

Supplementary Material

1 Supplementary Figures

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#
# Percent Identity Matrix - created by Clustal2.1
#
1: GEO1900      100.00   78.62   29.31   28.52   30.21   30.93   28.18   28.62   30.34   27.93
2: AF0933       78.62   100.00   32.29   31.49   33.45   31.83   29.76   30.21   30.90   29.86
3: ATA-117      29.31   32.29   100.00   91.82   37.92   40.00   43.12   43.75   42.01   42.99
4: ATA-117-Rd11 28.52   31.49   91.82   100.00   38.13   37.38   39.25   41.25   40.00   40.37
5: HoRTA        30.21   33.45   37.92   38.13   100.00   40.86   43.51   44.97   45.78   42.86
6: Aspergillus-fumigatus 30.93   31.83   40.00   37.38   40.86   100.00   68.42   69.06   72.98   72.36
7: Exophiala-xenobiotica 28.18   29.76   43.12   39.25   43.51   68.42   100.00   73.44   70.06   73.68
8: Nectria-haematococca  28.62   30.21   43.75   41.25   44.97   69.06   73.44   100.00   78.06   77.43
9: TsRTA         30.34   30.90   42.01   40.00   45.78   72.98   70.06   78.06   100.00   81.42
10: AtRTA        27.93   29.86   42.99   40.37   42.86   72.36   73.68   77.43   81.42   100.00

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Figure S1: Identity matrix of *TsRTA*, *HoRTA* with a variety of previously reported RTAs and two thermostable BCATs, created using MUSCLE (Madeira et al., 2019).

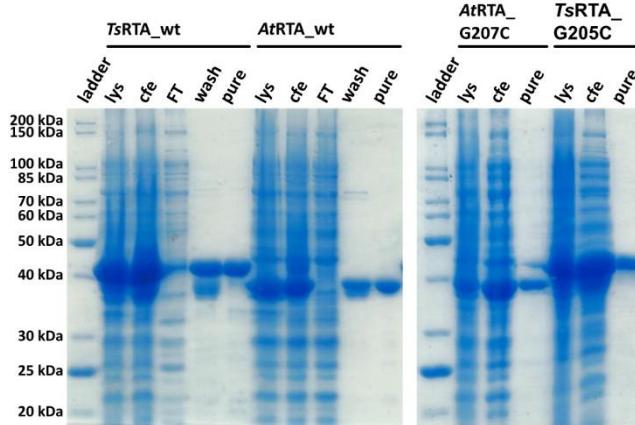


Figure S2: SDS-PAGE gels of *TsRTA*, *AtRTA*, *AtRTA_G207C*, *TsRTA_G205C*. lys: lysate, cfe: cell-free extract, FT: flow-through (IMAC purification), wash: eluted with 10% elution buffer, pure: eluted with 100% elution buffer.

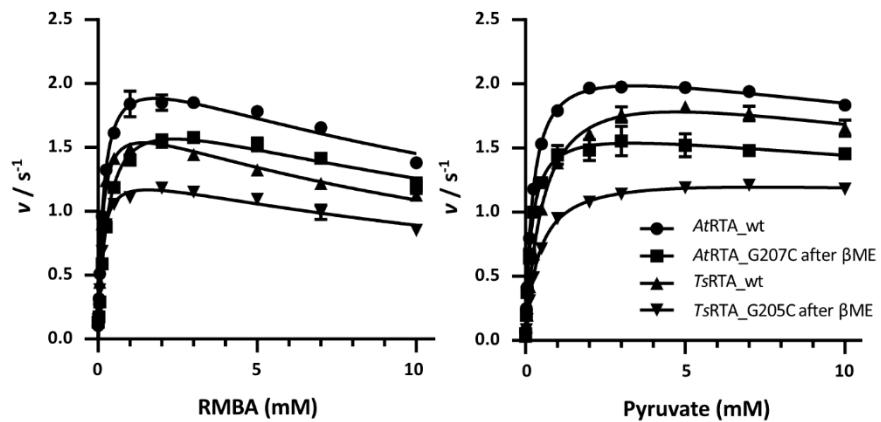


Figure S3: Substrate inhibition curves fitted to the reaction velocities obtained when RMBA and pyruvate were varied, respectively.

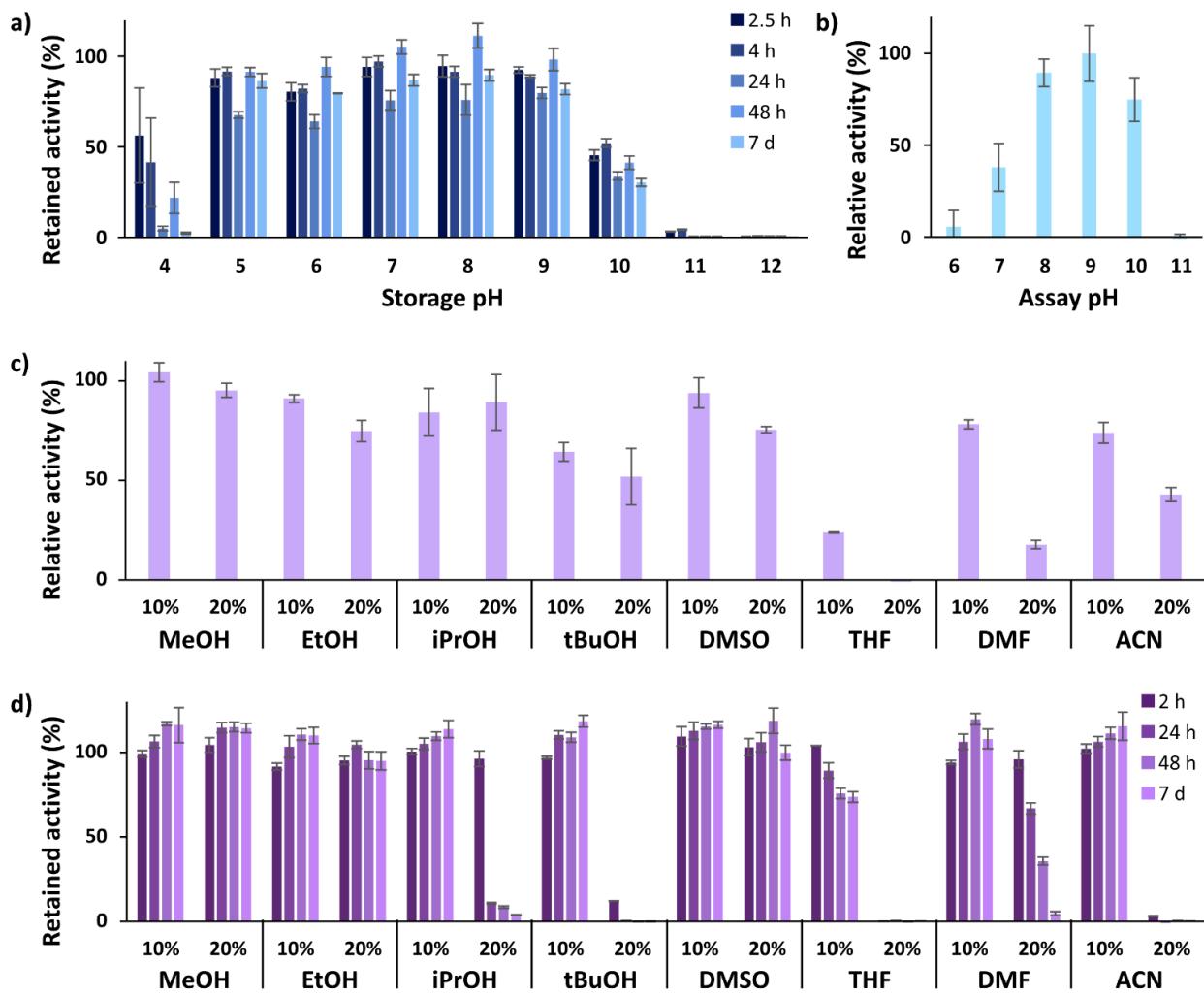


Figure S4: a) pH stability profile of *TsRTA*: retained activity after incubation at pH 4 – 12 for 2.5 h – 7 d (4 °C), relative to freshly purified enzyme. b) pH–activity relationship of *TsRTA*: relative activity at pH 6 – 11, relative to maximum activity at pH 9. c) Co-solvent–activity relationship of *TsRTA*: relative activity in the presence of 10 or 20 % (v/v) co-solvents, relative to activity without co-solvent. d) Co-solvent stability profile of *TsRTA*: retained activity after incubation with 10 or 20 % (v/v) co-solvents for 2 h – 7 d (25 °C, pH 8), relative to freshly purified enzyme. Error bars represent standard errors (n=3).

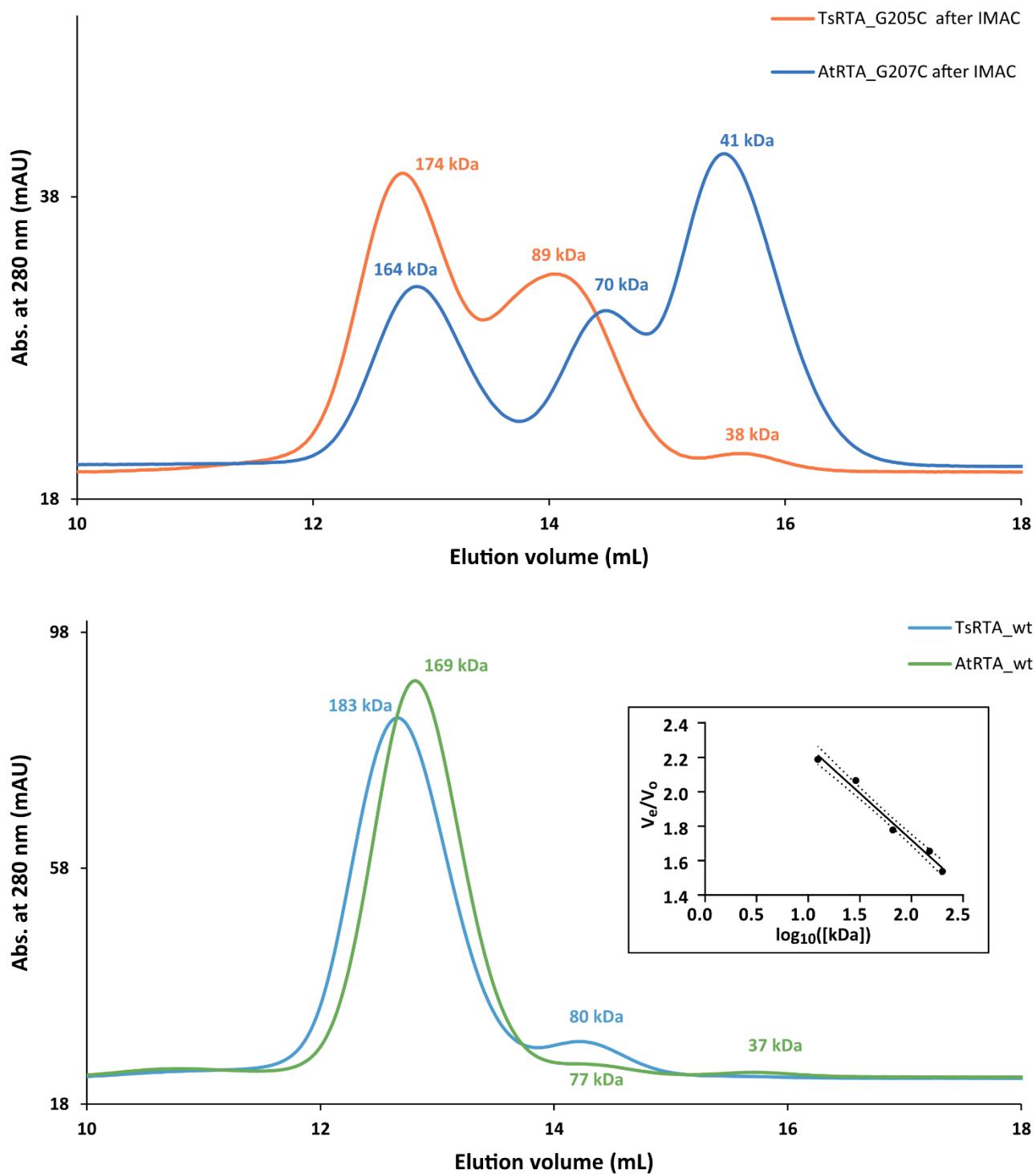


Figure S5: Gel filtration of TsRTA_G205C and AtRTA_G207C immediately after IMAC purification in contrast to the wild-type enzymes, showing the initially disrupted quaternary structure of the mutants. Insert: calibration curve using the Sigma Aldrich Gel Filtration Markers Kit for Protein Molecular Weights 12,000-200,000 Da (MWGF200).

