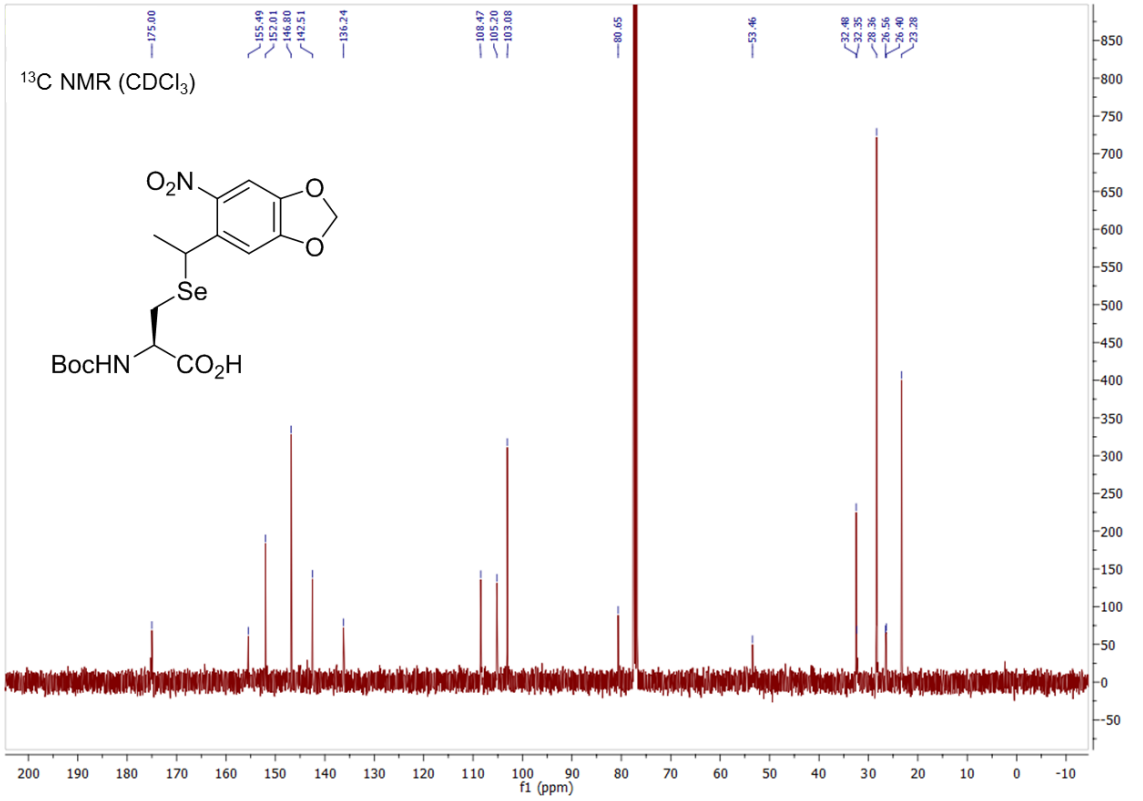
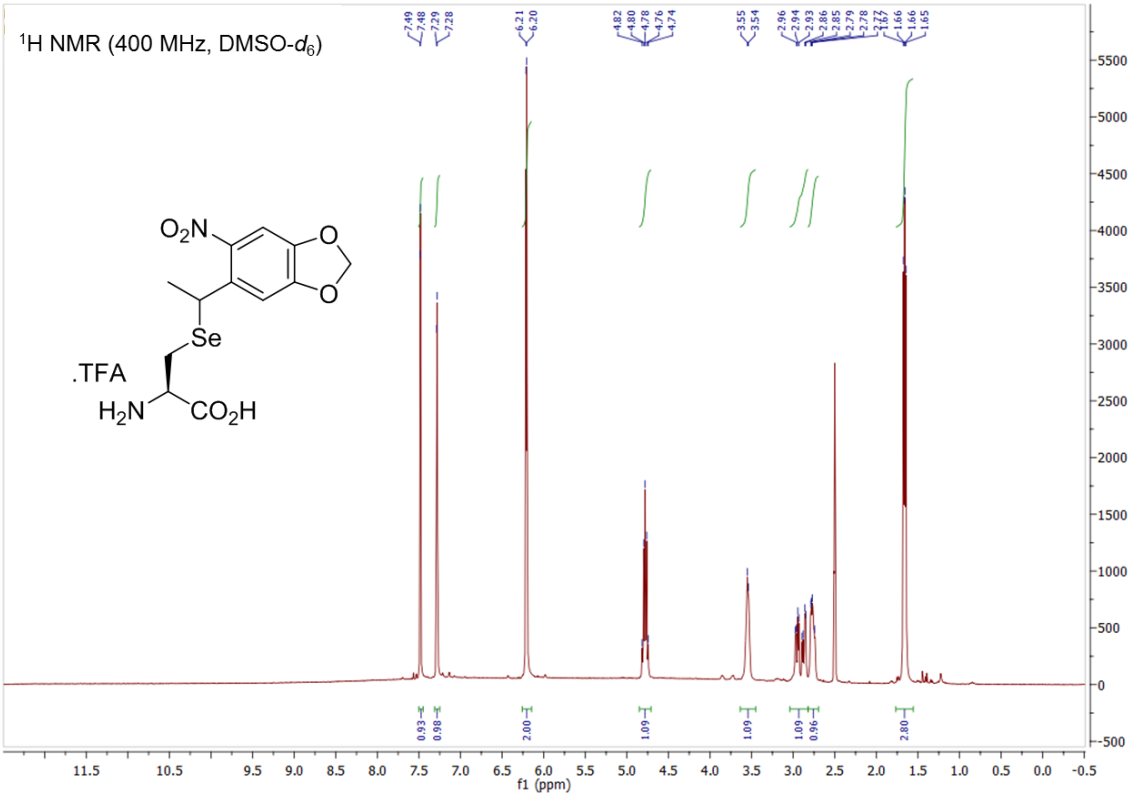


