

SUPPLEMENTARY INFORMATION

A noncanonical PLP-dependent enzyme catalyzes β -serine biosynthesis in the edeine antibiotic pathway

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* Shared contribution.

SUPPLEMENTARY TABLE 1. Predicted amino acid identities for edeine biosynthesis proteins from PARAS¹ and NRPS Transformer². Values in parentheses indicate prediction scores.

Ede	Paras ¹	NRPS Transformer ²		
I	Gly (0.852)	Gly (0.999)		
J	Glu (0.512)	Glu (0.690)	Gln (0.200)	Arg (0.081)
K	DAPA (0.782)	DAPA (0.954)		
L	β-Ala (0.161)	Gln (0.670)	Ala (0.195)	Gly (0.074)
N	Leu (0.123)	Tyr (0.854)	Leu (0.038)	Ala (0.024)
P (E domain)	Asn (0.949)	Asn (0.9999)		

SUPPLEMENTARY TABLE 2. X-ray diffraction data collection and refinement statistics for apo-EdeM

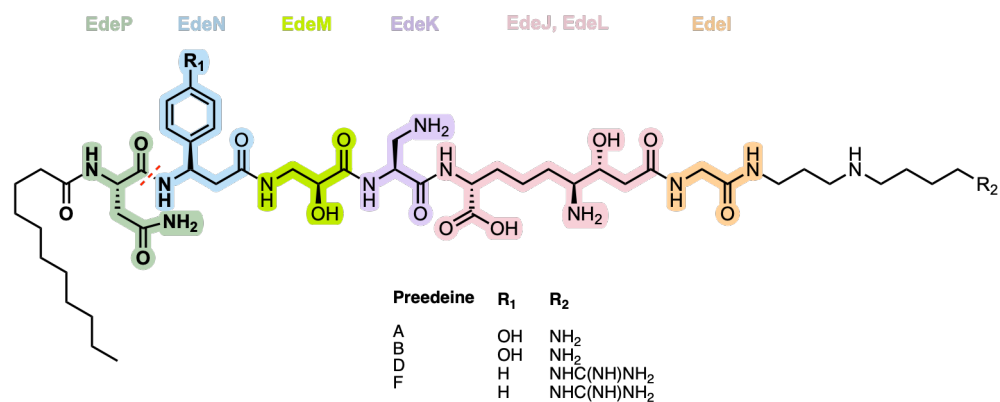
	Apo EdeM (native)	Apo EdeM (SeMet)
Wavelength	0.97928	0.97928
Resolution range	48.65 – 1.7 (1.76 – 1.7)	51.05 – 2.0 (2.05 – 2.0)
Space group	P 21 21 21	P 21 21 21
Unit cell	64.661 92.66 122.229 90 90 90	64.59 92.6 122.38 90 90 90
Total reflections	1078796	656078 (44584)
Unique reflections	80340 (7787)	96007 (6800)
Multiplicity	13.4	6.8 (6.6)
Completeness (%)	98.36 (96.9)	99.99 (100.00)
Mean I/σ(I)	25 (7.8)	12.72 (5.09)
Wilson B-factor	11.87	12.12
R-merge	0.086 (0.354)	0.1132 (0.346)
R-meas	0.090 (0.367)	0.1225 (0.376)
R-pim	0.024 (0.098)	0.04663 (0.1461)
CC1/2	0.999 (0.987)	0.997 (0.955)
CC*	–	0.999 (0.988)
Reflections used in refinement	80089 (5591)	50342 (3539)
Reflections used for R-free	1998 (140)	1999 (141)
R-work	0.1424 (0.1652)	0.1764 (0.1898)
R-free	0.1586 (0.2057)	0.2106 (0.2469)
Number of non-hydrogen atoms	7265	6918
macromolecules	6157	6099
ligands	0	0
solvent	1108	819
Protein residues	762	759
RMS (bond lengths, Å)	0.006	0.015
RMS (bond angles, °)	0.93	1.46
Ramachandran favored (%)	96.17	94.70
Ramachandran allowed (%)	3.30	4.11
Ramachandran outliers (%)	0.53	1.19
Rotamer outliers (%)	1.03	0.75
Clashscore	4.82	19.53
Average B-factor	17.57	15.48
macromolecules	15.84	14.24
solvent	27.21	24.69

Statistics for the highest-resolution shell are shown in parentheses.

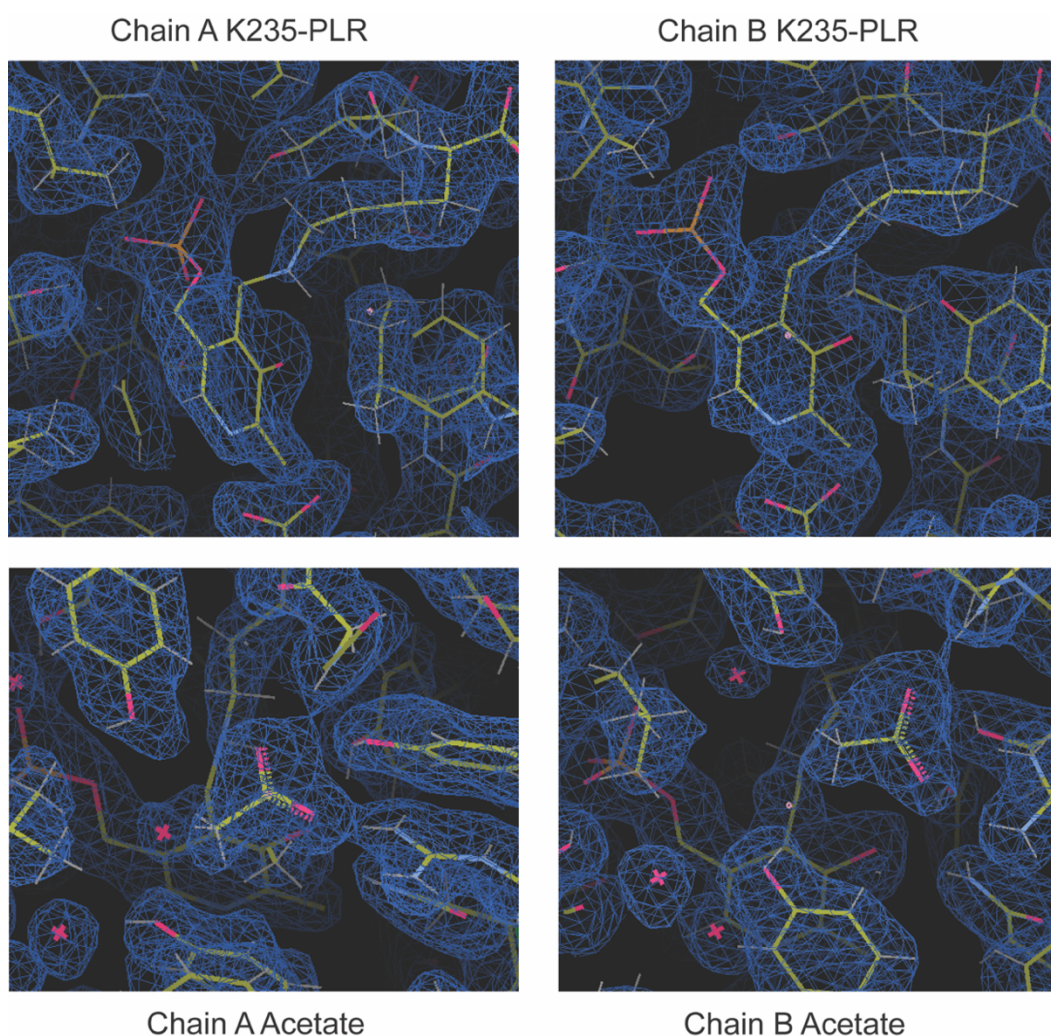
SUPPLEMENTARY TABLE 3. X-ray diffraction data collection and refinement statistics for EdeM in complex with reduced PLP (PLR) and acetate.

	EdeM-PLR + Acetate
Wavelength	1.512
Resolution range	62.51 - 2.3 (2.36 - 2.3)
Space group	P 21 21 21
Unit cell	60.78 66.1 192.18 90 90 90
Total reflections	226852 (14730)
Unique reflections	67738 (4664)
Multiplicity	3.3 (3.2)
Completeness (%)	95.68 (99.72)
Mean I/σ(I)	10.13 (1.64)
Wilson B-factor	25.79
R-merge	0.07569 (0.6304)
R-meas	0.09023 (0.7593)
R-pim	0.04861 (0.418)
CC1/2	0.997 (0.614)
CC*	0.999 (0.872)
Reflections used in refinement	33769 (2488)
Reflections used for R-free	1988 (141)
R-work	0.1666 (0.1902)
R-free	0.2074 (0.2283)
Number of non-hydrogen atoms	6675
macromolecules	6160
ligands	38
solvent	477
Protein residues	771
RMS (bond lengths, Å)	0.003
RMS (bond angles, °)	0.73
Ramachandran favored (%)	96.07
Ramachandran allowed (%)	3.90
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	4.63
Clashscore	3.84
Average B-factor	29.90
macromolecules	29.62
solvent	34.16
ligands	21.74

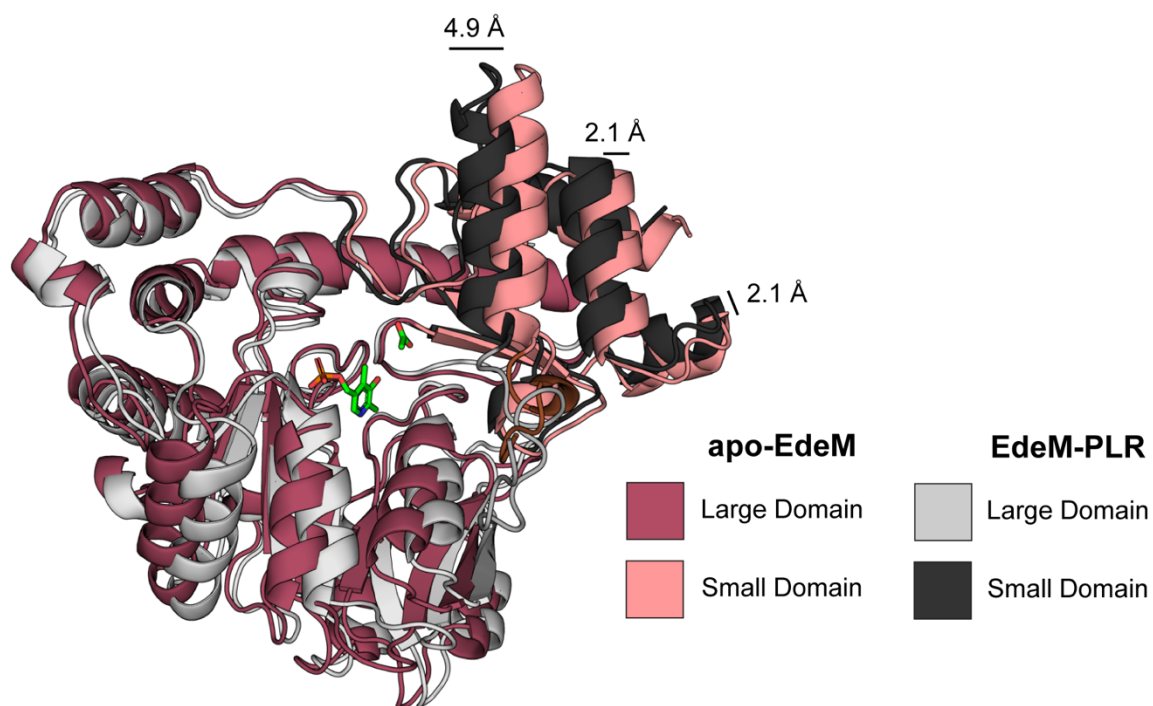
Statistics for the highest-resolution shell are shown in parentheses.



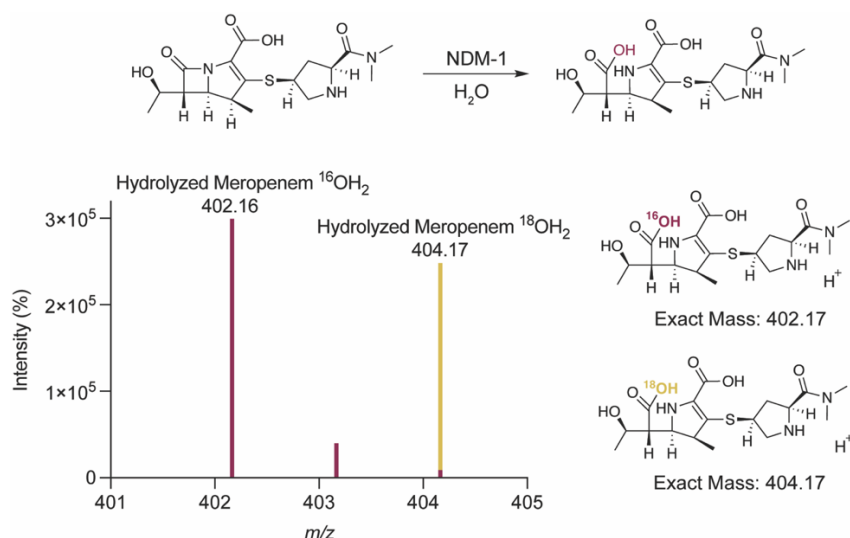
SUPPLEMENTARY FIGURE 1. Predeine chemical structure. Associated Ede proteins are highlighted with color for the substrate specificities and red dashed line indicates site of cleavage.