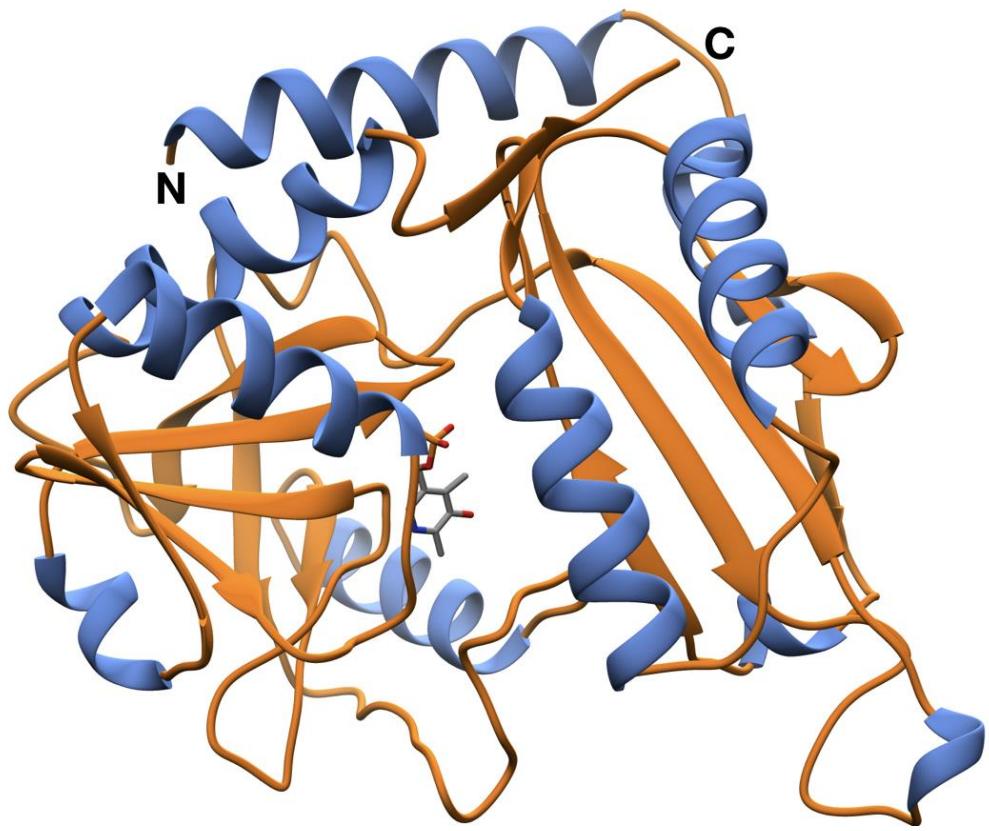


Figure S6: The 3D structure of the *TsRTA* monomer. Secondary structure ribbon representation of the overall fold of the *TsRTA* monomer (Chain A) bound to its PLP cofactor (sticks).

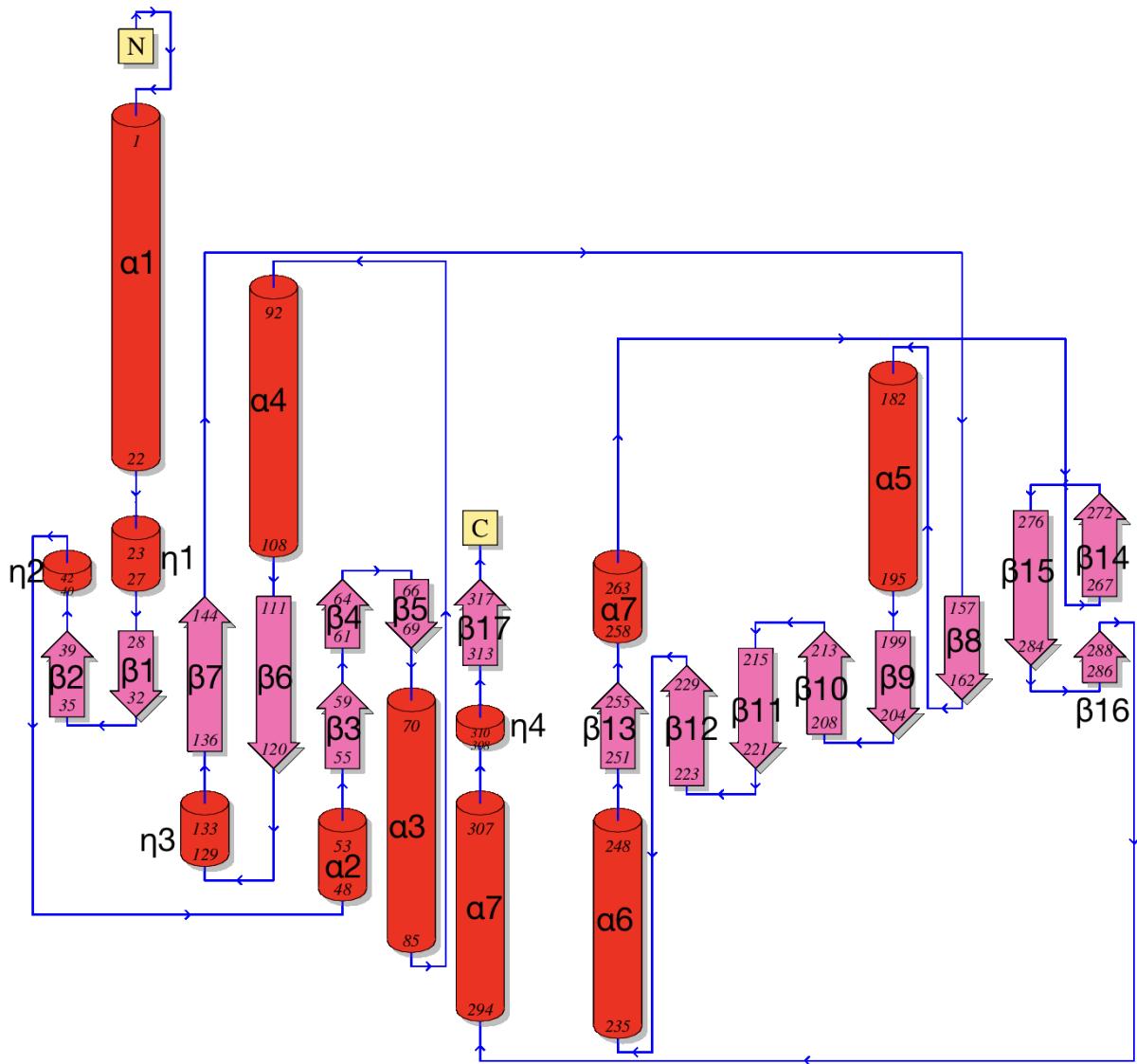


Figure S7: Secondary structure topology diagram of the secondary structure organization of the TsRTA monomer. This diagram generated by the PDBsum server indicates the diverse secondary structure elements (β -strands, α -helices and 3^{10} helices) present in the TsRTA monomer and clearly reveals the presence of two subdomains (Laskowski et al., 2018).

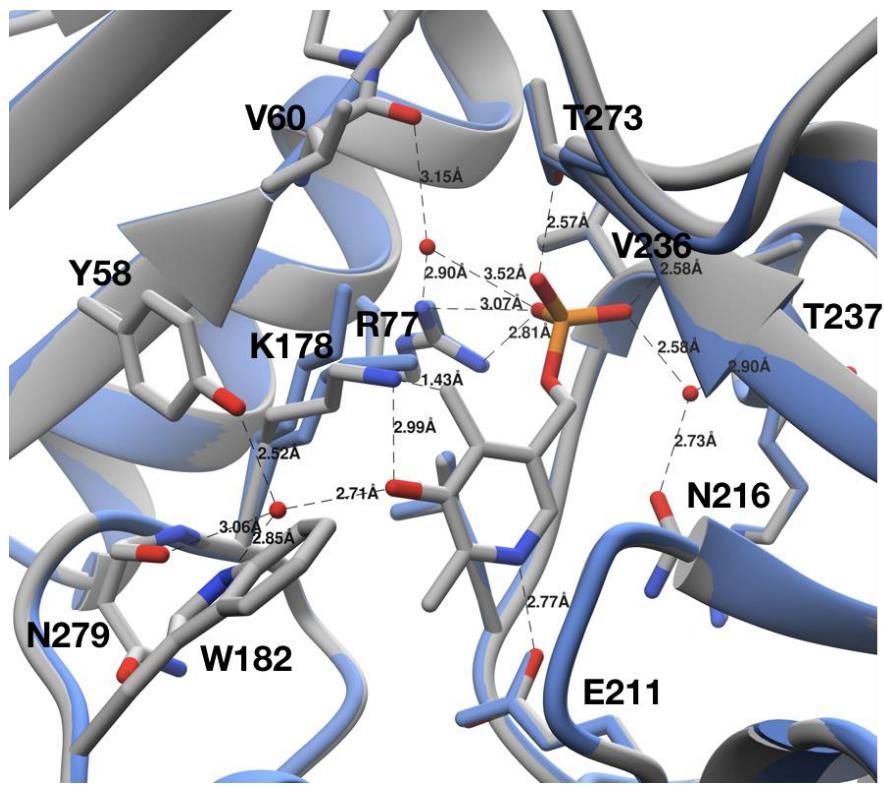


Figure S8: Detailed view of the interactions between PLP and *TsRTA*. The stabilizing hydrogen bonds formed between active site residues and water molecules and PLP are highlighted. To illustrate the high structural identity between *TsRTA* and *AtRTA*, the structure of the latter (PDB entry 4ce5; blue ribbons and sticks) is superposed with Chain A of *TsRTA* (grey ribbons and sticks) (Łyskowski et al., 2014). All panels were generated using Chimera (Pettersen et al., 2004).

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Figure S9: CLUSTAL multiple sequence alignment by MUSCLE (3.8) (Madeira et al., 2019): Bold red: residues differing from *At*RTA. Highlighted in blue: Cysteine forming a disulfide bridge in the tetrameric structure of ATA-117-RD11, and the corresponding glycine in ATA-117.

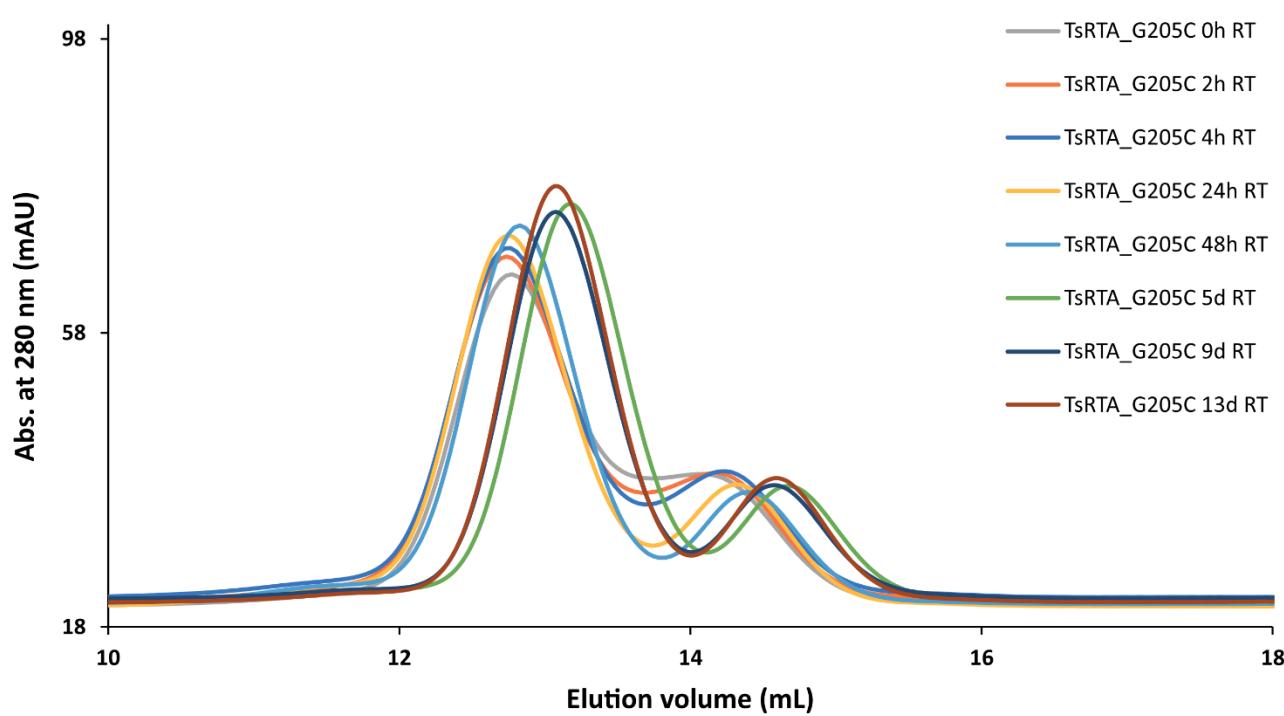
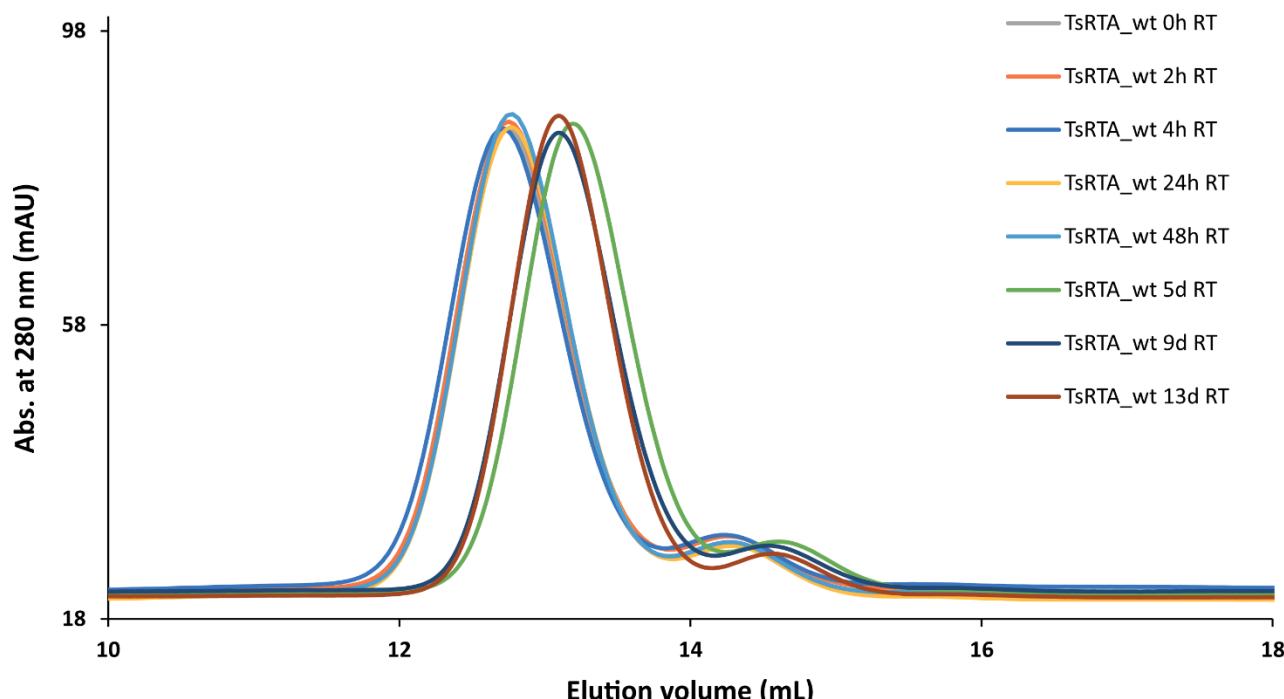


Figure S10: Gel-filtration chromatograms following the incubation of wild-type and mutant TsRTA at ambient temperature with gentle agitation (aeration).