Figure S5. 1D ^{1}H NMR spectra comparing the reactivity of TMS-1 at pH 7.5 and 5.1. PpiB H147 was incubated with TMS-1 overnight at room temperature. The signal of the TMS moiety (at about 0.03 ppm) was less intense when the reaction was performed at pH 5.1 rather than 7.5. The NMR spectra were recorded of 10 and 50 µM protein solutions in 50 mM HEPES, pH 8.0.

NMR spectra of the synthetic intermediate and PSc



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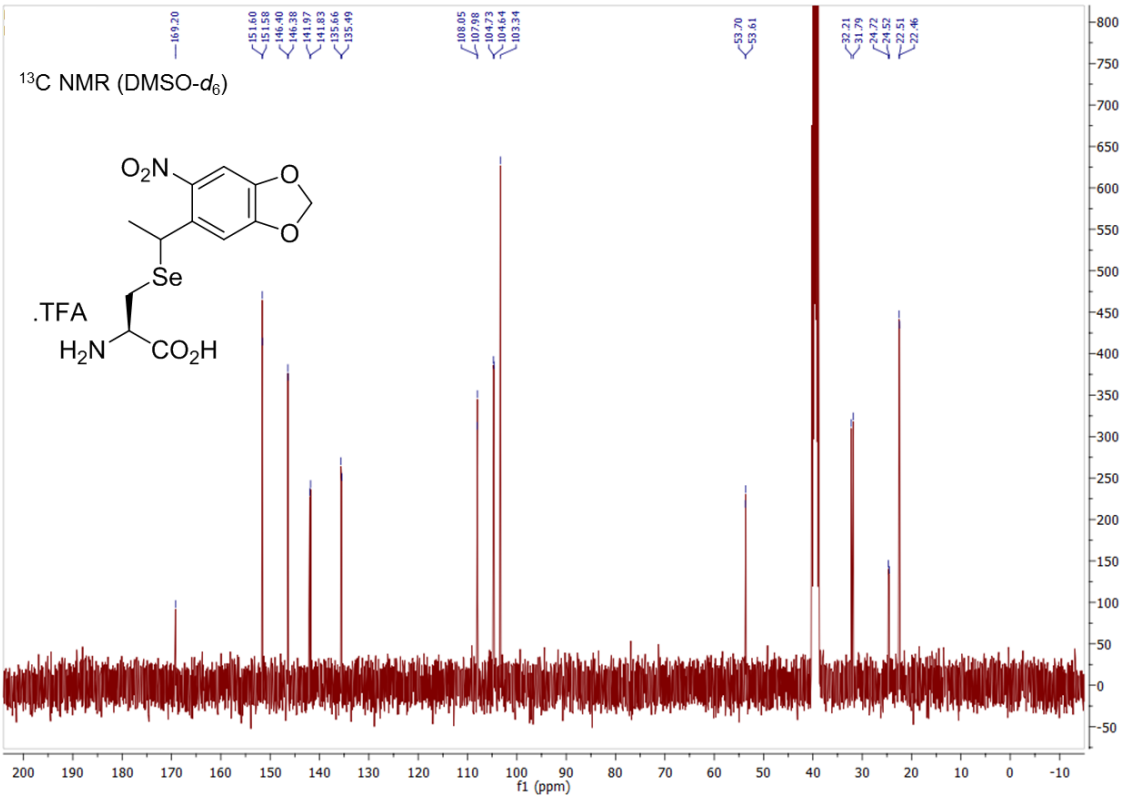
O_2N
O
O
Se
.TFA
H_2N
CO_2H
f1 (ppm)


^{13}C NMR (DMSO-d_6)
O_2N
O
O
Se
.TFA
H_2N
CO_2H
f1 (ppm)

References

(1) Konno, M., Ito, M., Hayano, T., and Takahashi, N. (1996) The substrate-binding site in *Escherichia coli* cyclophilin A preferably recognizes a *cis*-proline isomer or a highly distorted form of the *trans* isomer. *J. Mol. Biol. 256*, 897−908.

(2) 2) Lei, J., Hansen, G., Nitsche, C., Klein, C. D., Zhang, L., and Hilgenfeld, R. (2016) Crystal structure of Zika virus NS2B-NS3 protease in complex with a boronate inhibitor. *Science 353*, 503–505.