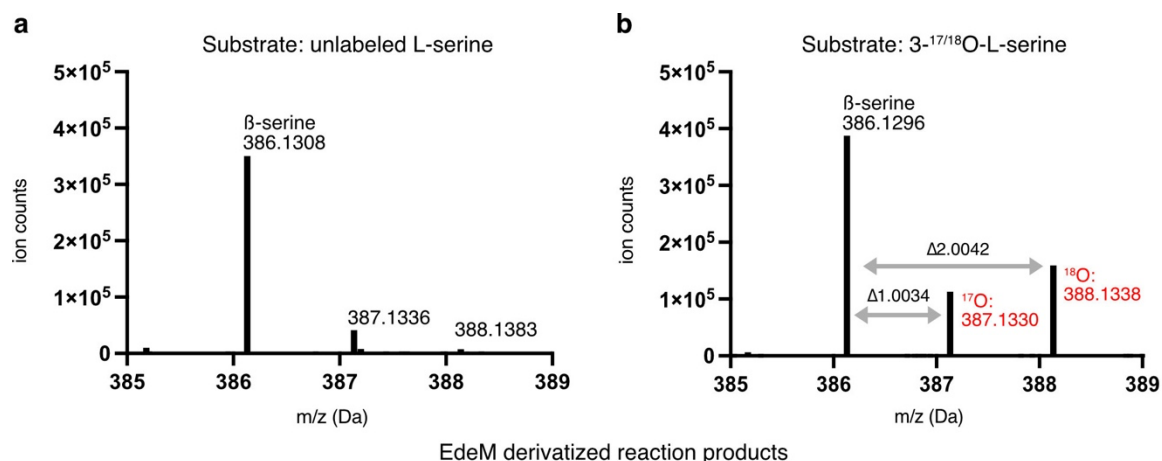
SUPPLEMENTARY FIGURE 2. 2Fo-Fc electron density map at RMSD at 1 Å of reduced PLP (PLR) and acetate in the active site of EdeM. Ligands are shown in Chain A and Chain B. Electron density surrounding the reduced Schiff base between K235 and PLR is partially complete in Chain A and complete in Chain B.



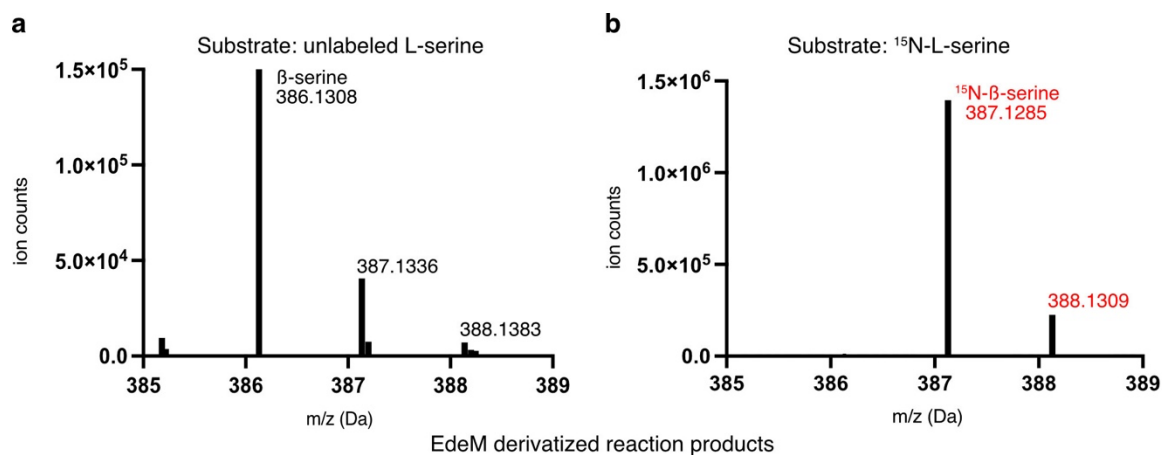
SUPPLEMENTARY FIGURE 3. Overlay of Chain B for apo-EdeM (open conformation) and EdeM-PLR (closed conformation) (RMSD 1.85 Å). The large and small domains of apo-EdeM are in dark pink and light pink, respectively. The large and small domains of EdeM-PLR are in black and grey, respectively. PLR and acetate are in green. The small domain of EdeM-PLR (grey) is positioned inwards toward the active site due to the presence of bound acetate. Distances between various α -helices of the small domain in both conformations are annotated.



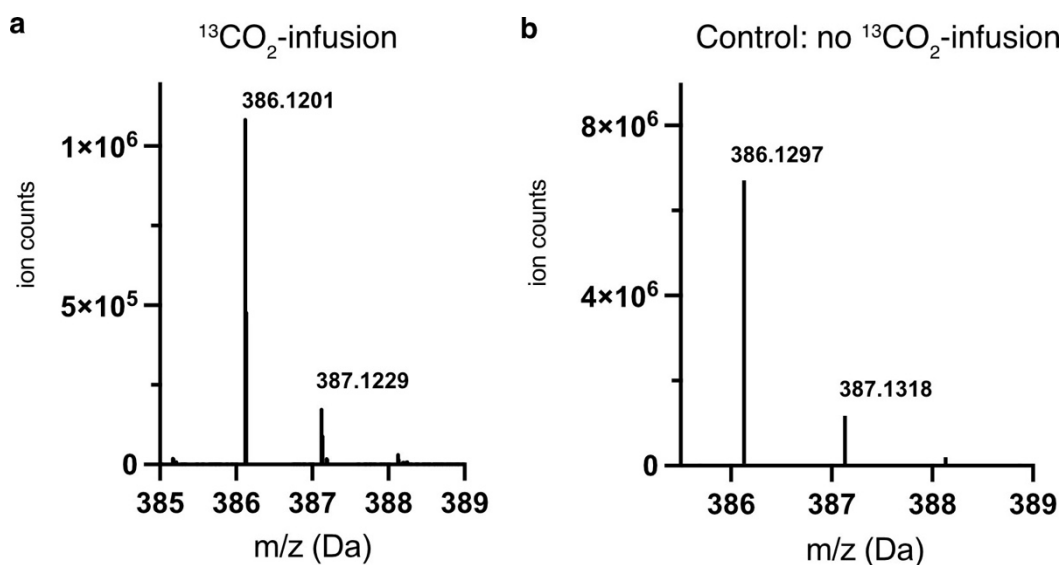
SUPPLEMENTARY FIGURE 4. NMD-1 and meropenem ^{18}O incorporation control. The incorporation of 2 Da is seen in the hydrolyzed meropenem reaction product when reactions are prepared in $^{18}\text{OH}_2$.



SUPPLEMENTARY FIGURE 5. LC-QToF-MS analysis of EdeM reaction products. a) Natural isotope abundance distribution of Marfey-derivatized β -serine from a control reaction using unlabeled L-serine as the substrate. b) Isotope enrichment in Marfey-derivatized β -serine from an EdeM reaction using 3- $^{17/18}\text{O}$ -L-serine (9% ^{17}O and 21% ^{18}O , synthesized by Huang et al.³) as the substrate. Double-headed arrows indicate the observed increased mass shifts ($\Delta m/z$) corresponding to the heavy oxygen isotopes.

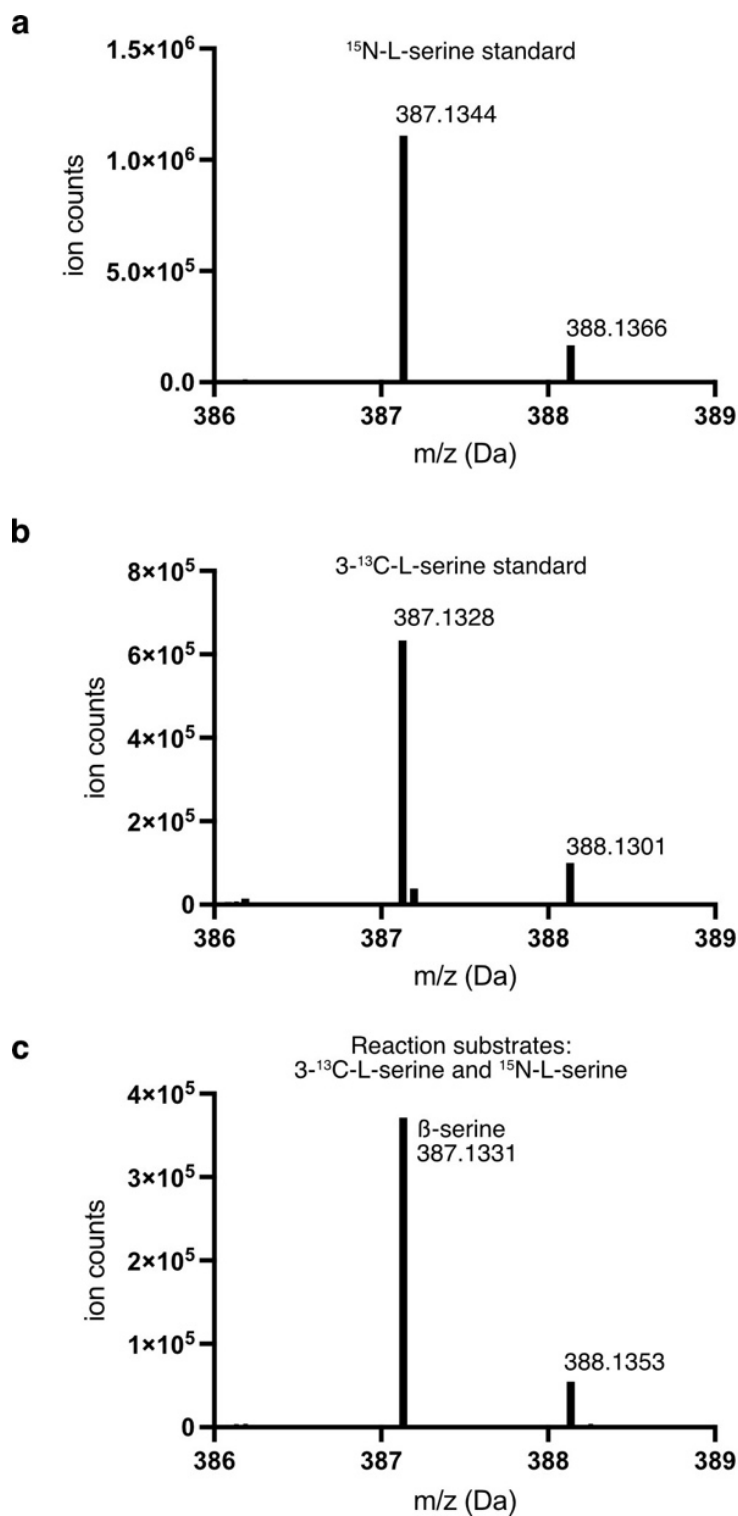


SUPPLEMENTARY FIGURE 6. LC-QToF-MS analysis of EdeM reaction products. a) Natural isotope abundance distribution of Marfey-derivatized β -serine from a control reaction using unlabeled L-serine as the substrate. b) Isotope enrichment in Marfey-derivatized β -serine from an EdeM reaction using 2- ^{15}N -L-serine (99% ^{15}N -L-serine) as the substrate.