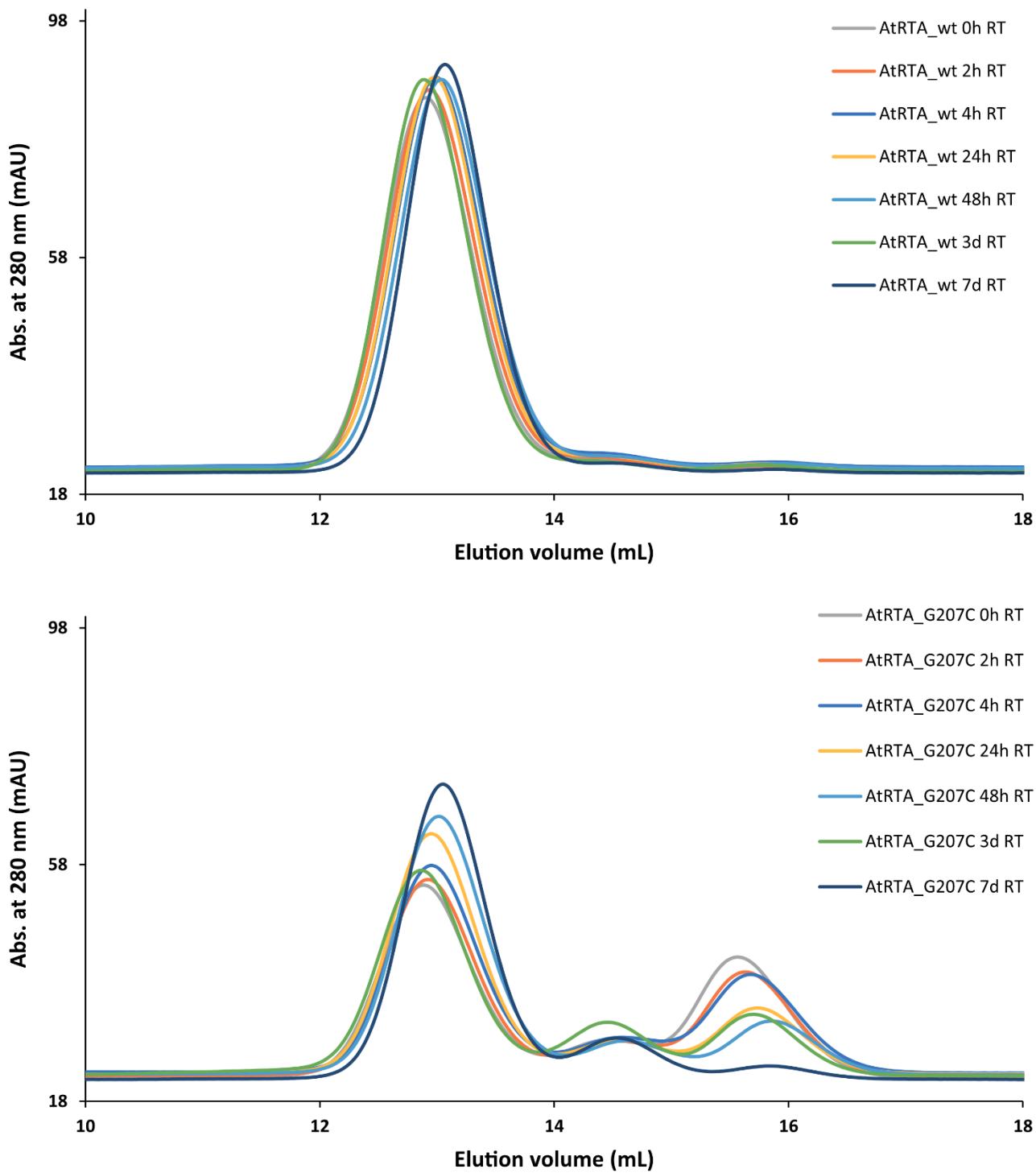


Figure S11: Gel-filtration chromatograms following the incubation of wild-type and mutant AtRTA at ambient temperature with gentle agitation (aeration).

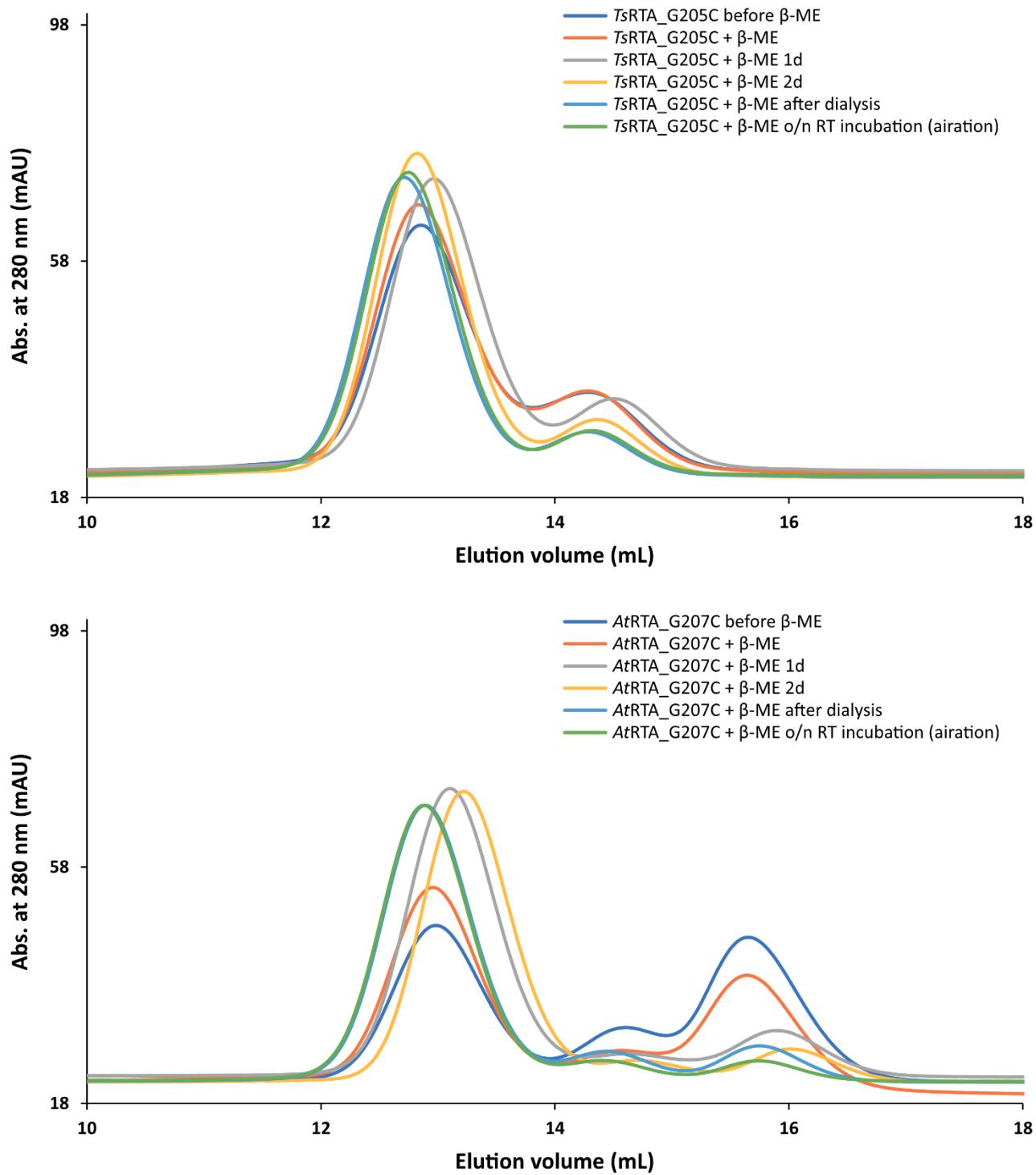


Figure S12: Gel-filtration chromatograms following the β -mercaptoproethanol treatment of *TsRTA_G205C* and *AtRTA_G207C*.

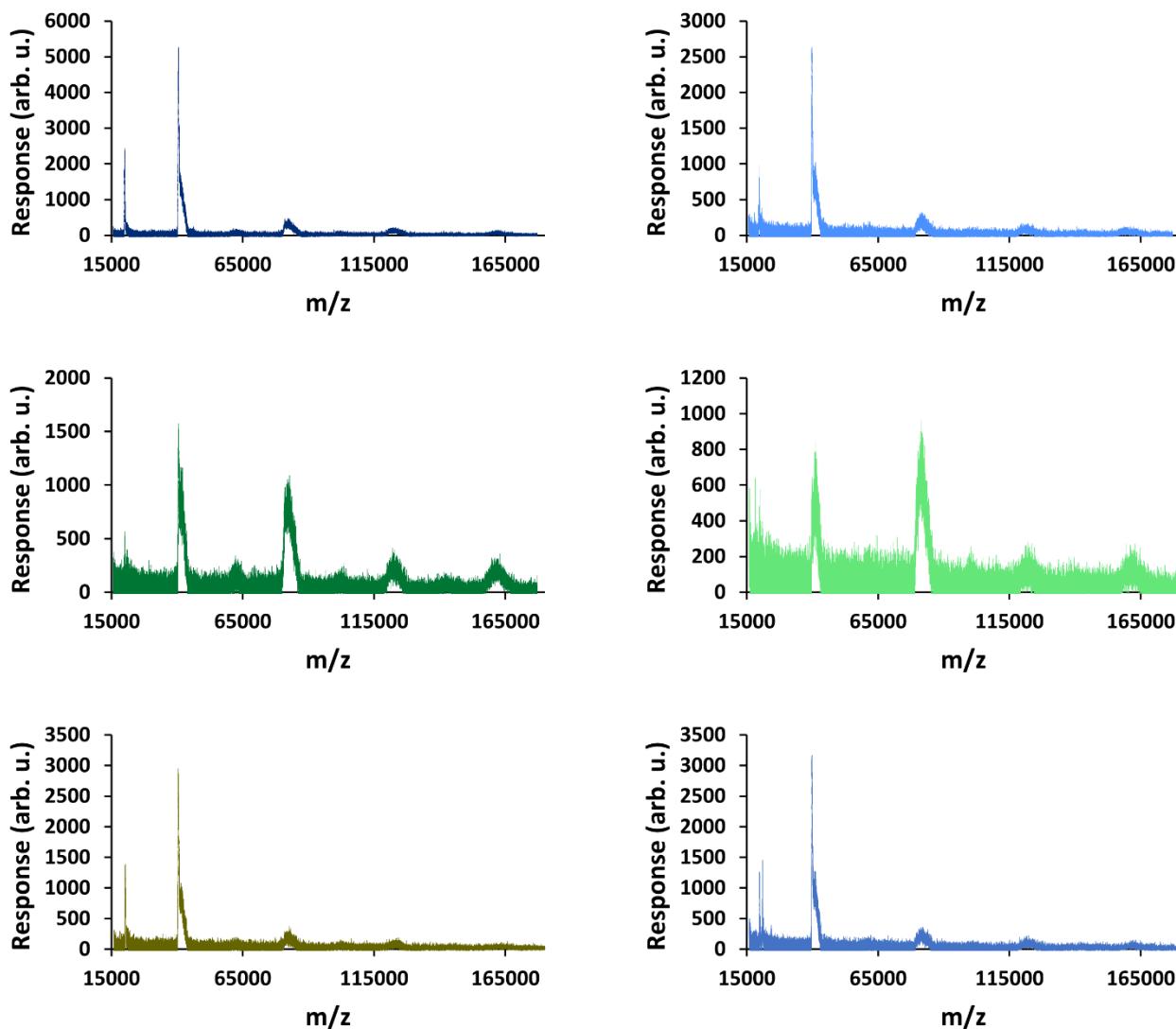


Figure S13: MALDI-TOF MS traces. Left: TsRTA, right: AtRTA. Top: wt. Middle: mutant. Bottom: mutant after incubation with TCEP.