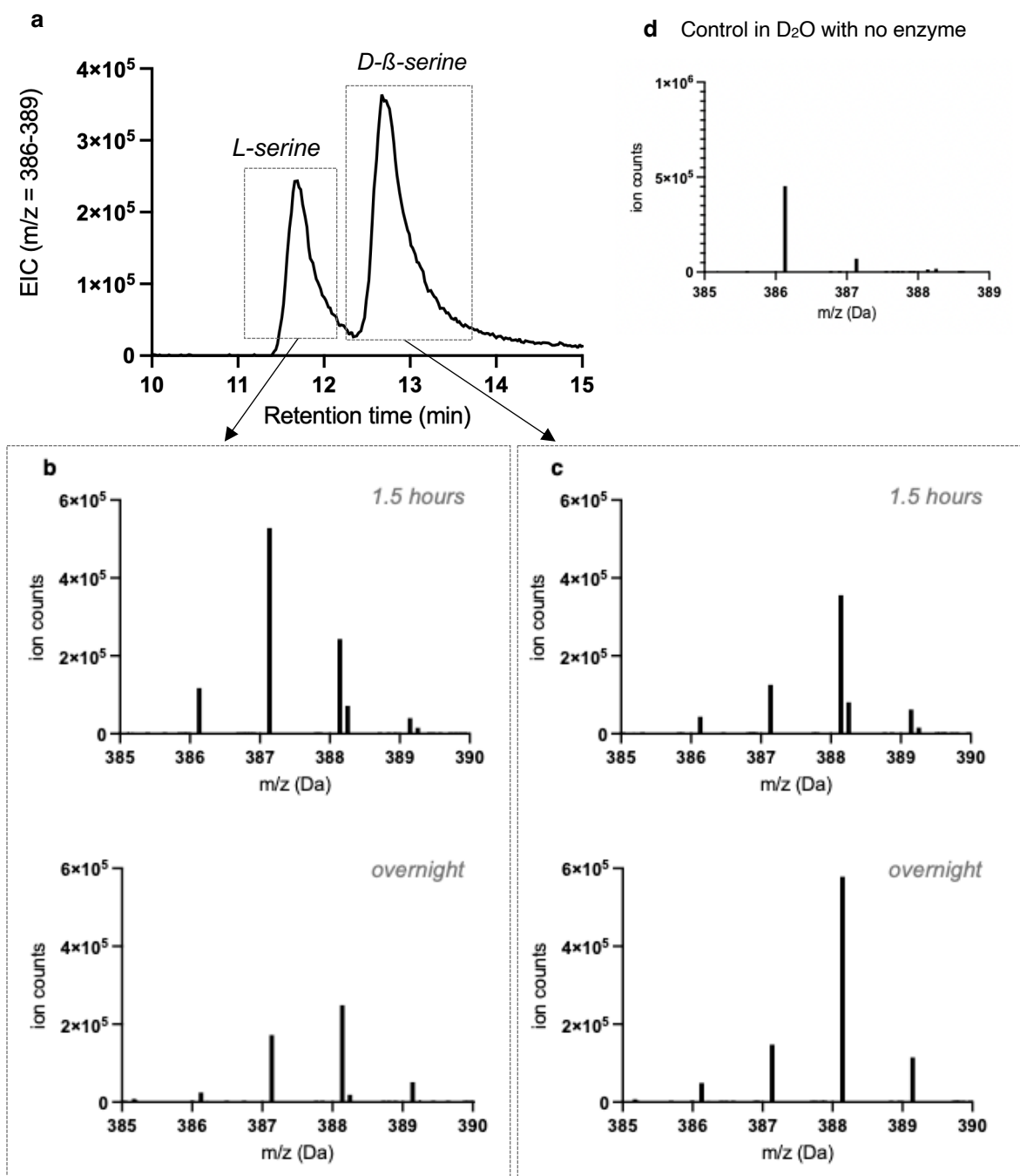


SUPPLEMENTARY FIGURE 7. LC-QToF-MS analysis of EdeM reaction under an atmosphere of isotopically labeled carbon dioxide ($^{13}\text{CO}_2$, 99.7% ^{13}C). Marfey's derivatized products after 4 hours.

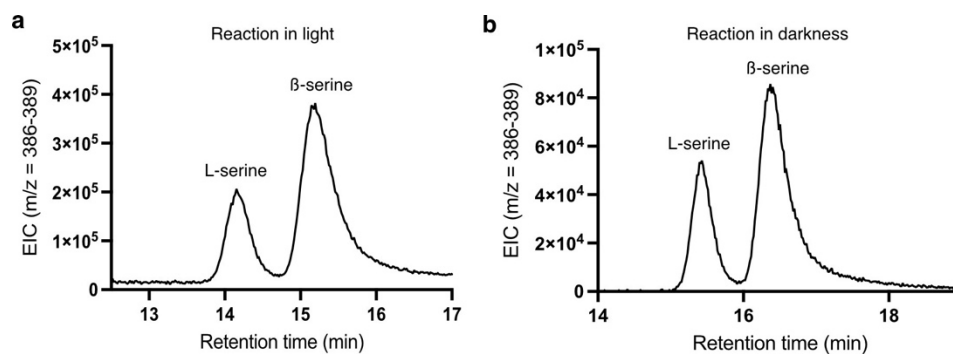
a) The mass-to-charge (m/z) ratio ion content of the beta-serine product peak showing no ^{13}C enrichment. This experiment was repeated five times, with degassing and gas exchange several times. A series of reaction were set up with low to increasing amount of CO_2 gas bubbled through the reaction and incubated at 25 °C and monitored after 1, 2 and 4 h before derivatizing reaction contents with Marfey's reagent, as previously described, all showing the same results, no ^{13}C enrichment. b) Control reaction with no labelled gas infusion.



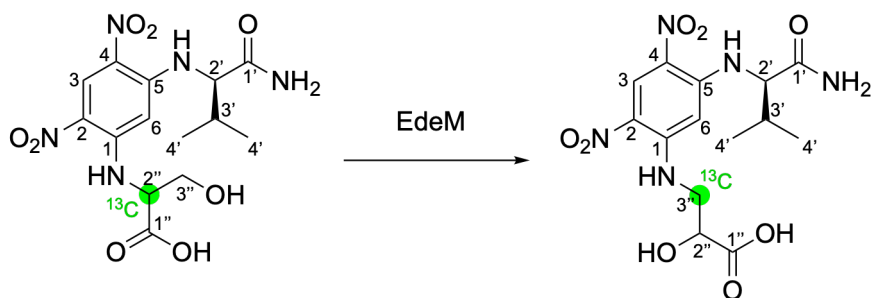
SUPPLEMENTARY FIGURE 8. LC-QToF-MS analysis of standard controls and EdeM reaction product of cross-over experiment. a) Marfey-derivatized ¹⁵N-L-serine (99% ¹⁵N) standard. b) Marfey-derivatized 3-¹³C-L-serine standard. c) Marfey-derivatized reaction product β-serine from an EdeM reaction using 2-¹⁵N-L-serine (99% ¹⁵N-L-serine) and 3-¹³C-L-serine as the substrate in 1:1 ratio. Reaction shows no signal of a crossover species.



SUPPLEMENTARY FIGURE 9. LC-QToF-MS of the EdEM reaction in D_2O a) Positive mode extracted ion chromatogram (EIC) for $m/z = 386-389$ showing Marfey's derivatized L-serine and D-β-serine. b) The mass-to-charge (m/z) ratio of ion content of the L-serine peak after 1.5 hour reaction (above) and overnight (below). c) The mass-to-charge (m/z) ratio of ions content of the D-β-serine peak after 1.5 hour reaction (above) and overnight (below). d) Control reaction in D_2O with no enzyme showing no incorporation of deuterium.

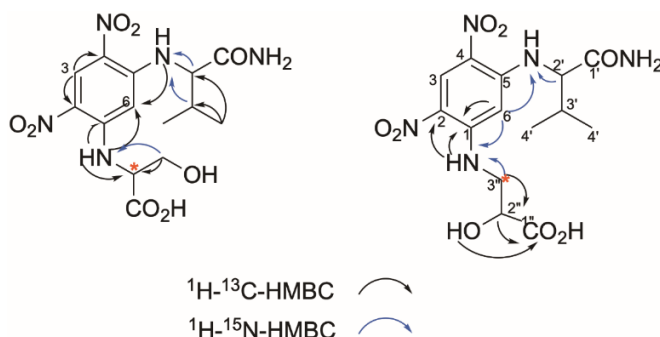


SUPPLEMENTARY FIGURE 10. LC-QToF-MS of the EdeM reaction in a) light vs. b) darkness. Showing the substrate L-serine and D- β -serine product is formed after 3 hours.

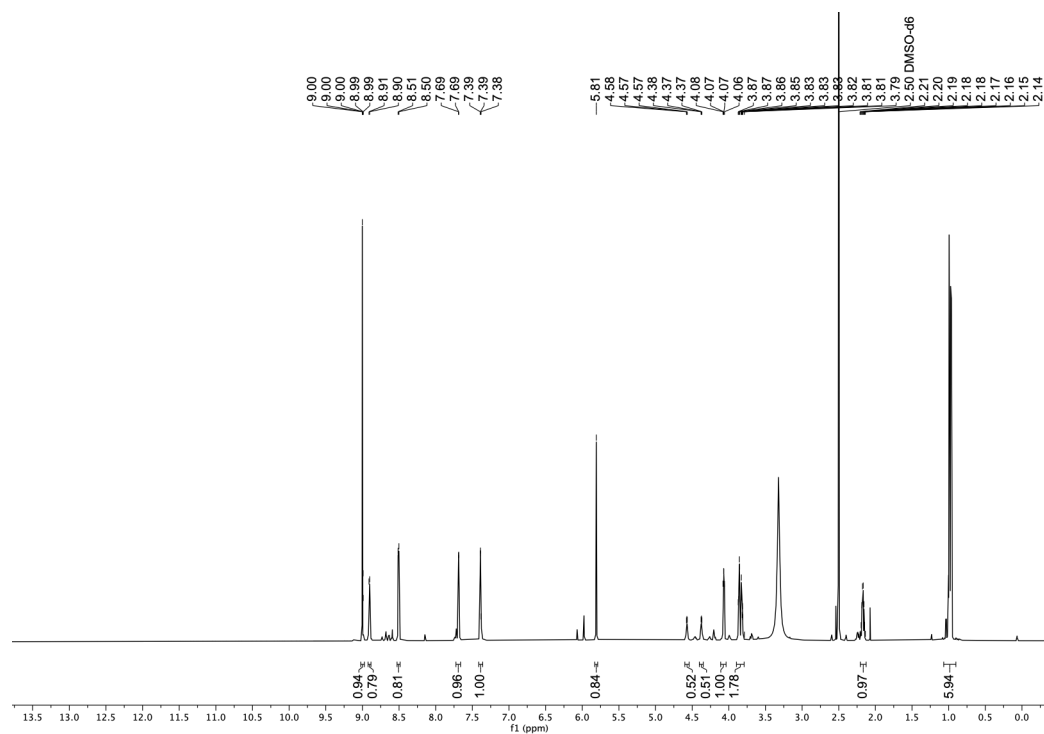


SUPPLEMENTARY TABLE 4. Complete NMR assignment of control L-serine-2-¹³C and the reaction product D-β-serine-3-¹³C after derivatization with Marfey's reagent and purification from large-scale EdeM reaction in dms-*d*₆.

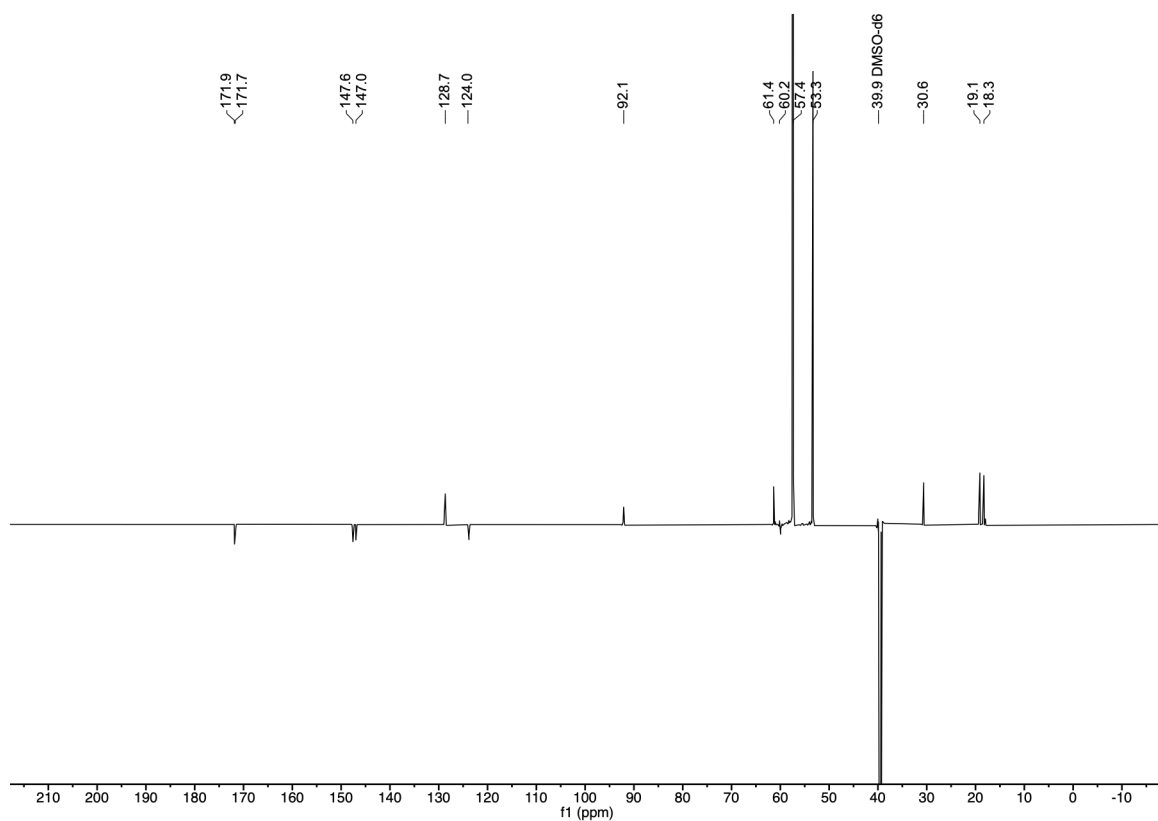
Atom #	¹ H (L-serine)	¹³ C (L-serine)	Atom #	¹ H (D-β-serine)	¹³ C (D-β-serine)
1	-	147.6	1	-	147.6
2	-	124.0	2	-	123.7
3	9.00 (d, <i>J</i> = 7.7 Hz, 1H)	128.7	3	8.97 (s, 1H)	128.3
4	-	124.0	4	-	123.6
5	-	147.0	5	-	148.2
6	5.81 (s, 1H)	92.1	6	6.10 (s, 1H)	91.8
L-Valinamide			L-Valinamide		
2'	4.07 (dd, <i>J</i> = 7.8, 6.1 Hz, 1H)	61.4	2'	4.16 (dd, <i>J</i> = 7.8, 5.5 Hz, 1H)	61.2
3'	2.29 – 2.06 (m, 1H)	30.6	3'	2.21 (dq, <i>J</i> = 13.5, 6.7 Hz, 1H)	30.7
4'	0.99 – 0.96 (m, 6H)	19.1	4'	0.99 (dd, <i>J</i> = 6.8, 2.3 Hz, 6H)	19.1
NH	8.51 (d, <i>J</i> = 7.8 Hz, 1H)	-	NH	8.48 (s, 1H)	-
CONH ₂ (1')	7.69 (m, 1H), 7.39 (m, 1H)	171.7	CONH ₂	7.77 (d, <i>J</i> = 2.1 Hz, 1H), 7.38 (d, <i>J</i> = 2.1 Hz)	171.6
L-Ser			D-β-serine		
2''	4.57 (m, 0.5H), 4.37 (m, 0.5H)	57.4	2''	4.21 (m, 1H)	72.5
3''	3.93 – 3.74 (m, 2H)	60.0	3''	3.59 (m, 1H) 3.22 (m, 1H)	45.9
NH	8.91 (d, <i>J</i> = 6.8 Hz, 1H)	-	NH	8.59 (t, <i>J</i> = 4.8 Hz, 1H)	-
CO ₂ H (1'')		171.9	CO ₂ H	-	176.3



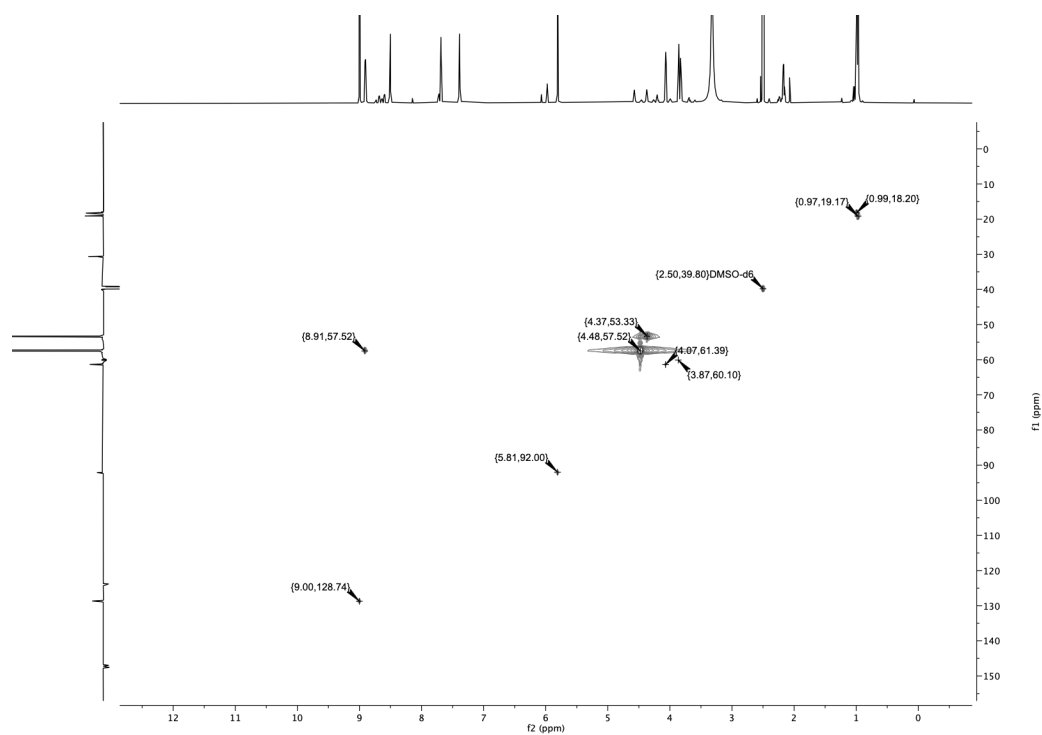
SUPPLEMENTARY FIGURE 11. HMBC diagram of Marfey's labelled L-serine-2-¹³C control and D-β-serine-3-¹³C reaction product.



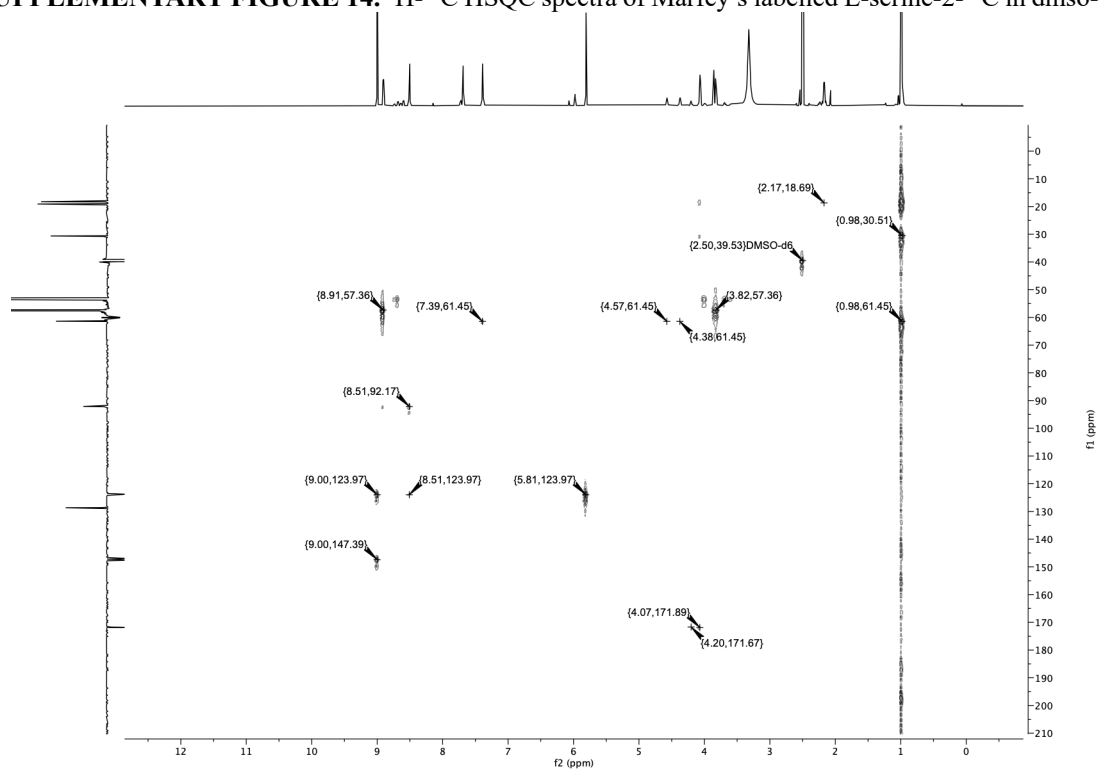
SUPPLEMENTARY FIGURE 12. ^1H -NMR spectra of Marfey's labelled L-serine-2- ^{13}C in $\text{dmsO-}d_6$.



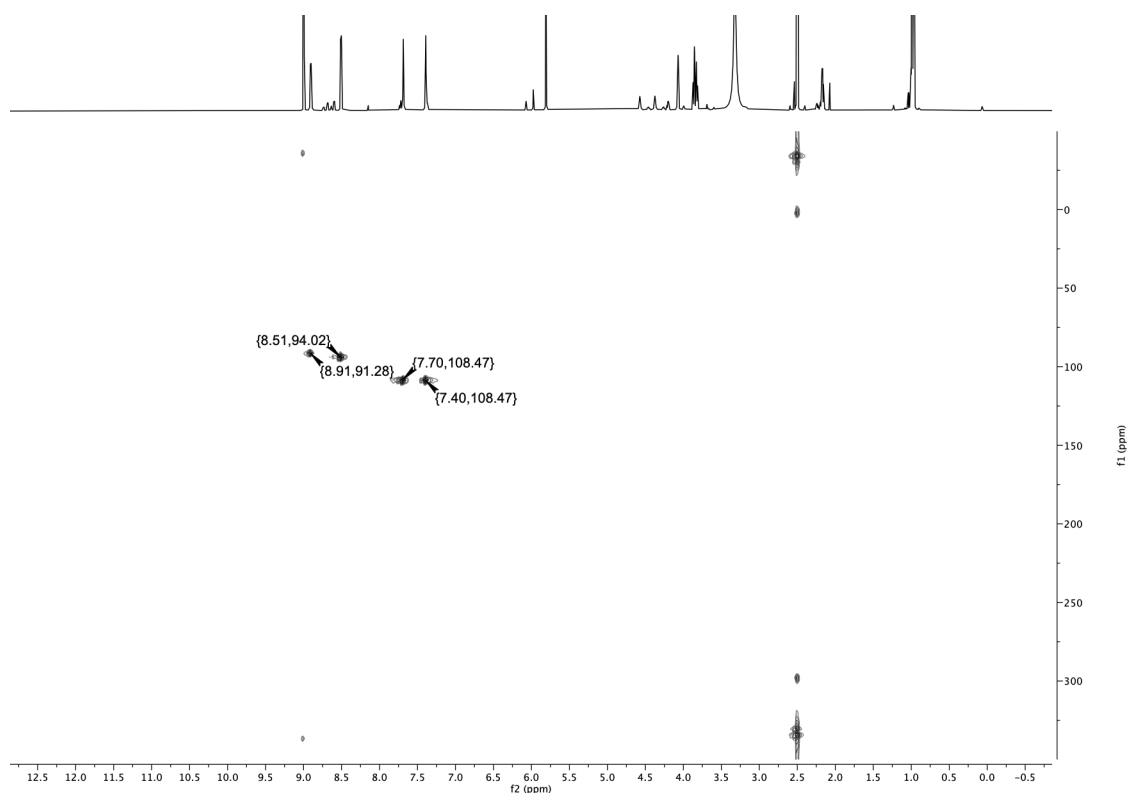
SUPPLEMENTARY FIGURE 13. ^{13}C NMR DEPTQ-135 spectra of Marfey's labelled L-serine-2- ^{13}C in $\text{dmsO-}d_6$. The CH α -carbon is in the same phase as the CH_3 groups of the Marfey's valinamide group. DEPTQ-135 shows CH/ CH_3 (methine/methyl) with a positive phase and CH_2 with negative quaternary carbons



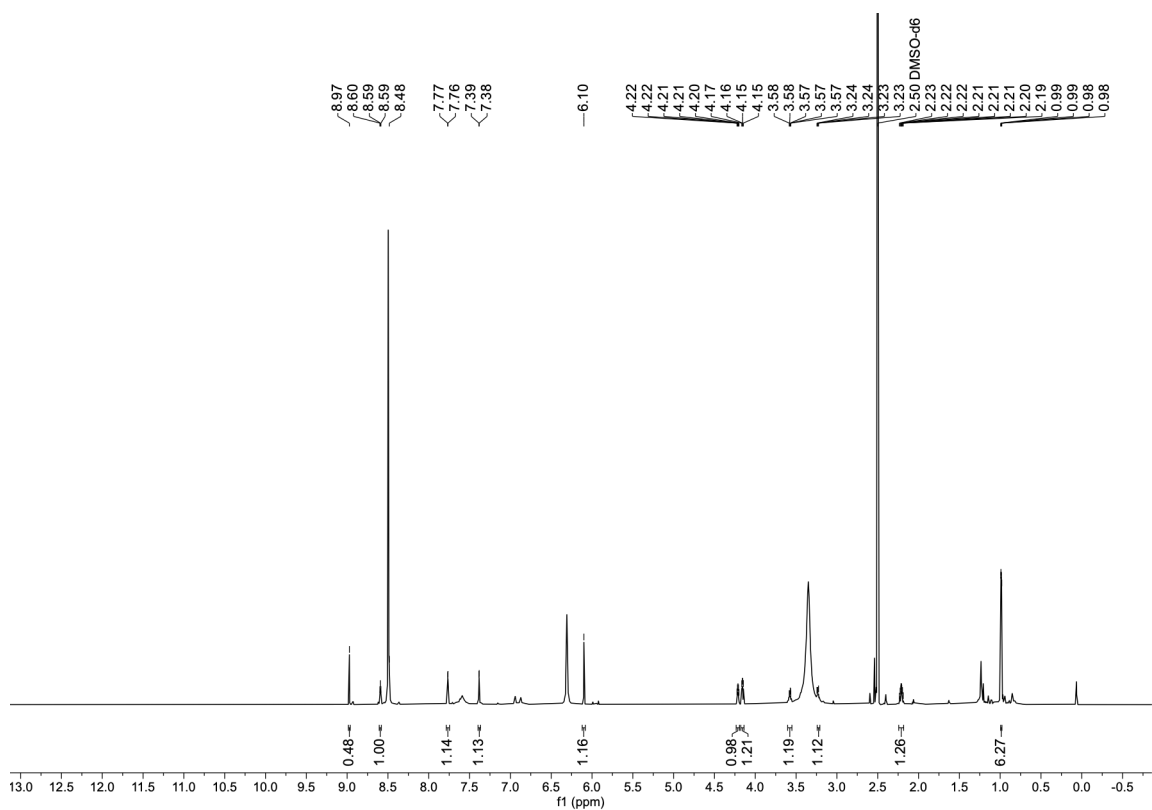
SUPPLEMENTARY FIGURE 14. ^1H - ^{13}C HSQC spectra of Marfey's labelled L-serine-2- ^{13}C in dmsd6.