2 Supplementary Tables

Table S1: Data collection statistics and refinement parameters for TsRTA.^a

TsRTA	
Data collection	
Space group	I ₁ 2 ₁
Cell dimensions	
a, b, c (Å)	67.8, 98.0, 117.3
α, β, γ (°)	90.0, 91.2, 90.0
Resolution (Å)	59-2.2 (2.2-2.27)
^a R _{merge}	0.112 (0.526)
I / σI	10.2 (2.7)
Completeness (%)	100 (100)
Redundancy	6.1(6.2)
^b CC ^{1/2}	100 (91.2)
Refinement	
Resolution (Å)	2.2-59.0
No. unique reflections	38887
^c R _{work} / ^d R _{free}	20.7/23.4
No. atoms	
Protein	2482(A)2496(B)
Ethylene glycol	114
PLP	30
Sodium ion	2
Water	165
B-factors (Å ²)	
Protein	29 (A) 31 (B)
Water	27 (A) 26 (B)
Ethylene glycol	32
PLP	23
Sodium ion	26
Water	25
RMSD:	
Bond lengths (Å)	0.003
Bond angles (°)	0.598
Ramachandran Plot (%)	
Allowed Regions	100

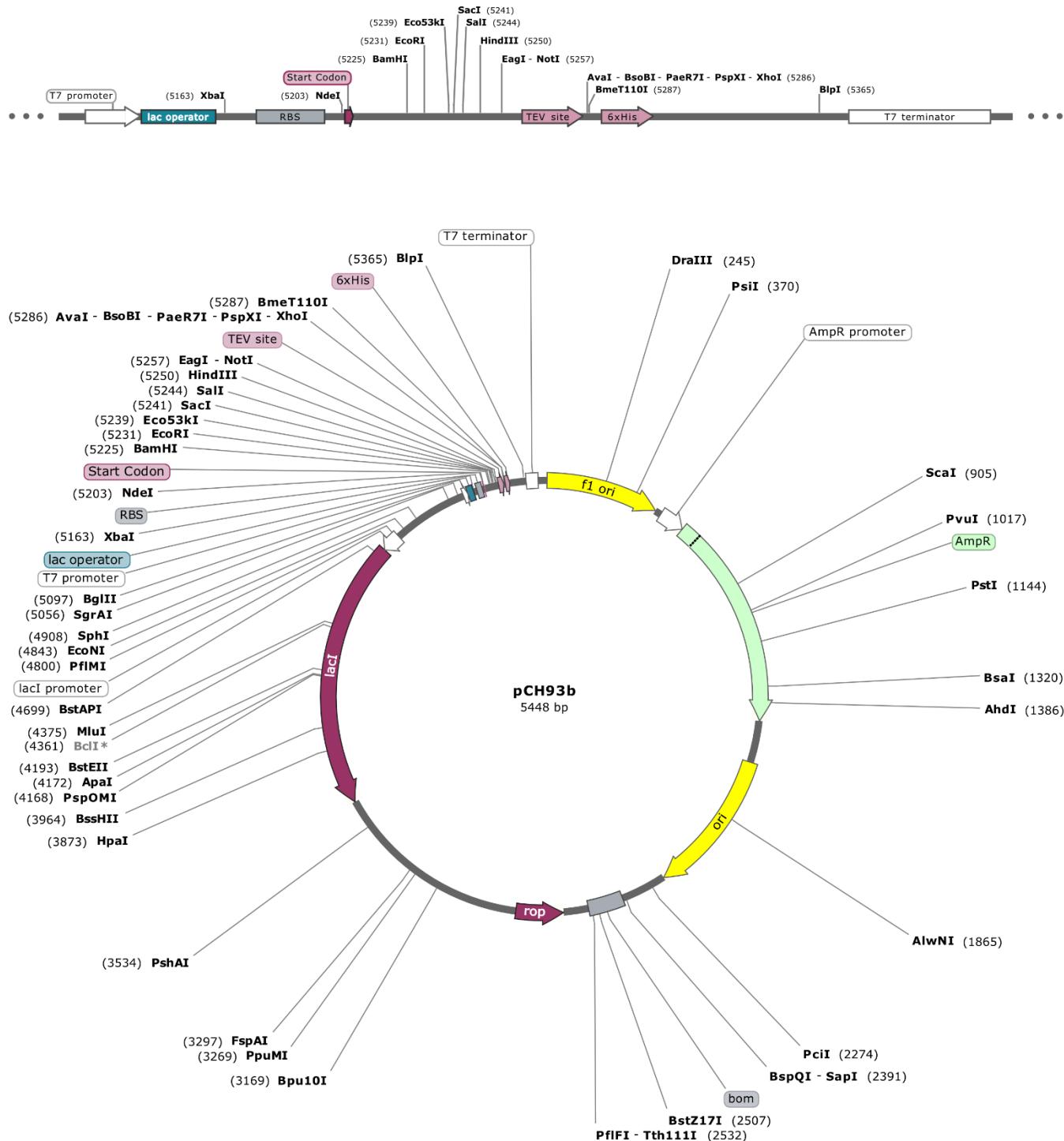
^aData were collected AT 2.2Å on a single TsRTA crystal. Parentheses indicate parameters related to the high-resolution cell (2.2 – 2.27 Å). ^aR_{merge} = $\sum |I - \langle I \rangle| / \sum I \times 100$, where I is the intensity of a reflection and $\langle I \rangle$ is the average intensity. ^bCC^{1/2} is the correlation between random half-sets of data. ^cR_{work} = $\sum |F_o - F_c| / \sum F_o \times 100$; ^dFor cross-validation, 10 % experimental reflections were randomly selected to calculate the R_{free}.

3 Supplementary References

- Laskowski, R. A., Jabłońska, J., Pravda, L., Vařeková, R. S., and Thornton, J. M. (2018). PDBsum: Structural summaries of PDB entries. *Protein Sci.* 27, 129–134. doi:10.1002/pro.3289.
- Łyskowski, A., Gruber, C., Steinkellner, G., Schürmann, M., Schwab, H., Gruber, K., et al. (2014). Crystal structure of an (R)-selective ω -transaminase from *Aspergillus terreus*. *PloS one* 9, e87350. doi:10.1371/journal.pone.0087350.
- Madeira, F., Park, Y. mi, Lee, J., Buso, N., Gur, T., Madhusoodanan, N., et al. (2019). The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* 47, W636–W641. doi:10.1093/nar/gkz268.
- Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., et al. (2004). UCSF Chimera?A visualization system for exploratory research and analysis. *J. Comput. Chem.* 25, 1605–1612. doi:10.1002/jcc.20084.

4 Sequences

4.1 pCH93b



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Legend: ACGCGC...CGCTTACAATT

f1 ori

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ATGAGTATTCAAC...GGTAA	AmpR
TTGAGATCCTT...TGGAA	pBR322/pUC ori
TCAGAGGTTTC...GGTCAC	rop (anti-sense)
CACTG...TAACGGGTTTCAC	LacI (anti-sense)
ATTCACCA...CGATGGTGT	LacI promoter ("antisense")
TAATACGACTCACTATAGG	T7 promoter
GGAATTGT...AACAAATTCC	lac operator
AAGGAG	RBS
ATG	Start
CACCACCACCACCAACAC	His-tag
TGA	Stop
CTAGCAT...GGGGTTTTTG	T7 terminator
<u>GAGAACCTCTATTCCAAGGG</u>	
	TEV recognition sequence

4.2 TsRTA

DNA sequence:

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In **bold**: the synthetic gene. Underlined: first and last codon of gene. **Red**: mutagenesis site.

Translated protein:

MDIGINSDPMATMDKVFAGYAARQKAMEAAGNPLSEGIAWVEGEMVPLHEARIPLMD
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ENLYFO/GLEHHHHHH

In **bold**: the wild-type protein. In *italics*: additional amino acids from the expression vector.
Underlined: TEV cleavage site. **Red**: G205.

4.3 AtRTA

DNA sequence:

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In **bold**: the synthetic gene. Underlined: first and last codon of gene. **Red**: mutagenesis site.

Translated protein:

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PVELAYRCDEIFMCTTAGGI
MPIITLDGMPVNGGQIGPITKKIWDGYWAMHYDAAYSFEIDYNERNKLAAAEONLYFO/GLEHHHHHH

In **bold**: the wild-type protein. In *italics*: additional amino acids from the expression vector.

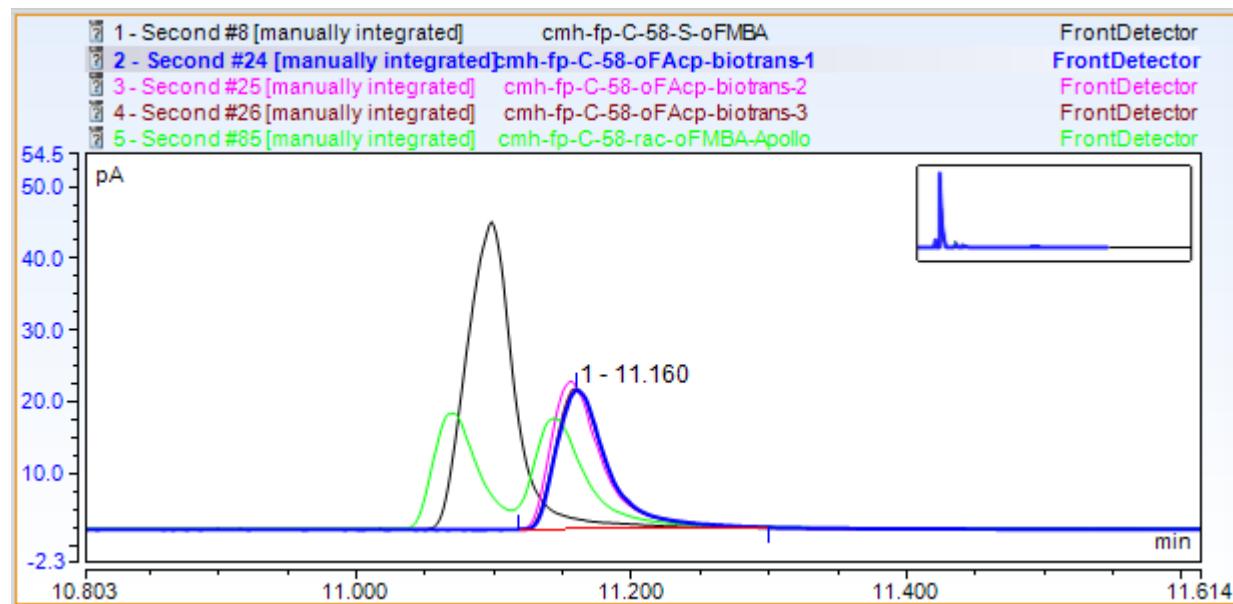
Underlined: TEV cleavage site. **Red**: G207.