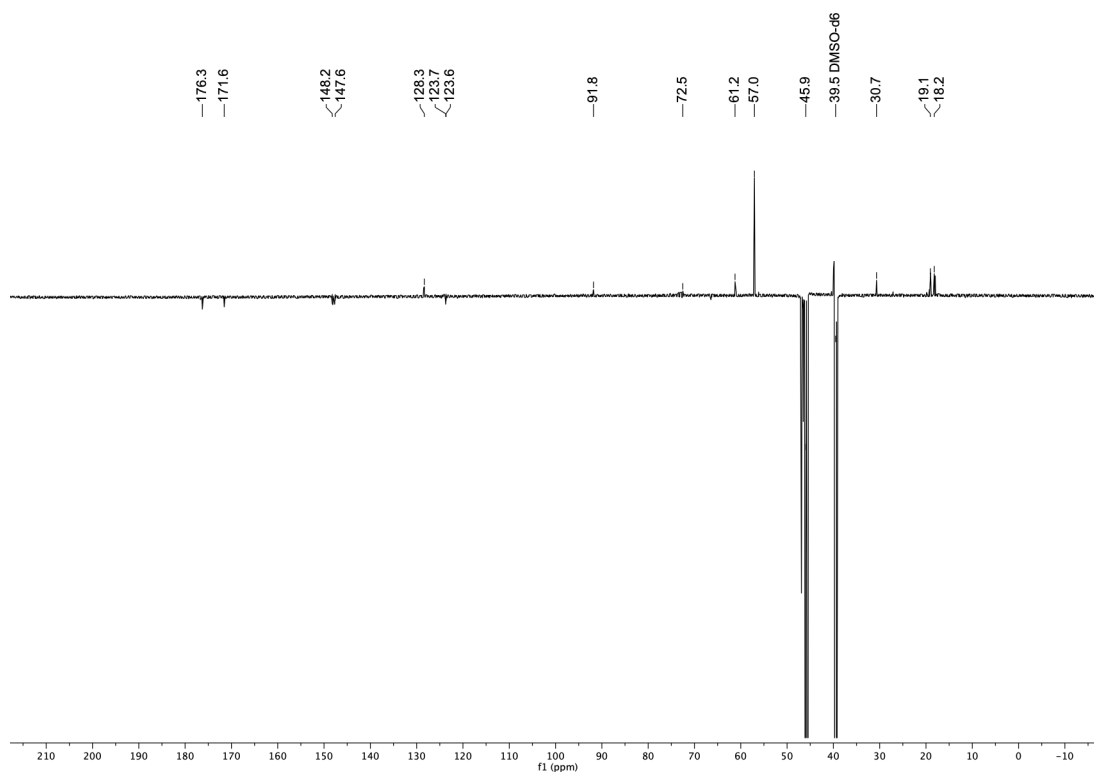
SUPPLEMENTARY FIGURE 15. ^1H - ^{13}C HMBC spectra of Marfey's labelled L-serine-2- ^{13}C in dmsd6.



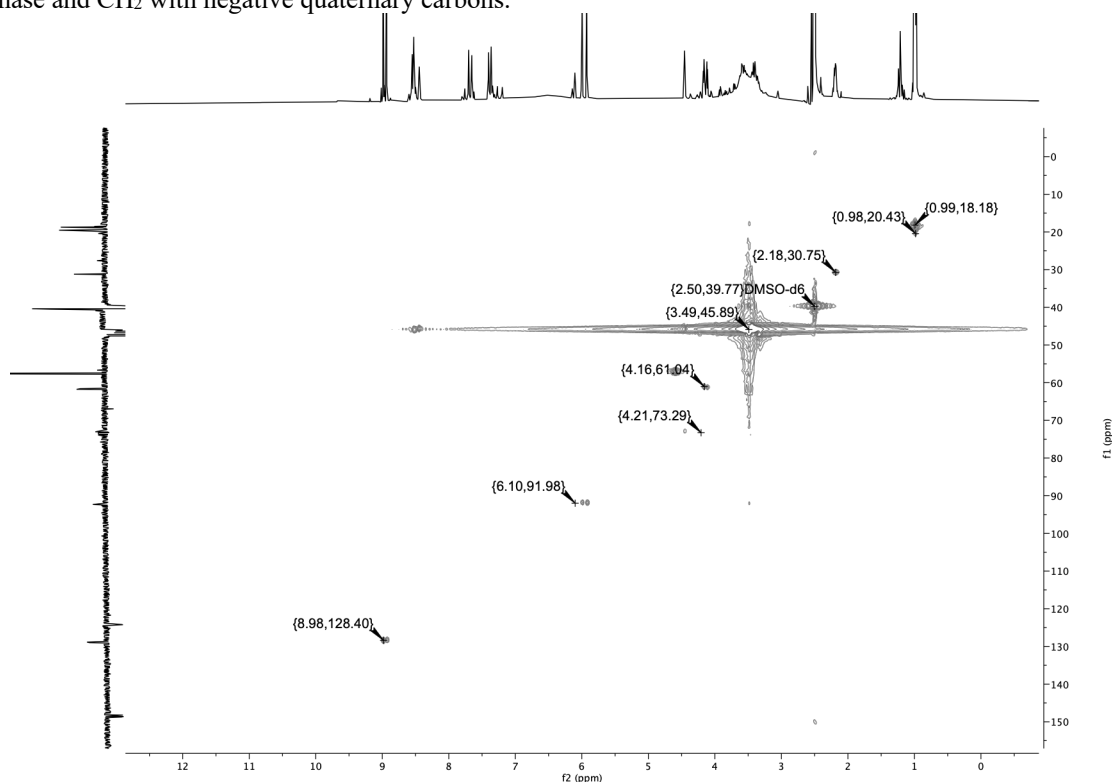
SUPPLEMENTARY FIGURE 16. ^1H - ^{15}N HSQC spectra of Marfey's labelled L-serine-2- ^{13}C in $\text{dms0-}d_6$.



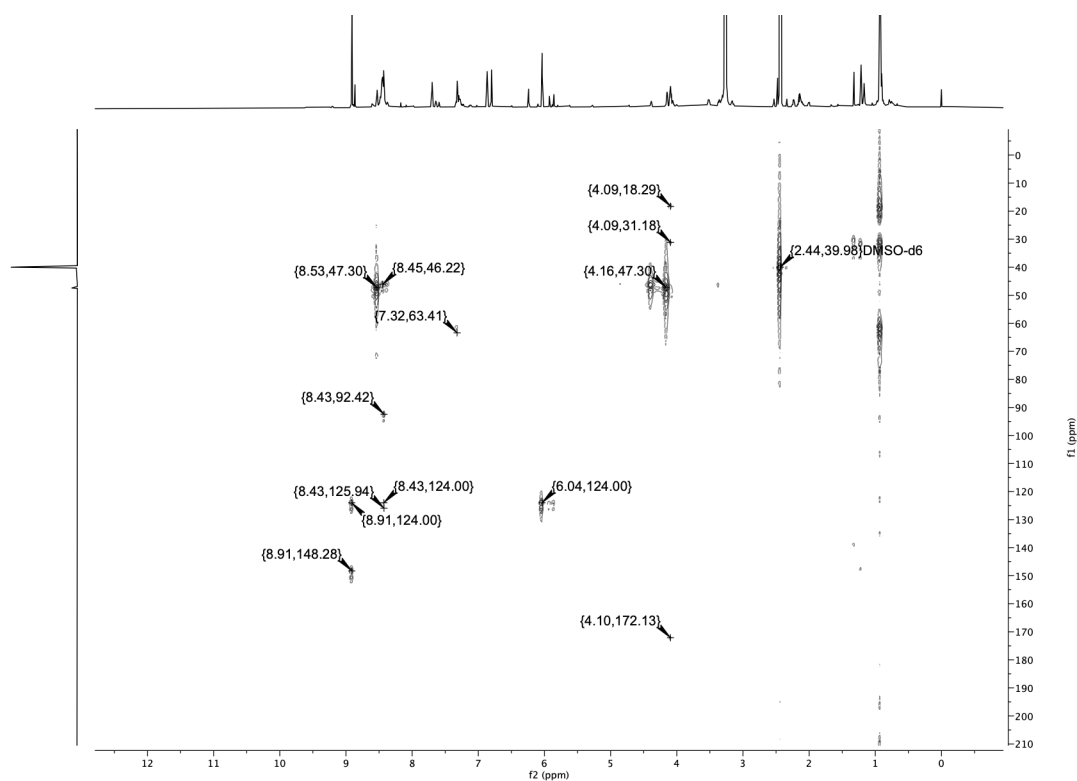
SUPPLEMENTARY FIGURE 17. ^1H NMR spectra of Marfey's labelled D- β -serine-3- ^{13}C reaction product in $\text{dms0-}d_6$.



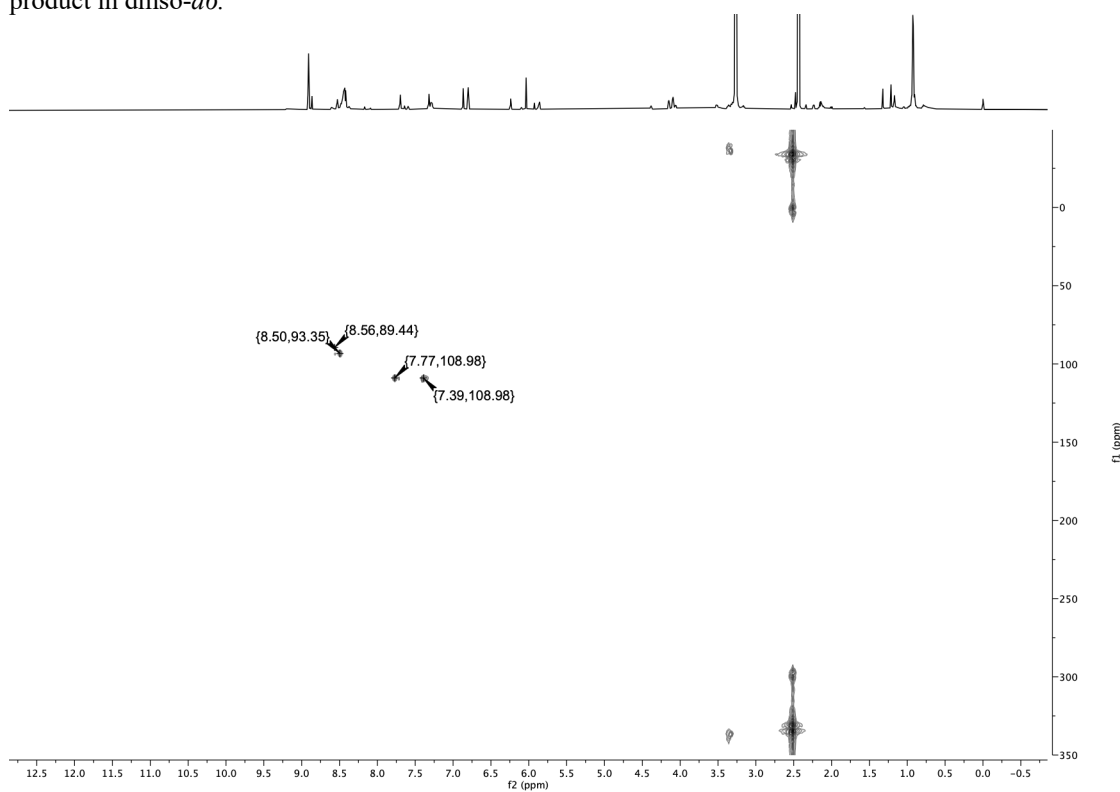
SUPPLEMENTARY FIGURE 18. ^{13}C NMR DEPTQ-135 spectra of Marfey's labelled D- β -serine-3- ^{13}C reaction product in dmsO- d_6 . The carbon bearing the ^{13}C label is now located on the CH_2 group and situated on the opposite plane of CH_3 and CH groups. DEPTQ-135 shows CH/CH_3 (methine/methyl) with a positive phase and CH_2 with negative quaternary carbons.



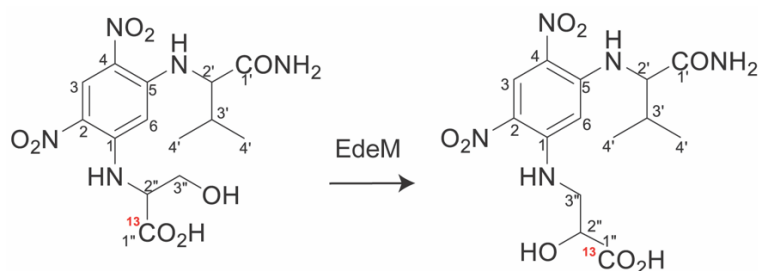
SUPPLEMENTARY FIGURE 19. ^1H - ^{13}C HSQC spectra of Marfey's labelled D- β -serine-3- ^{13}C reaction product in dmsO- d_6 .



SUPPLEMENTARY FIGURE 20. ^1H - ^{13}C HMBC spectra of Marfey's labelled D- β -serine-3- ^{13}C reaction product in dmsol- d_6 .

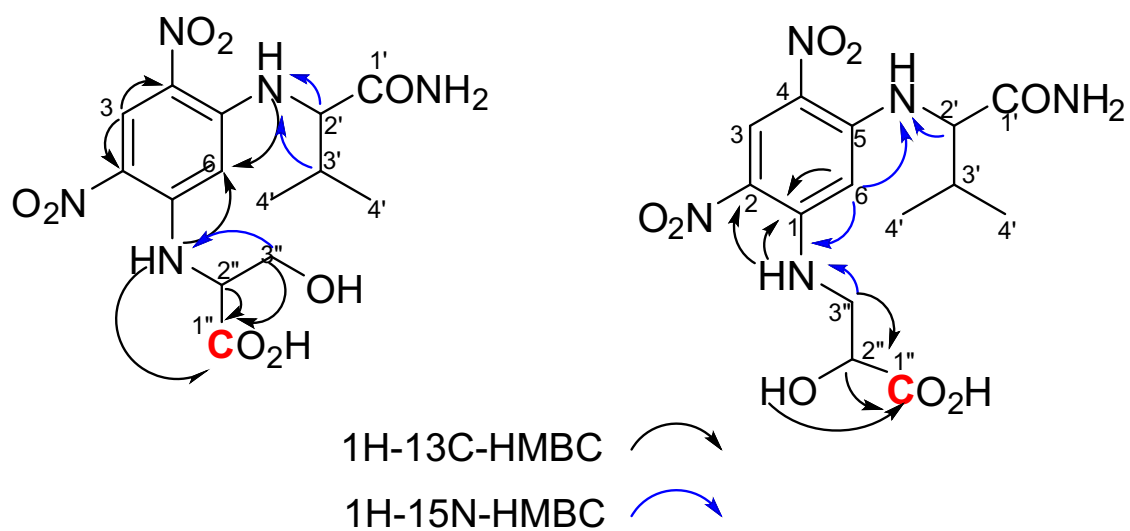


SUPPLEMENTARY FIGURE 21. ^1H - ^{15}N HSQC spectra of Marfey's labelled D- β -serine-3- ^{13}C reaction product in dmsol- d_6 .

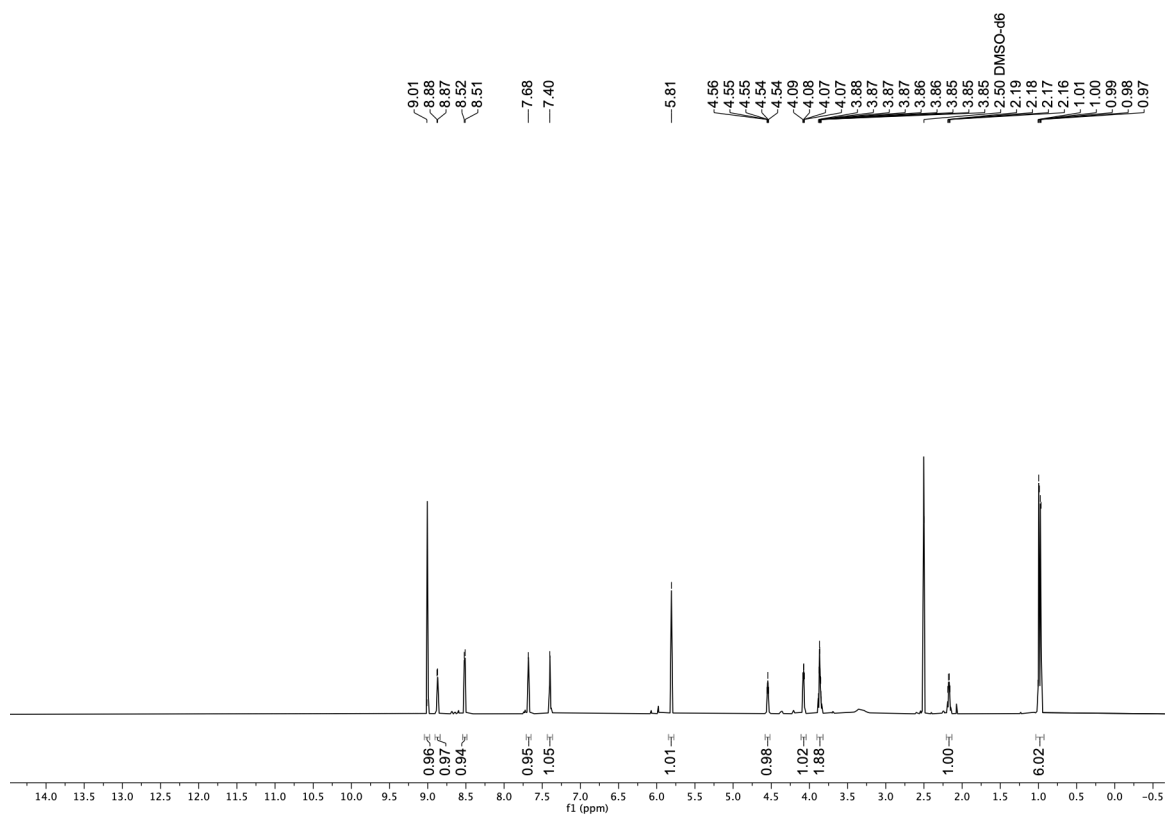


SUPPLEMENTARY TABLE 5. Complete NMR assignment in dmso-*d*₆ of control L-serine-1-¹³C and the reaction product D-β-serine-1-¹³C after derivatization with Marfey's reagent and purification from large-scale EdeM reactions.

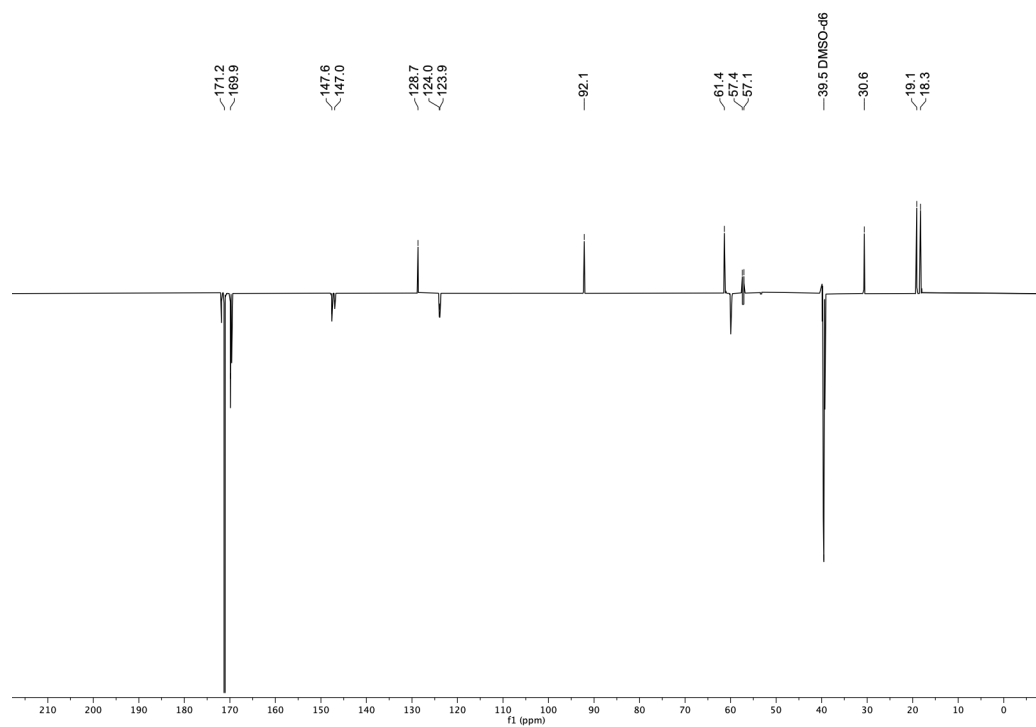
Atom #	¹ H (Ser)	¹³ C (Ser)	Atom #	¹ H (D-Ise)	¹³ C (D-Ise)
1	-	147.0	1	-	147.2
2	-	123.9	2	-	123.2
3	9.01 (s, 1H)	128.7	3	8.97 (s, 1H)	128.0
4	-	124.0	4	-	123.3
5	-	147.6	5	-	147.9
6	5.81 (s, 1H)	92.1	6	6.10 (s, 1H)	91.7
L-Valinamide			L-Valinamide		
NH	8.52 (d, <i>J</i> = 7.8 Hz, 1H)	-	NH	8.59 (m, 1H)	-
1'CONH2	7.68 (s, 1H) 7.40 (s, 1H)	169.9	1'CONH2	7.77 (s, 1H) 7.38 (s, 1H)	171.2
2'	4.08 (m, 1H)	61.4	2'	4.16 (m, 1H)	60.5
3'	2.17 (m, 1H)	30.6	3'	2.22 (m, 1H)	30.3
4' (CH3)	0.98 (dd, <i>J</i> = 15.1, 6.8 Hz, 6H)	19.1 18.3	4' (CH3)	0.99 (dd, <i>J</i> = 6.8, 2.3 Hz, 6H)	17.4 18.8
L-Ser			D-Ise		
NH	8.87 (d, <i>J</i> = 5.0 Hz, 1H)	-	NH	8.49 (m, 1H)	-
1'' (¹³ CO ₂ H)	-	171.2	1'' (¹³ CO ₂ H)	-	176.2
2''	4.55 (m, 1H)	57.1	2''	4.21 (m, 1H)	73.2
3''	3.88 – 3.85 (m, 2H)	57.4	3'' (β)	3.49 (m, 1H) 3.33 (m, 1H)	46.4



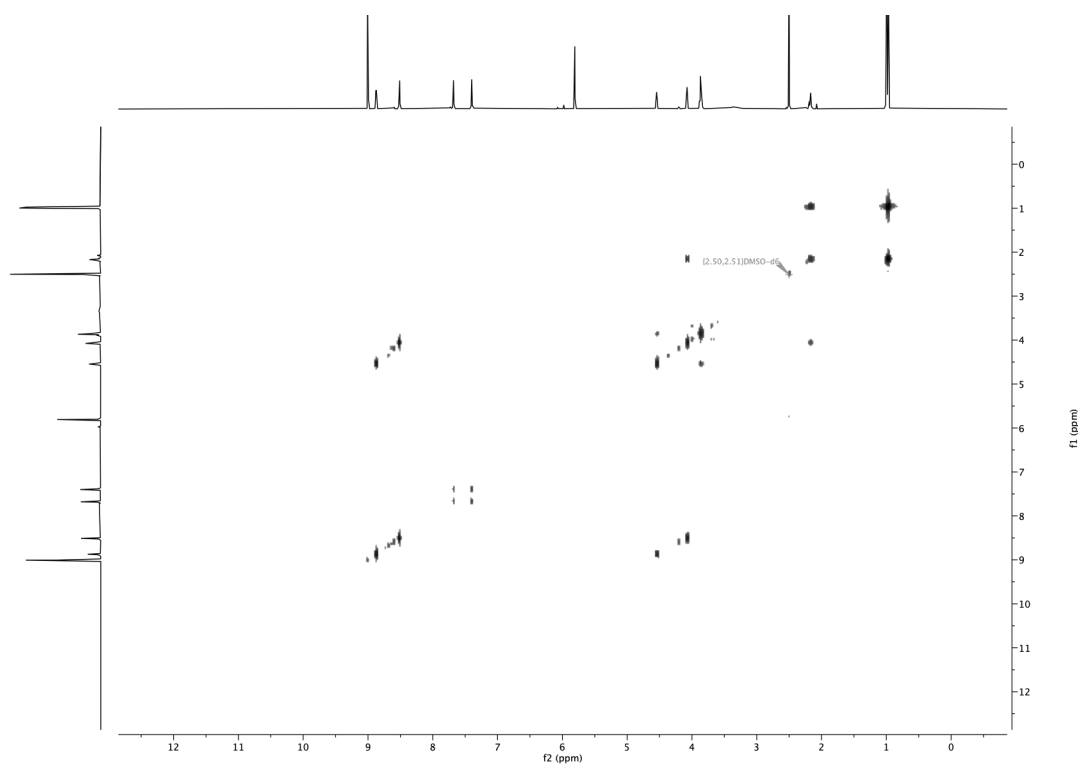
SUPPLEMENTARY FIGURE 22. HMBC diagram of Marfey's labelled L-serine-1- ^{13}C control and D- β -serine-1- ^{13}C reaction product.