5 Enantiomeric excess chromatograms

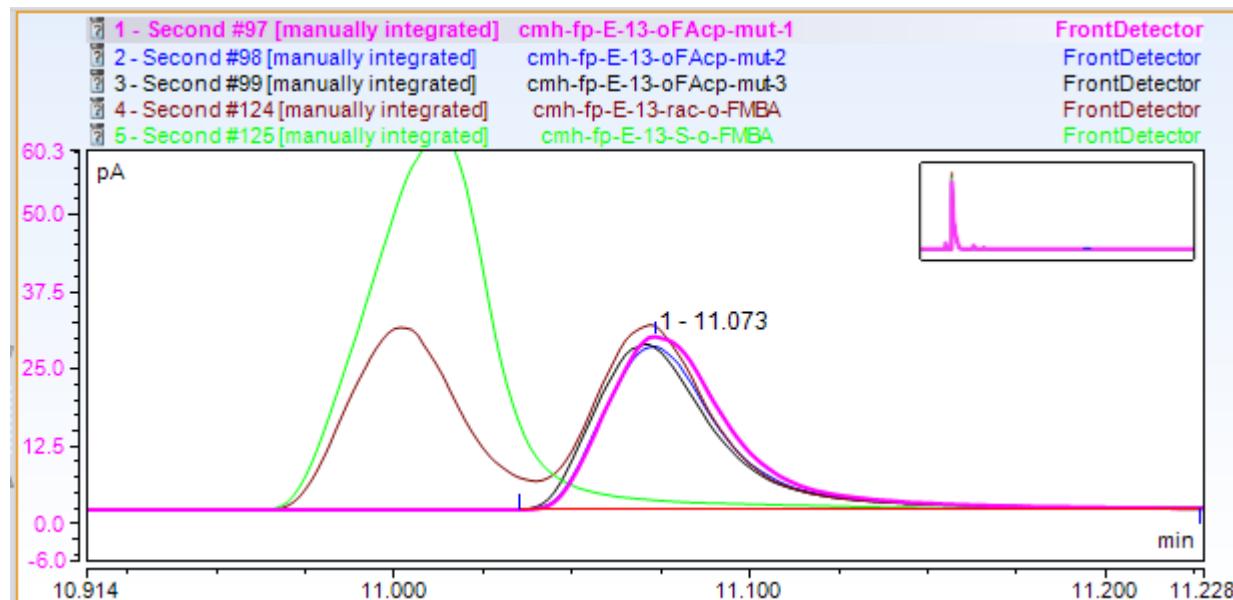
5.1 GC-FID

o-fluoro- α -methylbenzylamine

TsRTA_wild-type

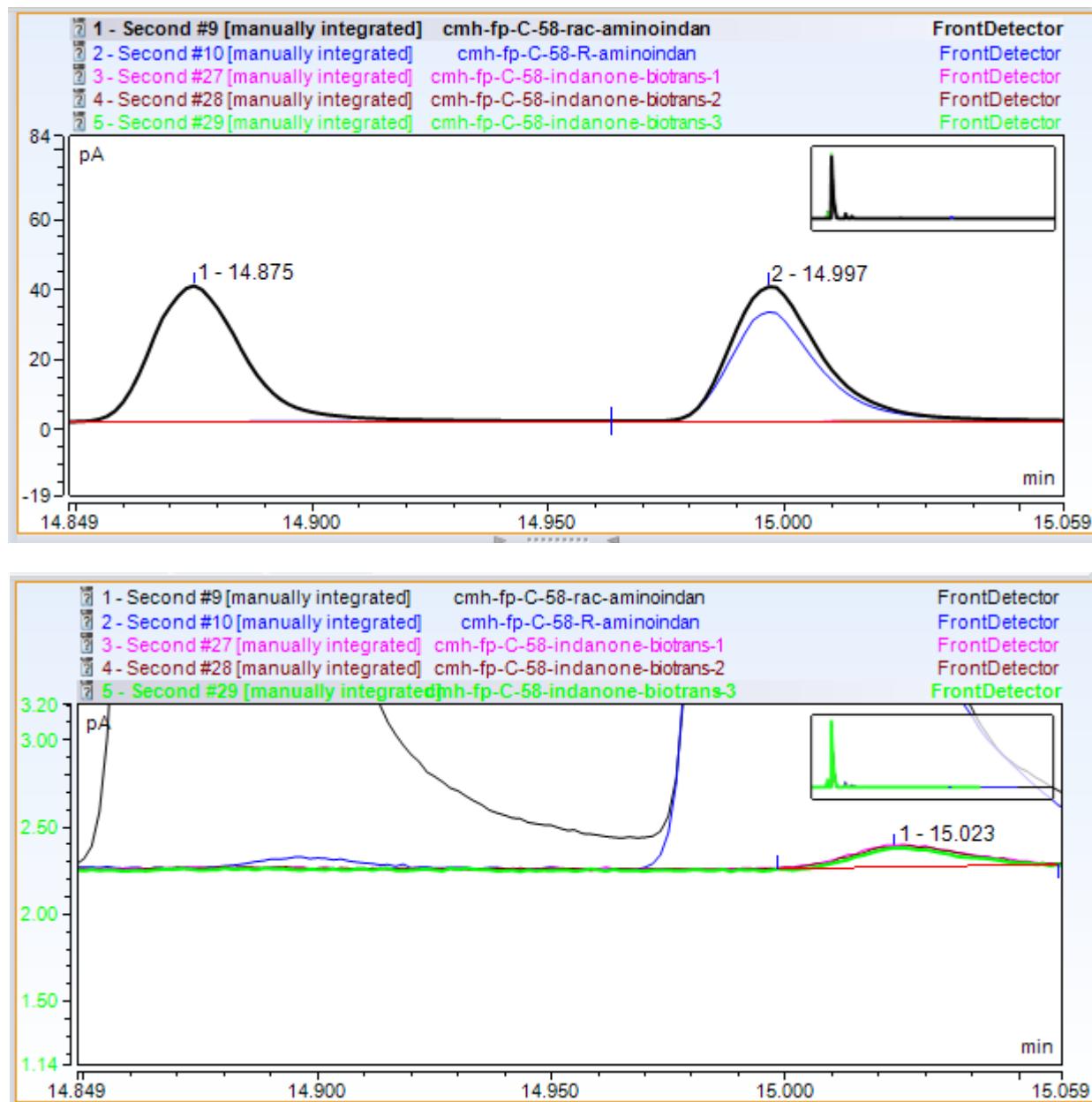


TsRTA_G205C



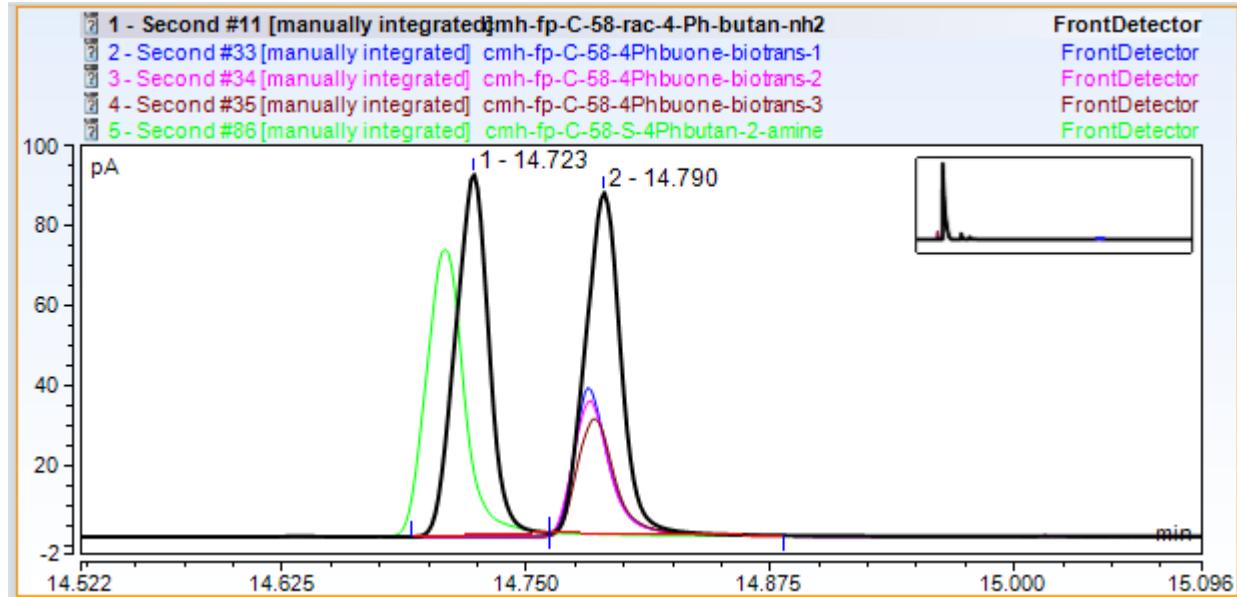
1-aminoindan

TsRTA_wild-type

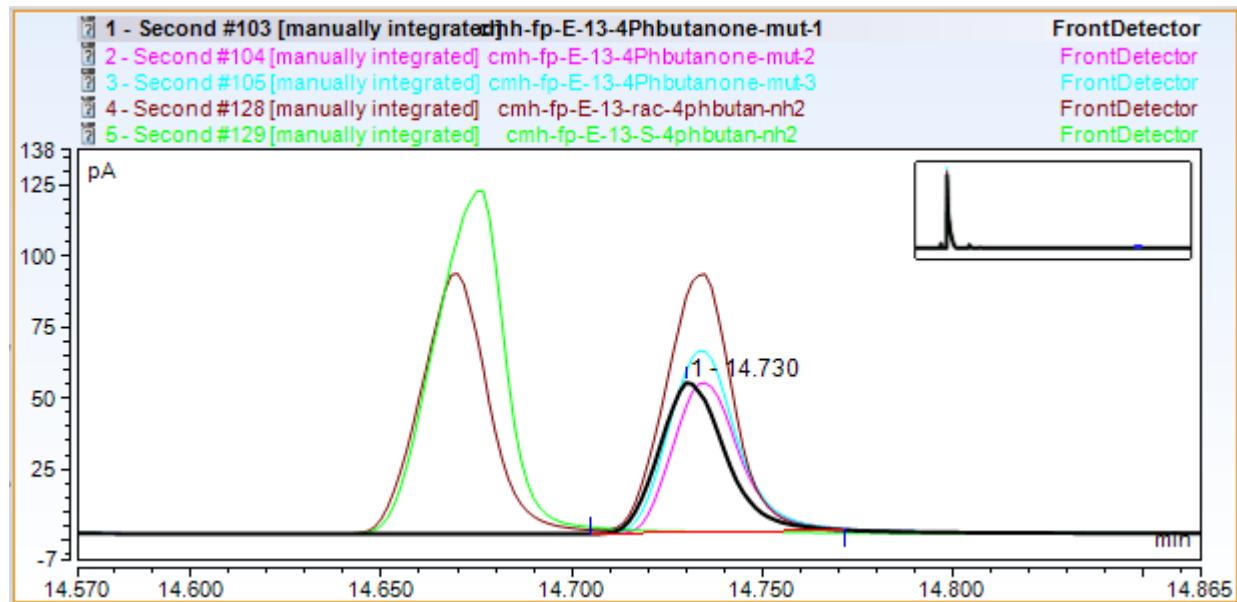


4-phenylbutan-2-amine

TsRTA_wild-type

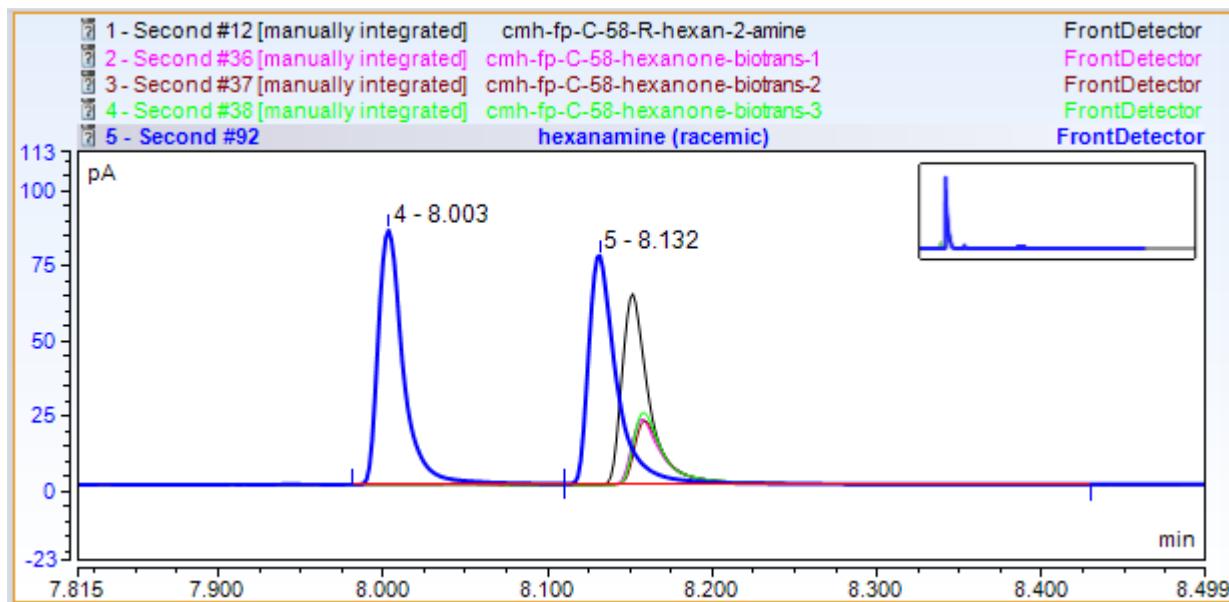


TsRTA_G205C

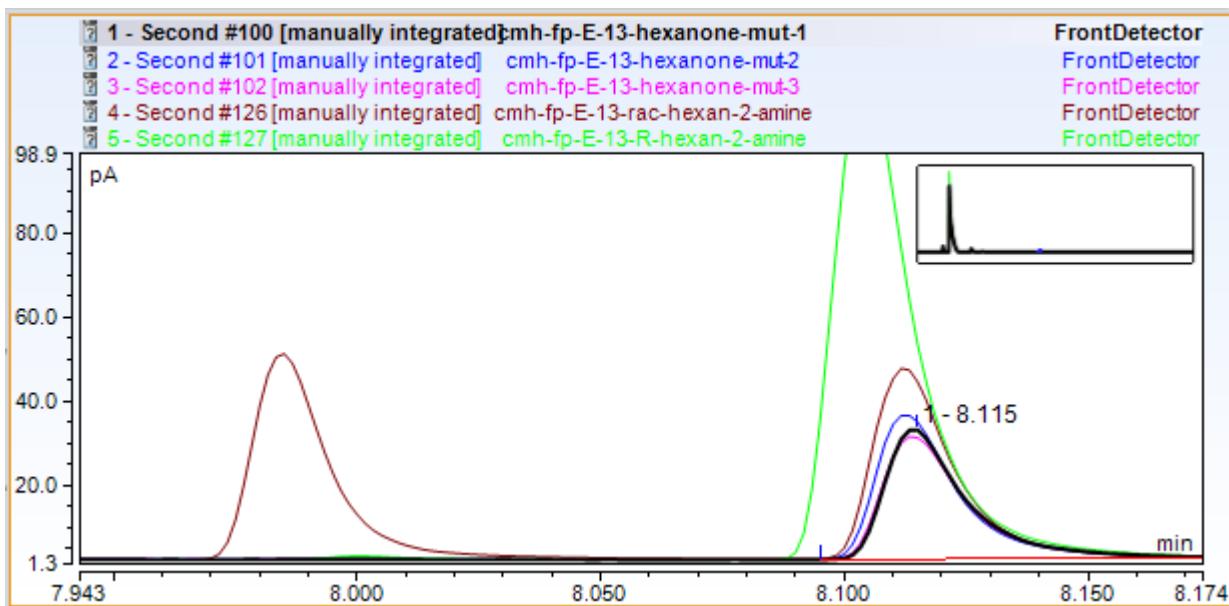