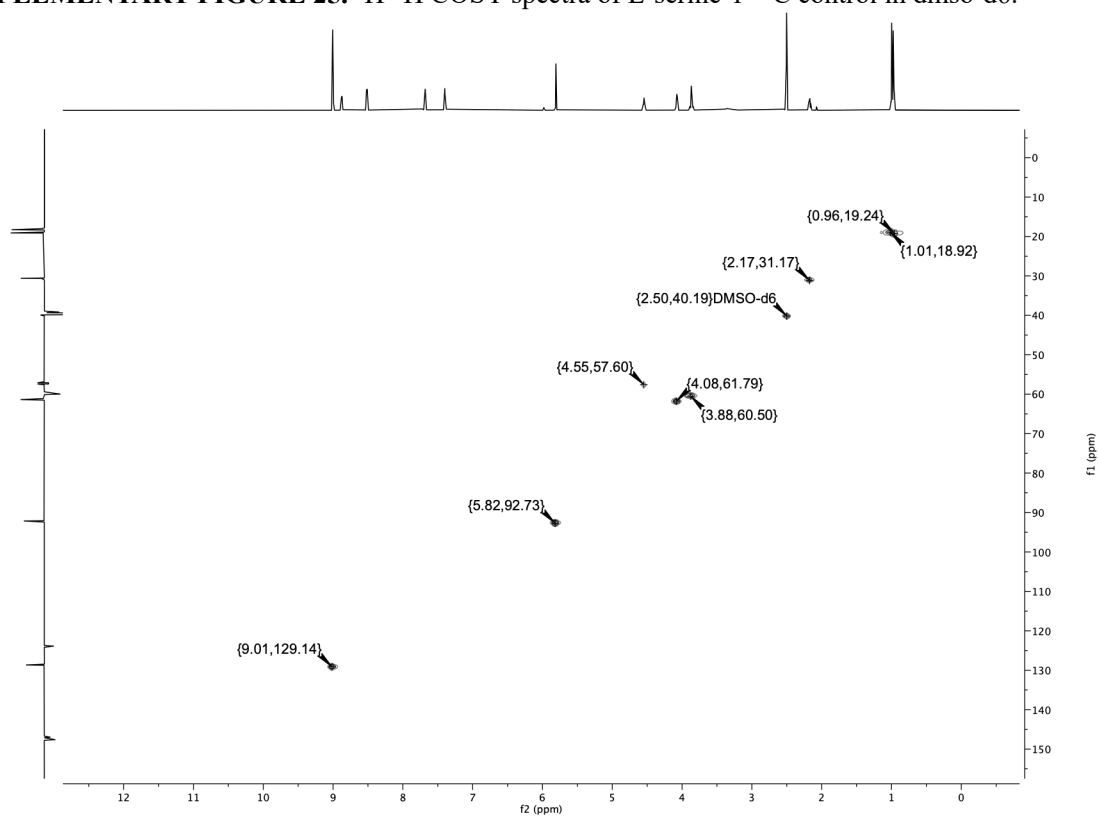
SUPPLEMENTARY FIGURE 23. ¹H NMR spectra of L-serine-1-¹³C control in dmsO-d₆.



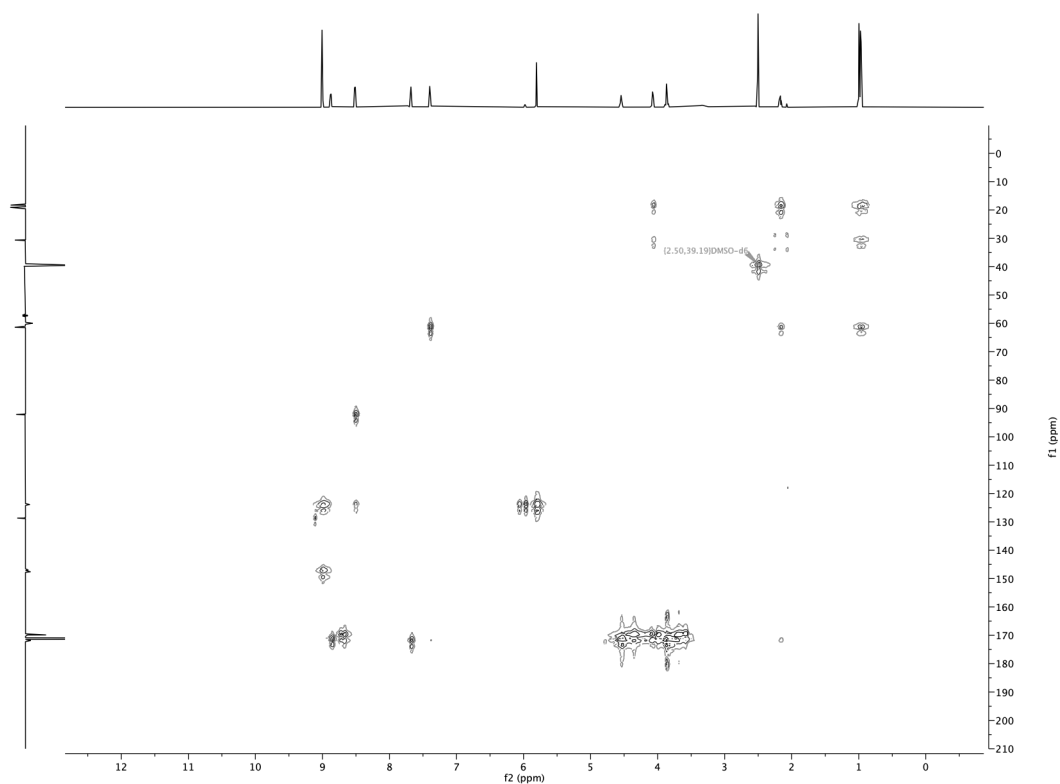
SUPPLEMENTARY FIGURE 24. ¹³C NMR DEPTQ-135 spectra of L-serine-1-¹³C control in dmsO-d₆.



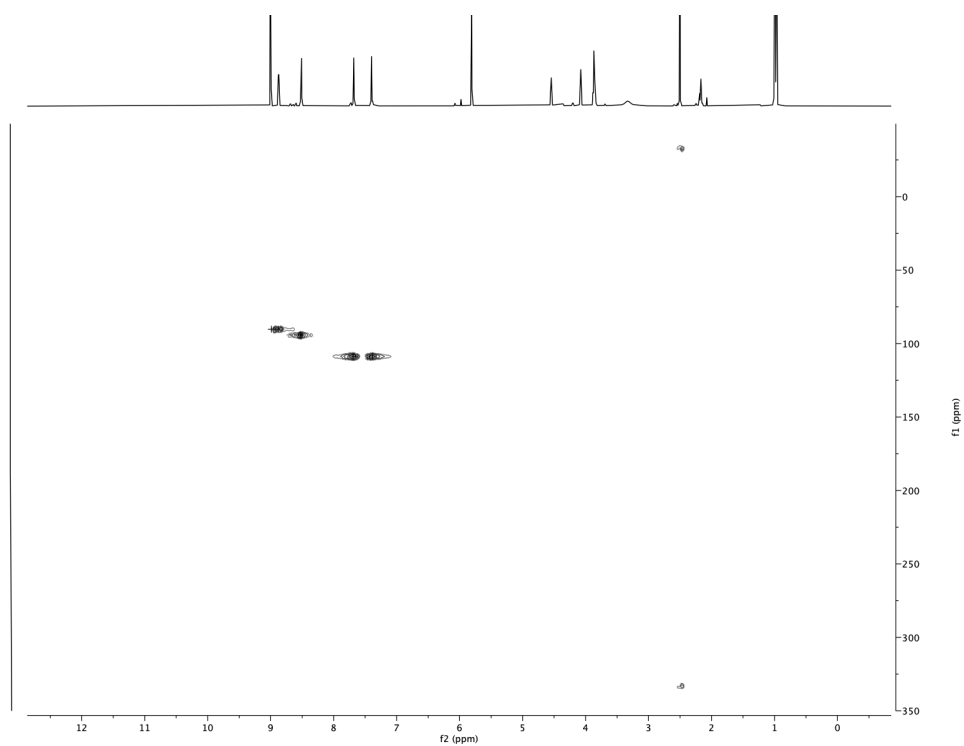
SUPPLEMENTARY FIGURE 25. ^1H - ^1H COSY spectra of L-serine-1- ^{13}C control in dmsd6.



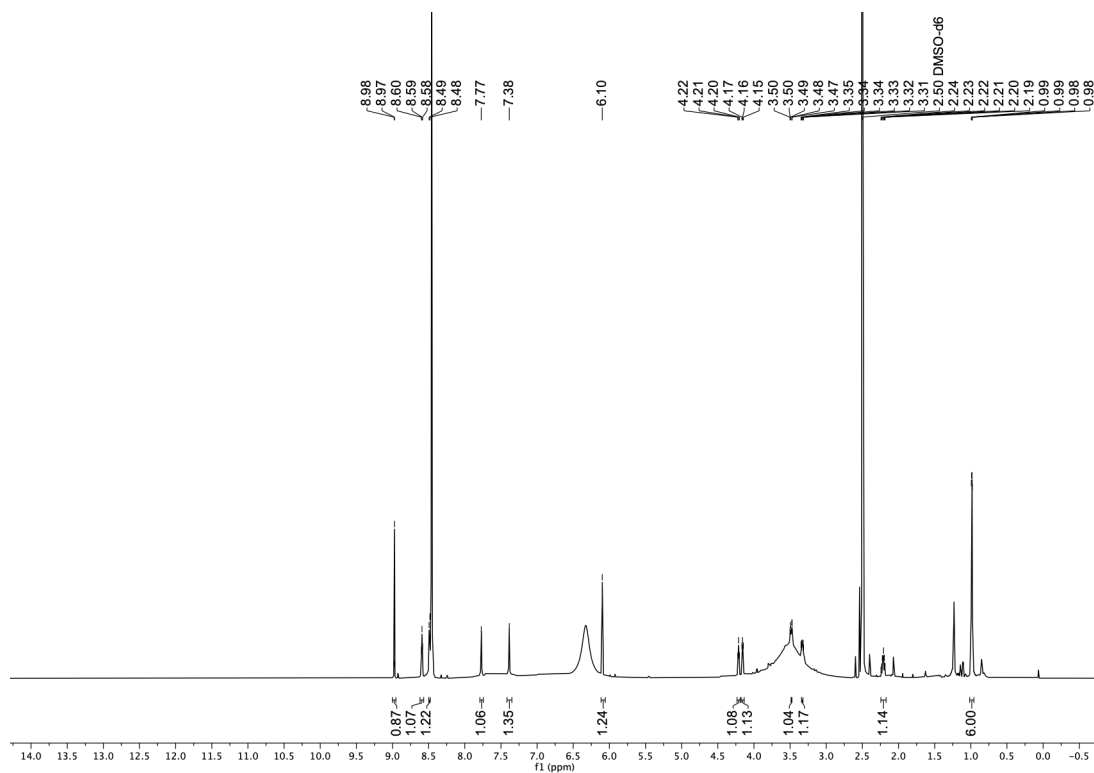
SUPPLEMENTARY FIGURE 26. ^1H - ^{13}C HSQC spectra of L-serine-1- ^{13}C control in dmsd6.