hexan-2-amine

TsRTA_wild-type

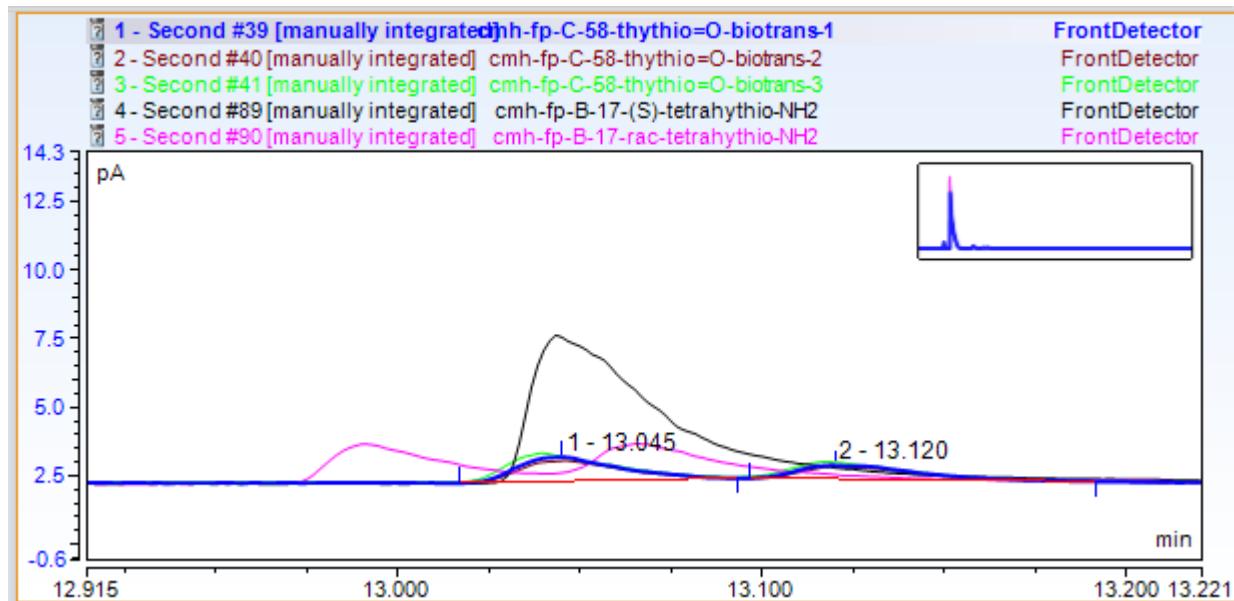


TsRTA_G205C



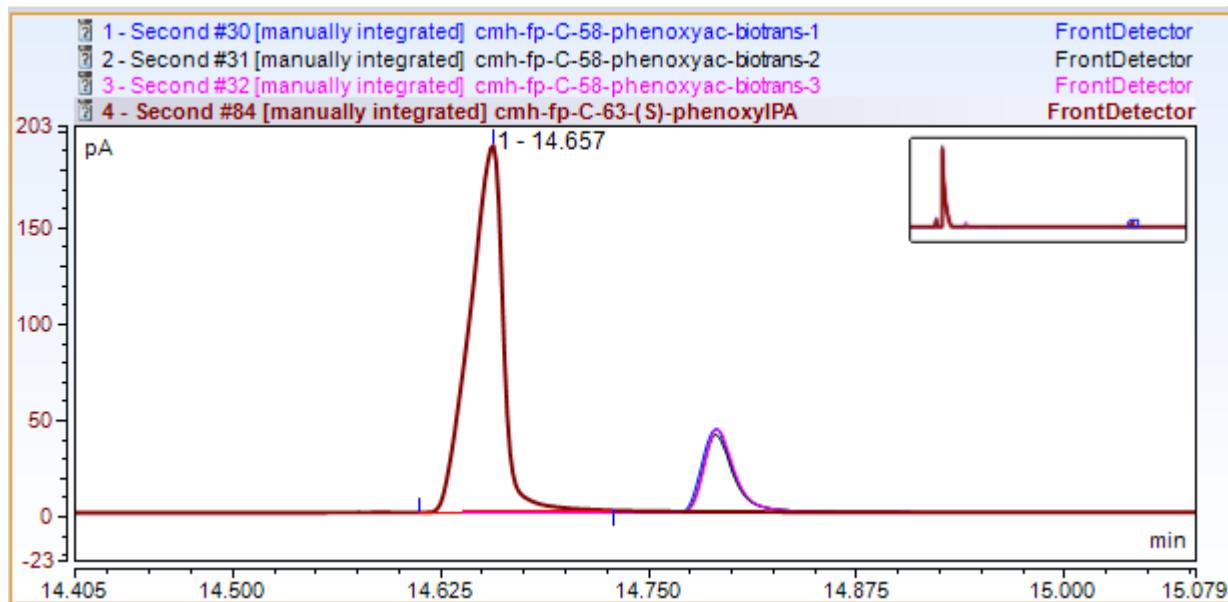
tetrahydrothiophene-3-amine

TsRTA_wild-type

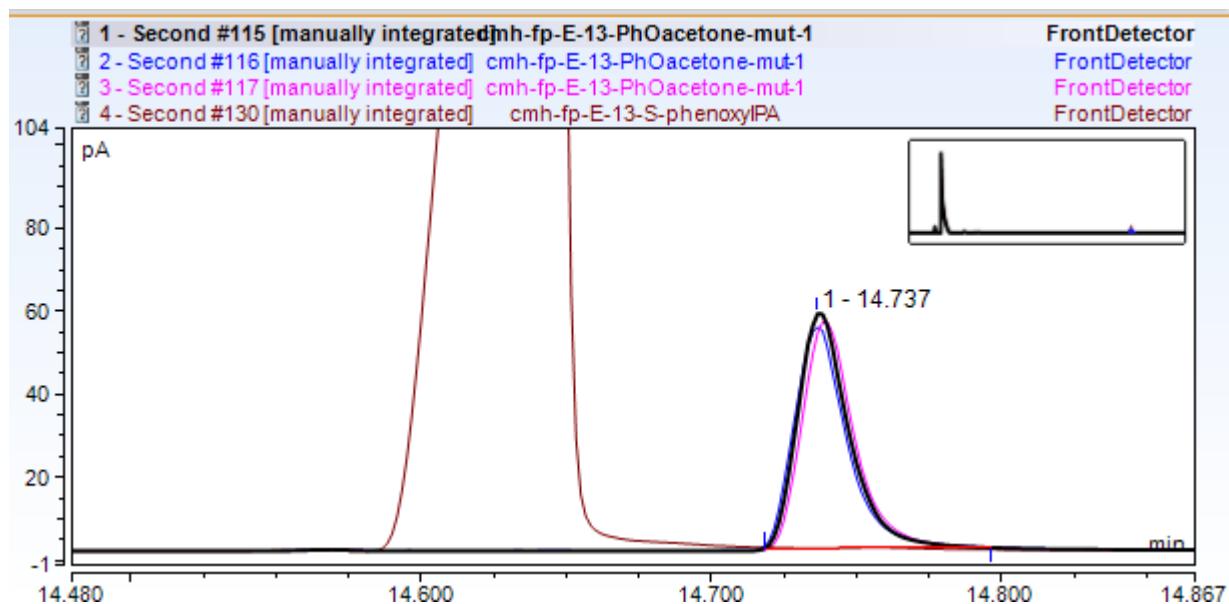


phenoxypropan-2-amine

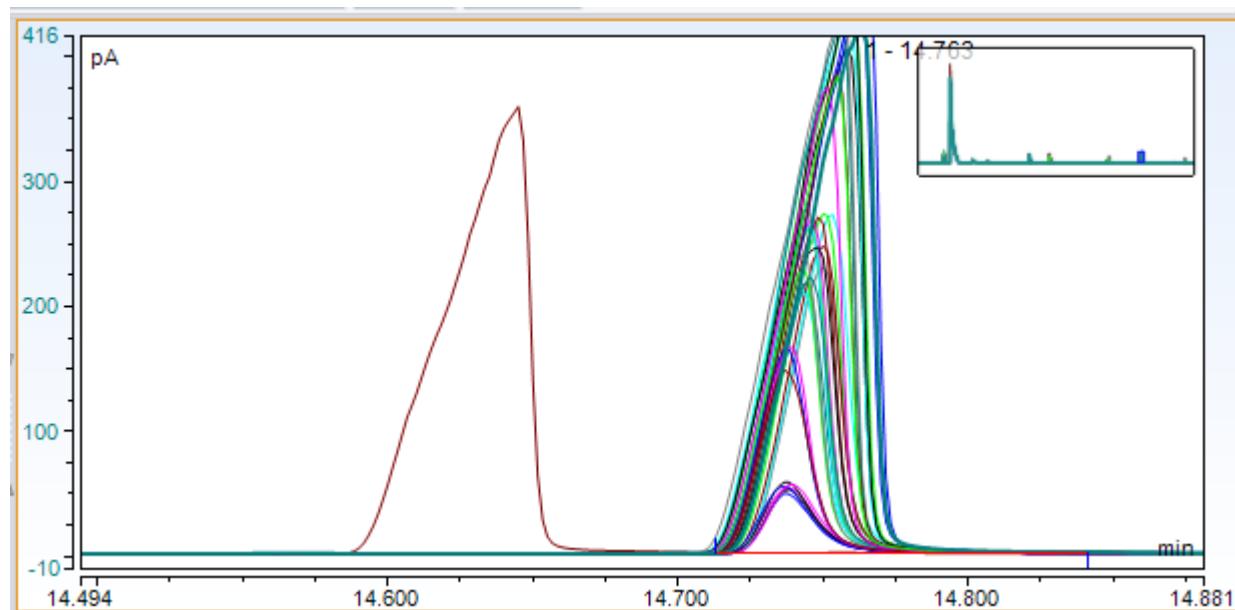
TsRTA_wild-type



TsRTA_G205C

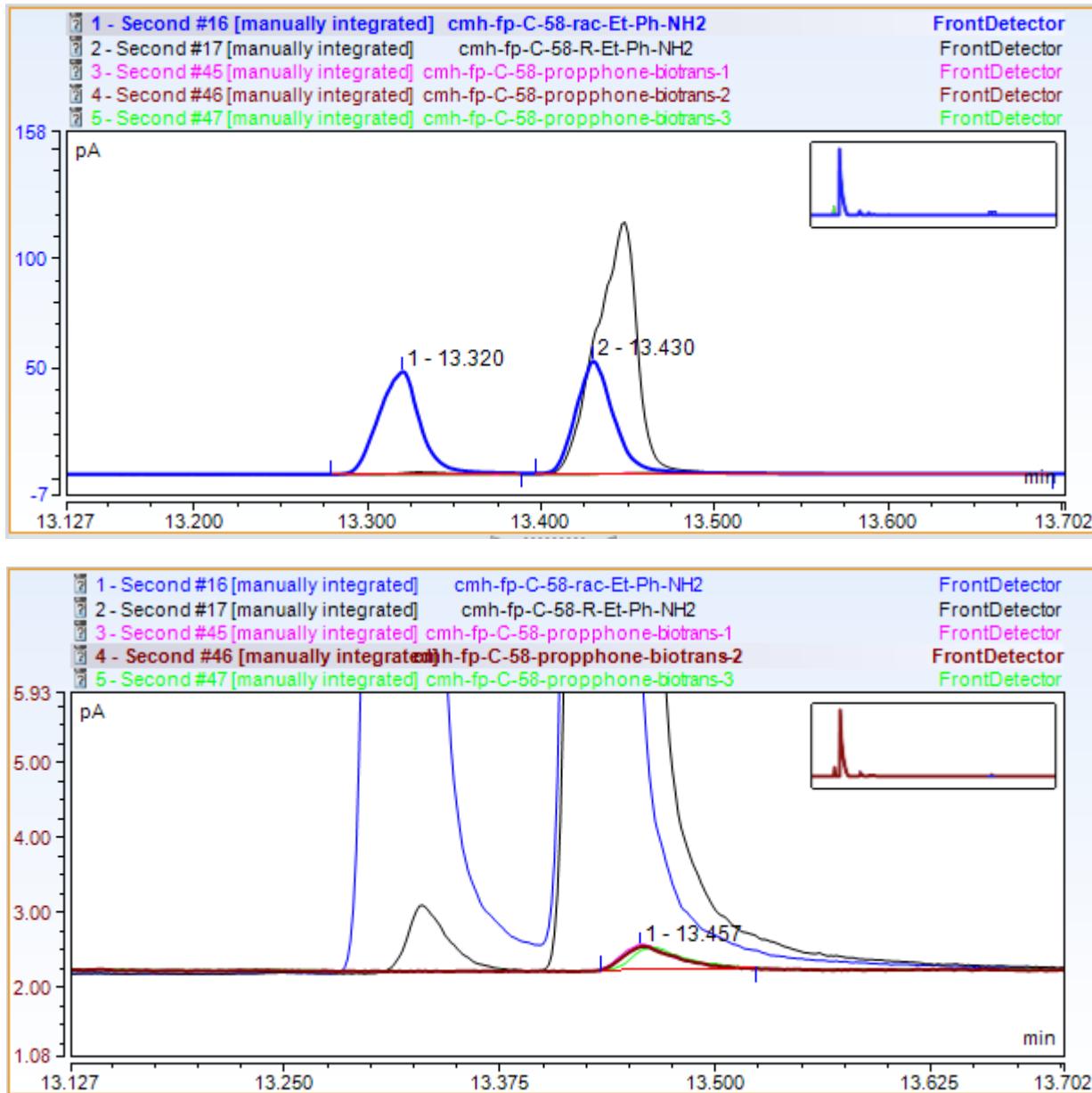


All intensification biotransformations of wild-type and G205C vs (*S*)-phenoxypropan-2-amine



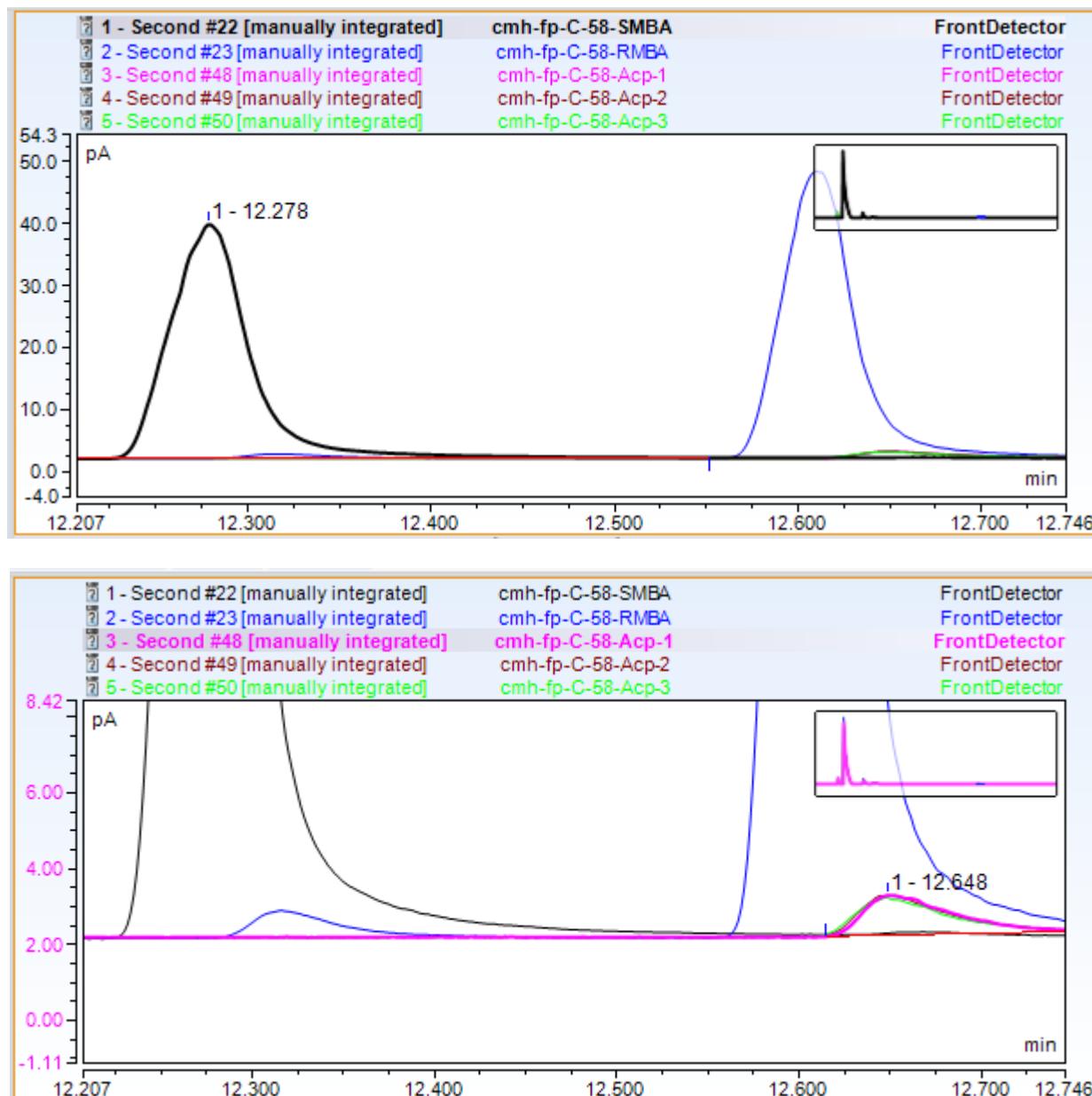
α -Ethylbenzylamine

TsRTA_wild-type



α -Methylbenzylamine

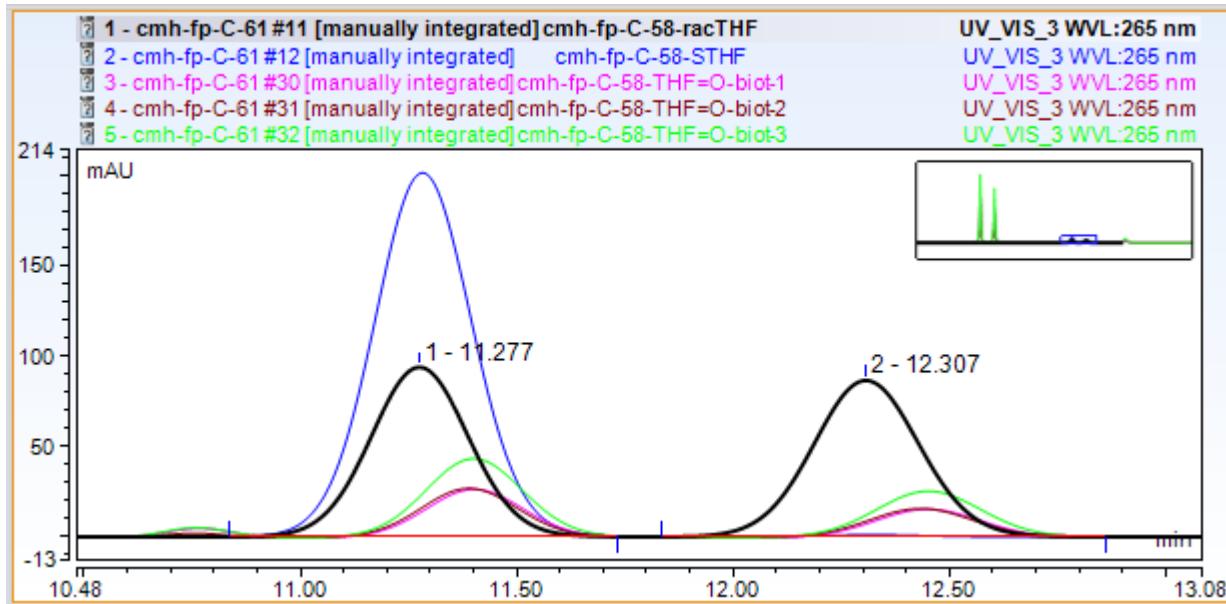
TsRTA_wild-type



5.2 RP-HPLC

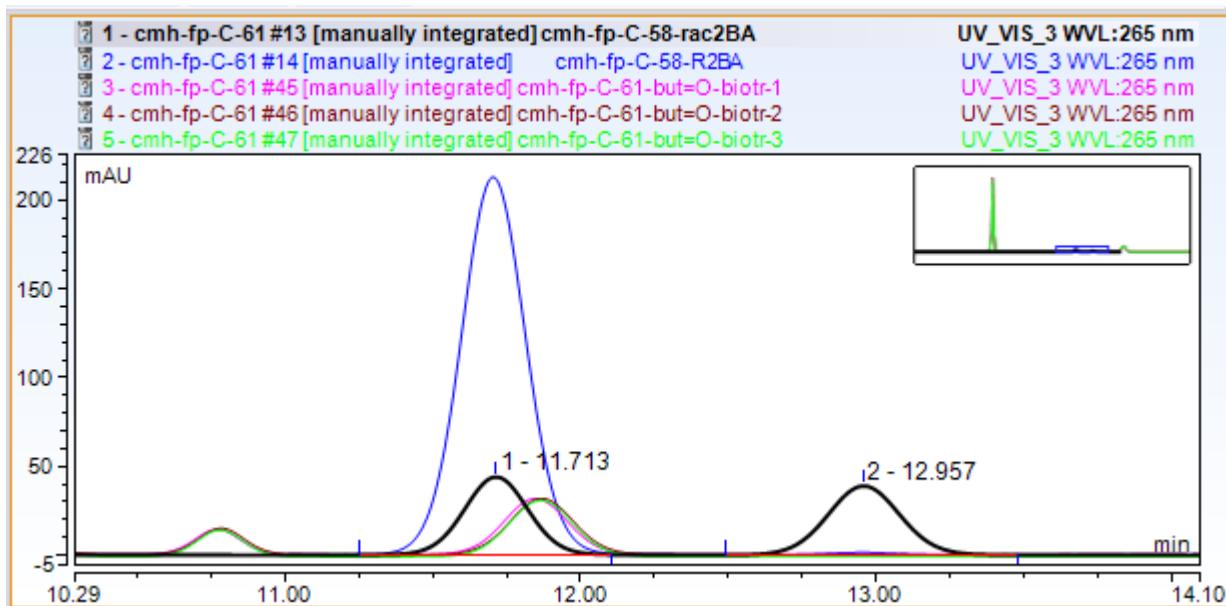
tetrahydrofuran-3-amine

TsRTA_wild-type



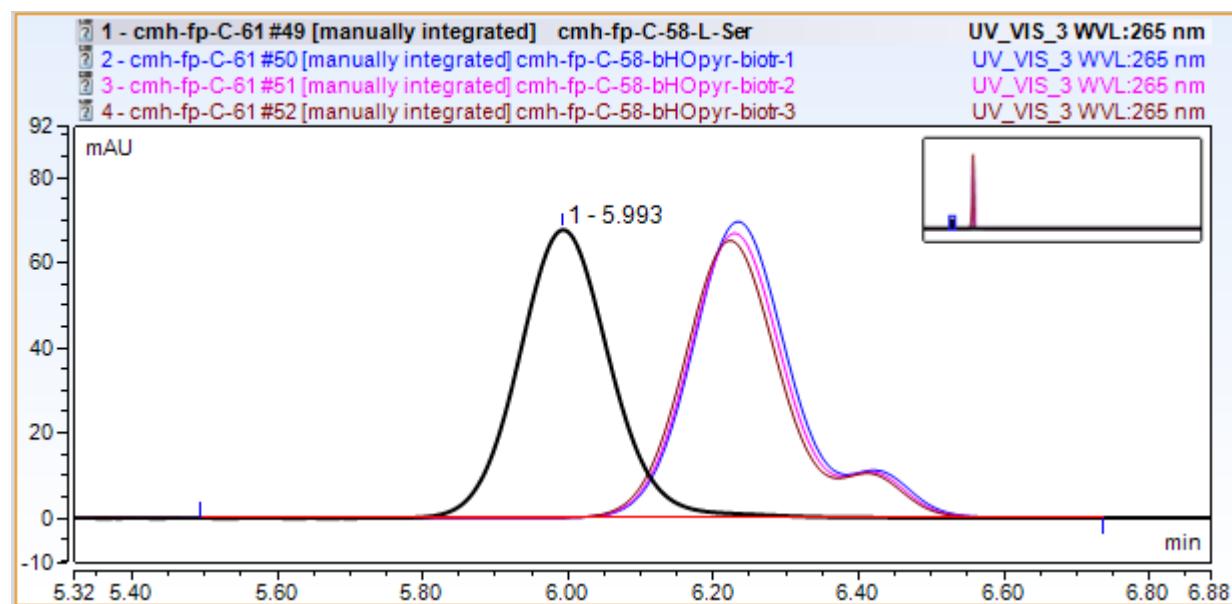
butan-2-amine

TsRTA_wild-type

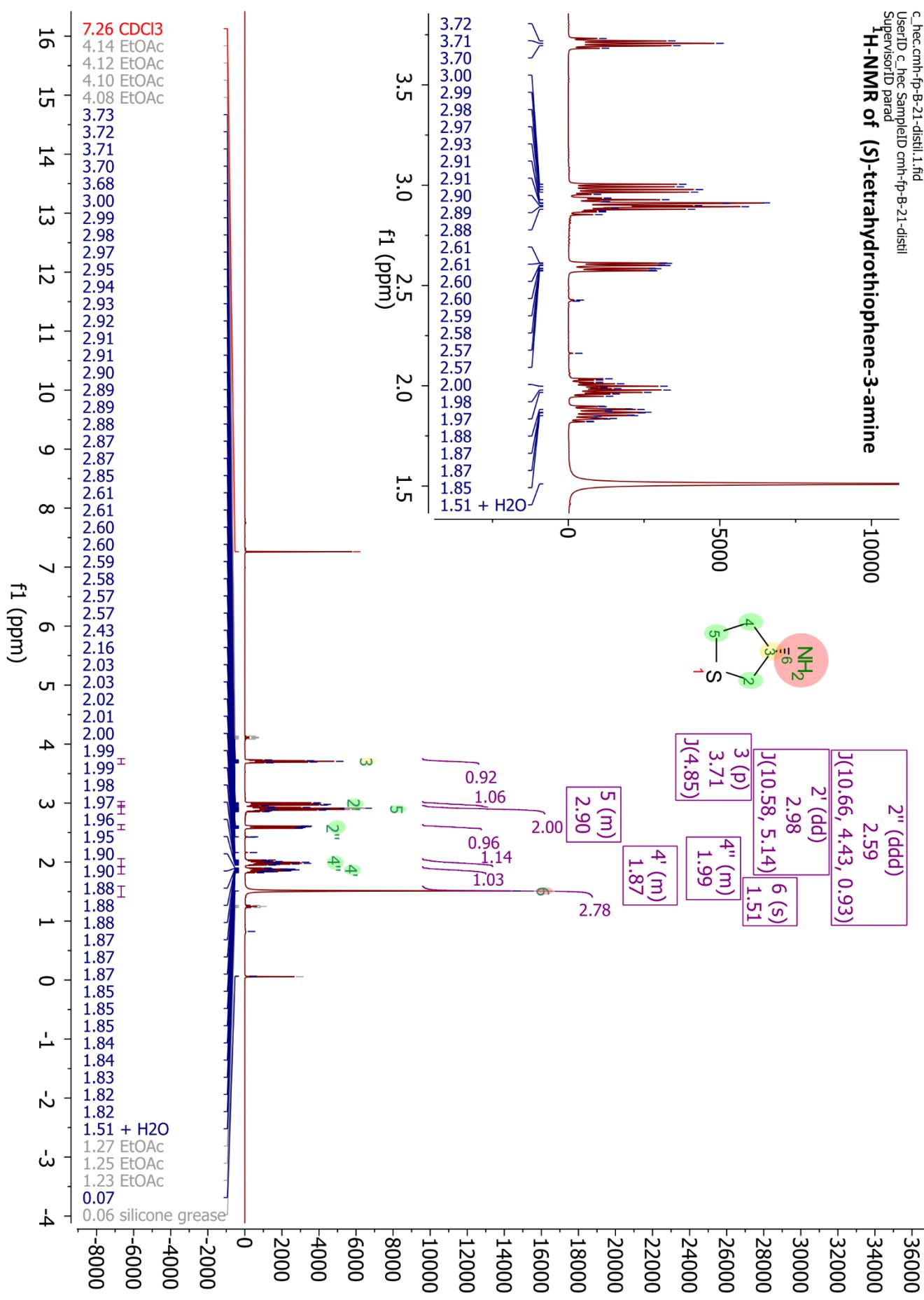


Serine

TsRTA_wild-type

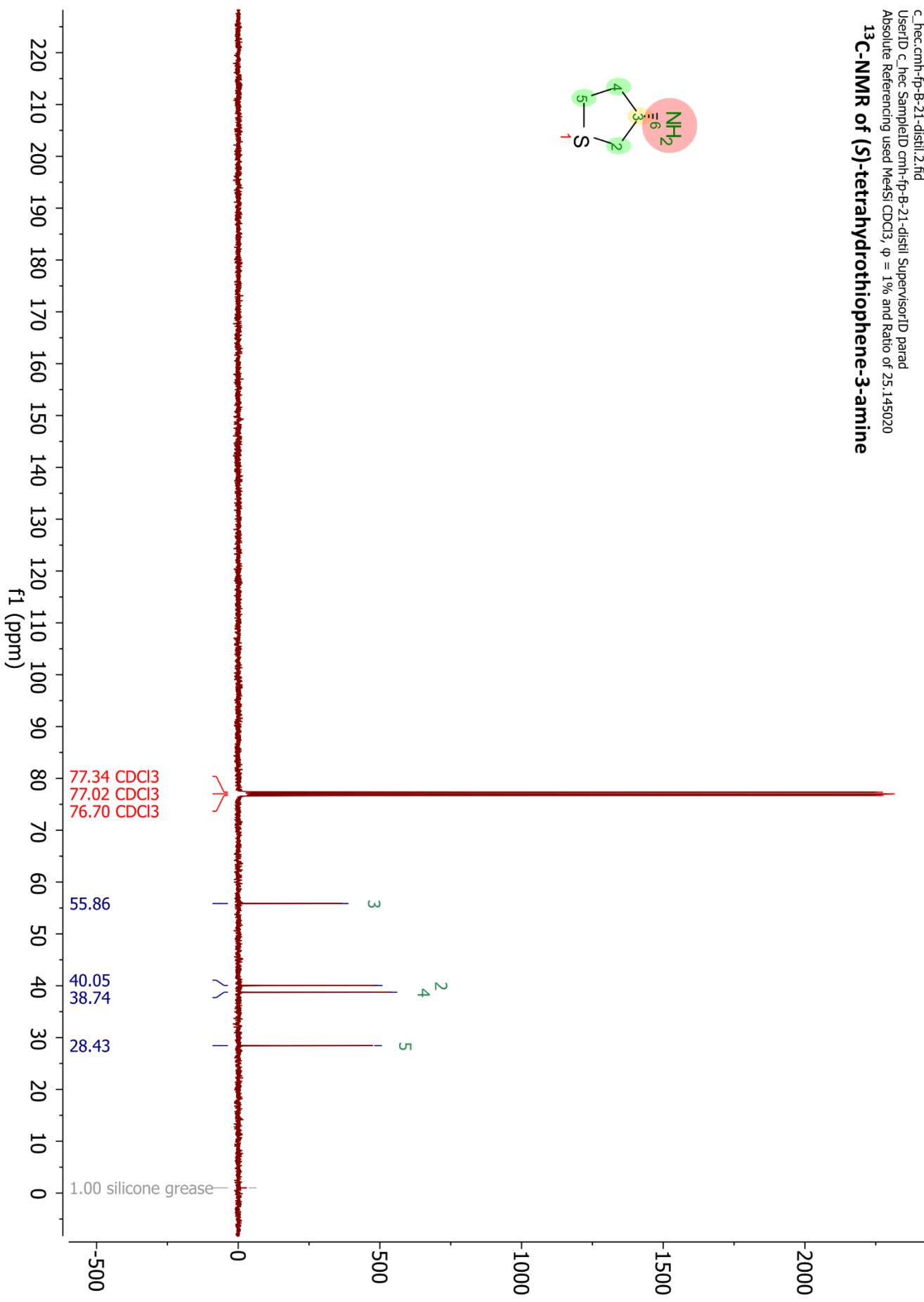
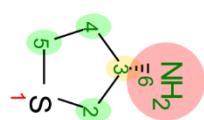


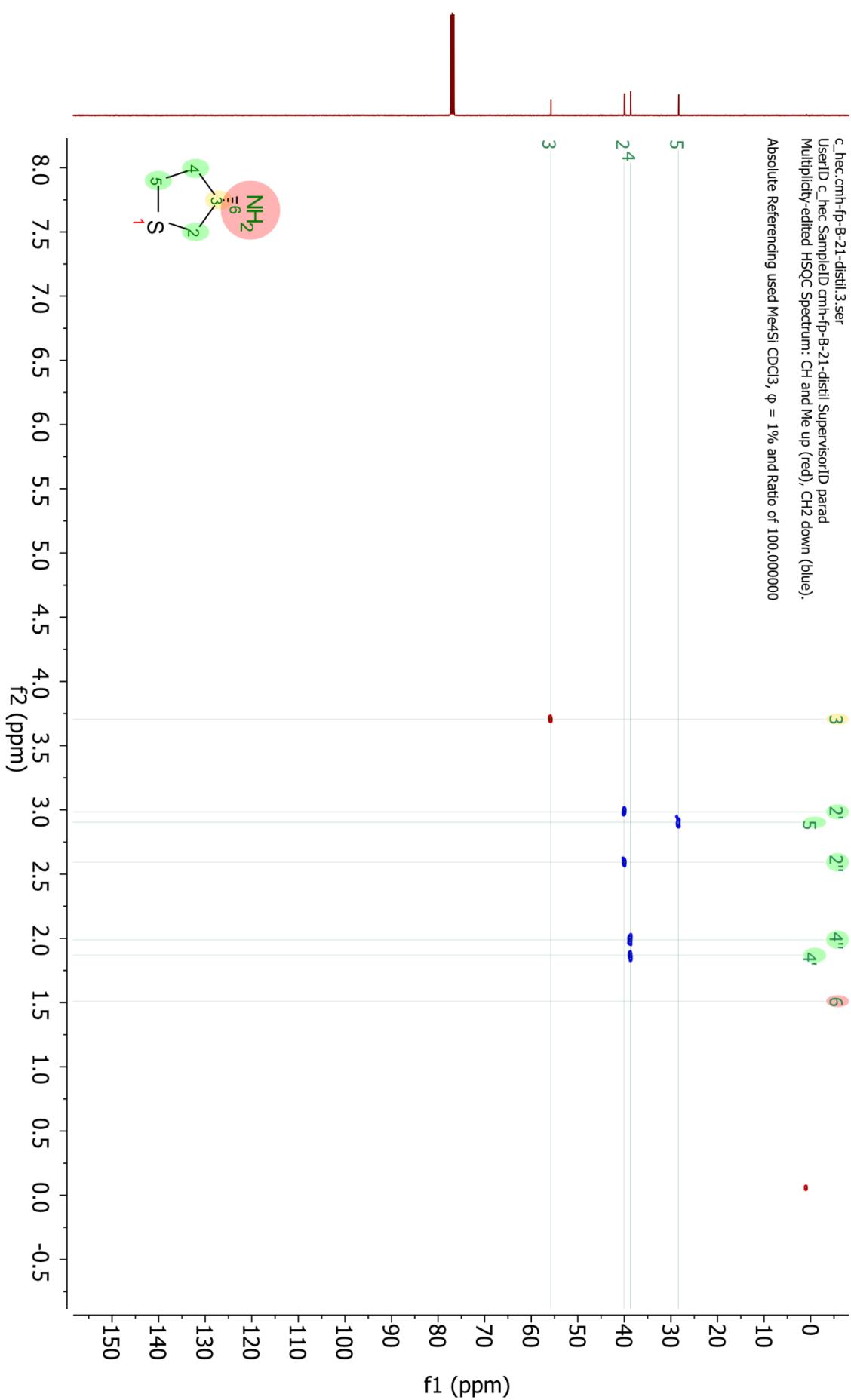
6 NMR spectra



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Absolute Referencing used Me4Si CDCl₃, φ = 1% and Ratio of 25.145020

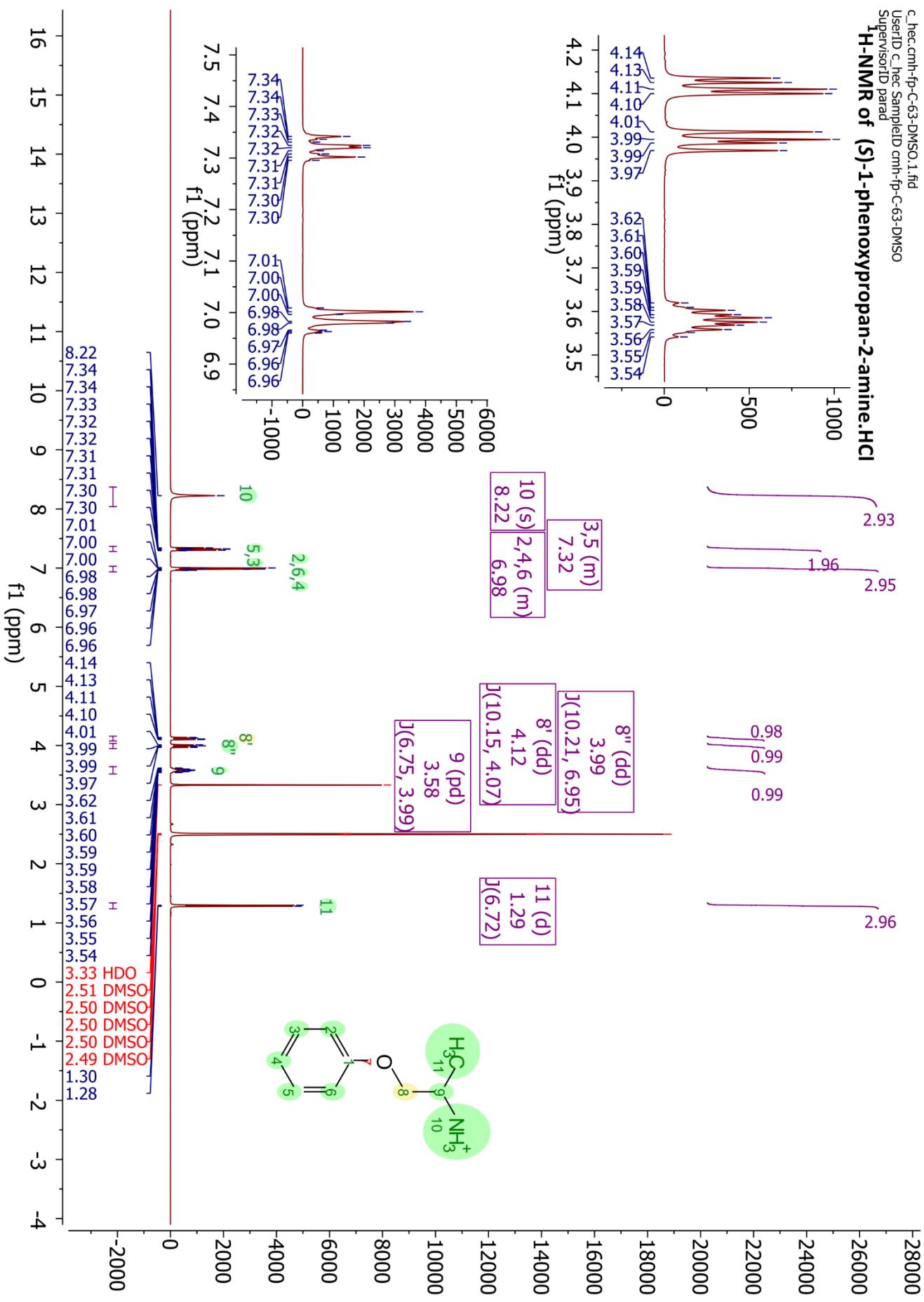
¹³C-NMR of (S)-tetrahydrothiophene-3-amine



¹H-¹³C-HSQC-ME of (S)-tetrahydrothiophene-3-amine

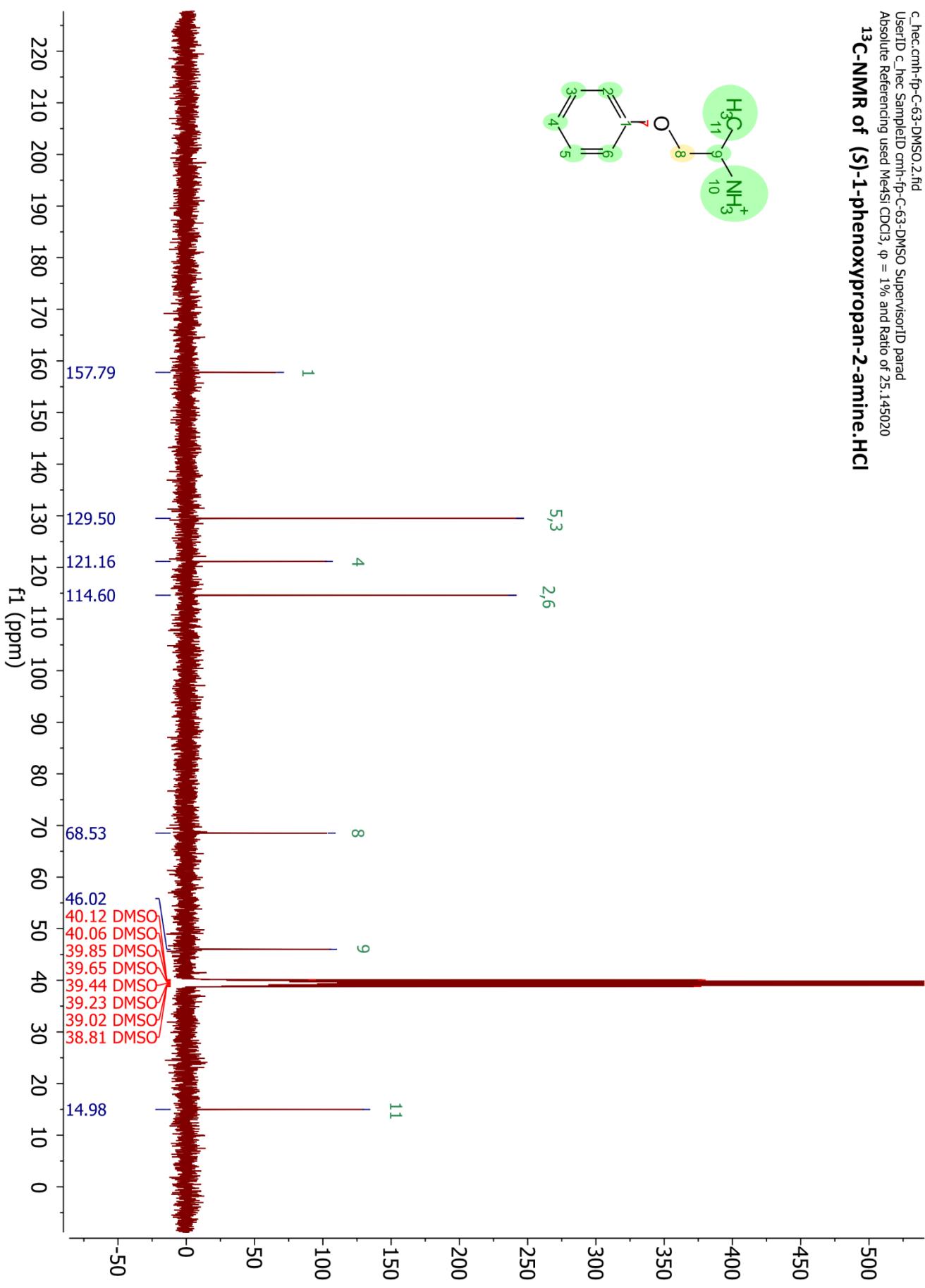
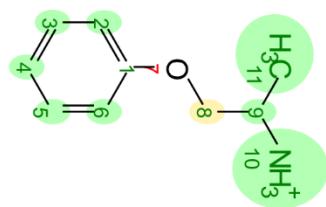
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SupervisorID parad

¹H-NMR of (S)-1-phenoxypropan-2-amine.HCl



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 Absolute Referencing used Me4Si:CDCl₃, φ = 1% and Ratio of 25.145020

¹³C-NMR of (S)-1-phenoxypropan-2-amine.HCl



¹H-¹³C-HSQC-ME of (S)-1-phenoxypropan-2-amine.HCl