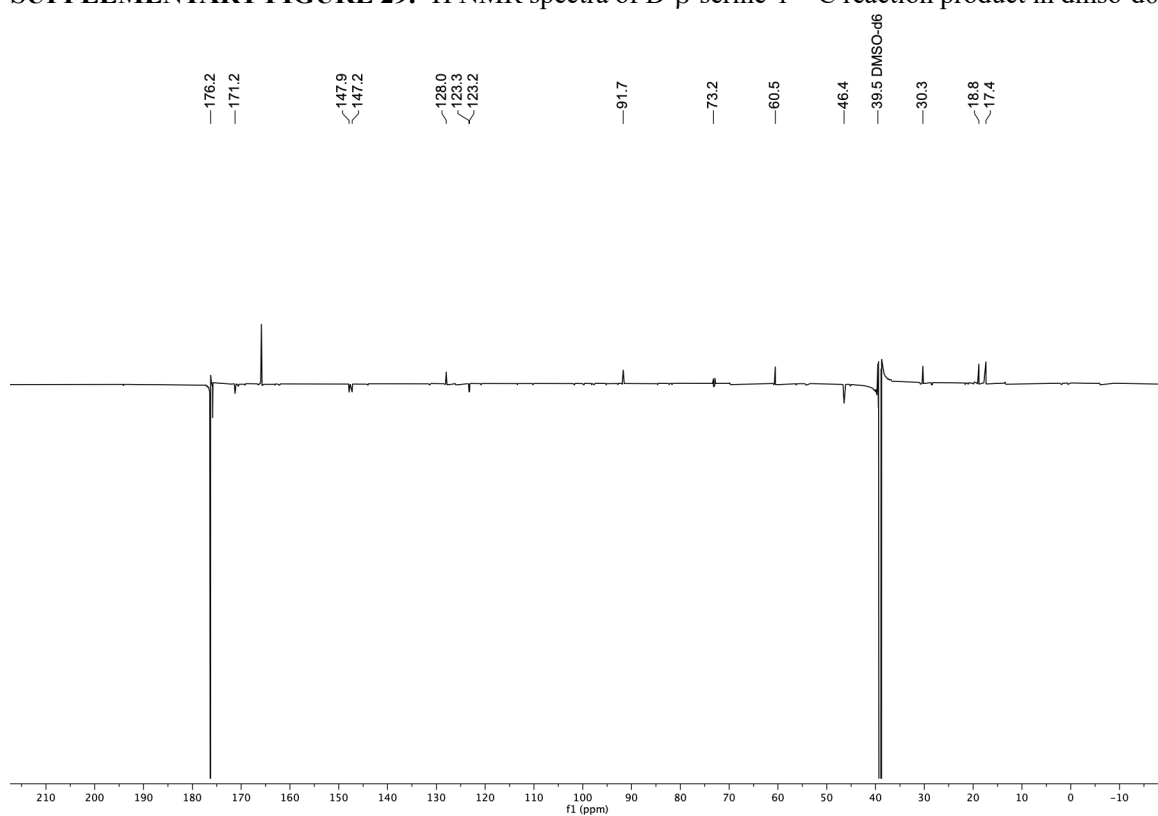
SUPPLEMENTARY FIGURE 27. ^1H - ^{13}C HMBC spectra of L-serine-1- ^{13}C control in dmsd-d6.



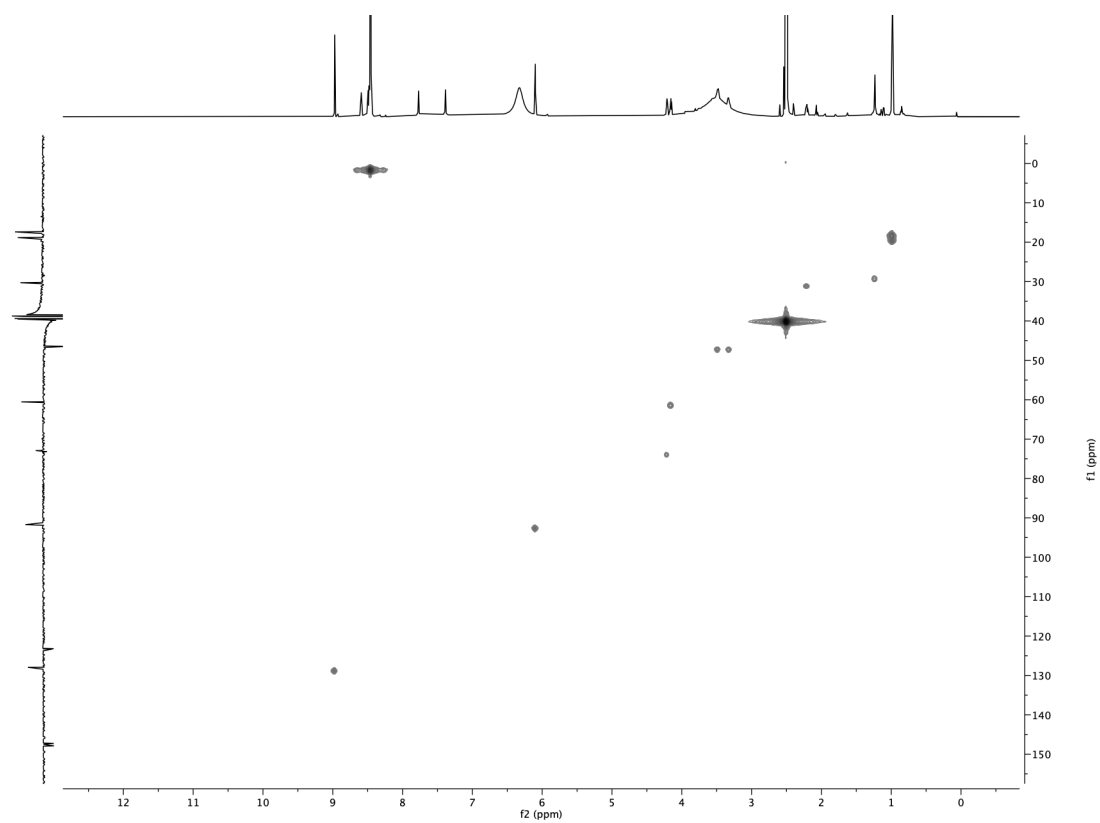
SUPPLEMENTARY FIGURE 28. ^1H - ^{15}N HSQC spectra of L-serine-1- ^{13}C control in dmsd-d6.



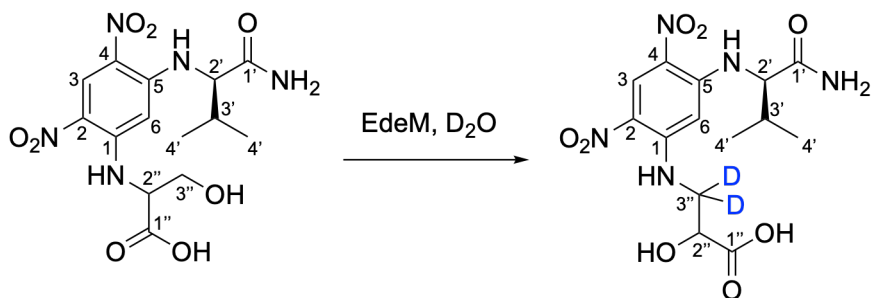
SUPPLEMENTARY FIGURE 29. ^1H NMR spectra of D- β -serine-1- ^{13}C reaction product in dmsd-d6.



SUPPLEMENTARY FIGURE 30. ^{13}C NMR DEPTQ-135 spectra of D- β -serine-1- ^{13}C reaction product in dmsd-d6.

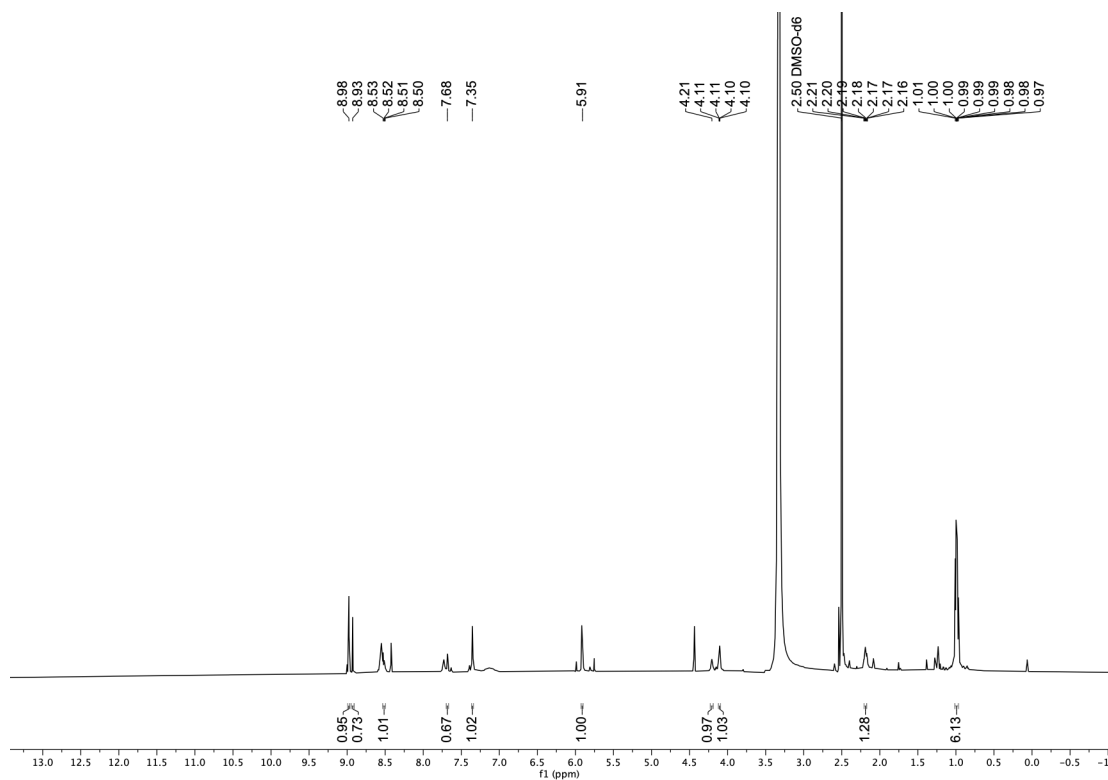


SUPPLEMENTARY FIGURE 31. ^1H - ^{13}C HSQC spectra of D- β -serine-1- ^{13}C reaction product in dms0-d6.

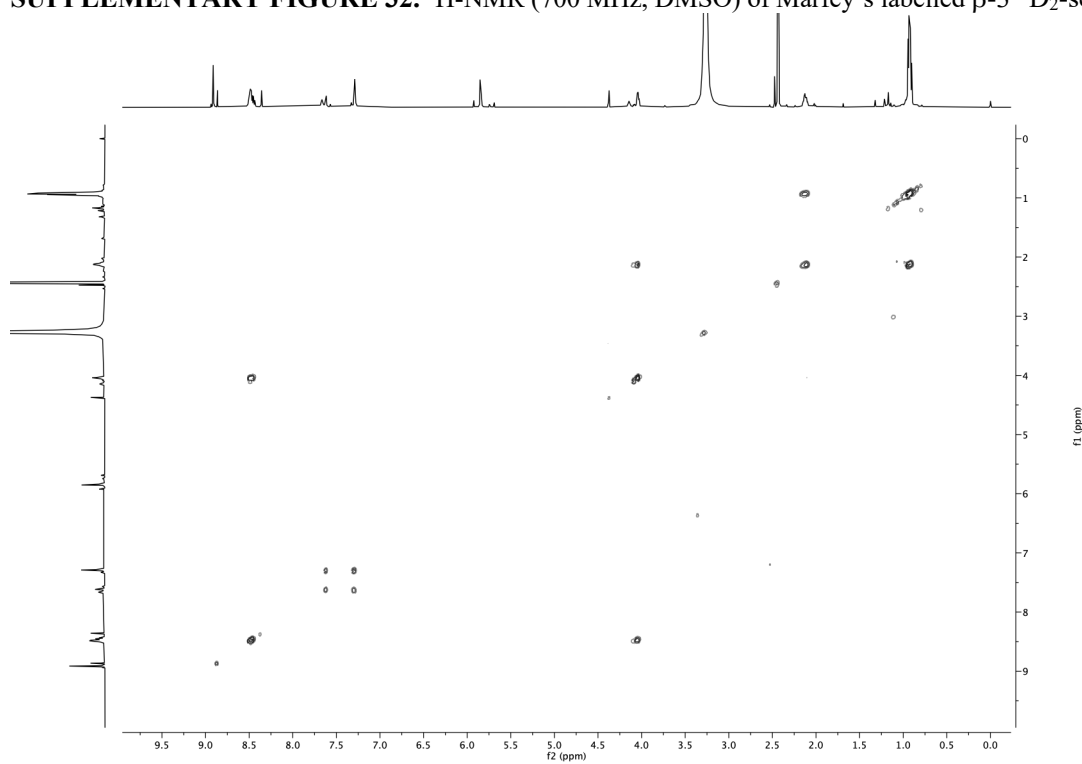


SUPPLEMENTARY TABLE 6. Complete NMR assignment of β -3''D₂-serine from large-scale EdeM-D₂O reactions in dms-*d*6.

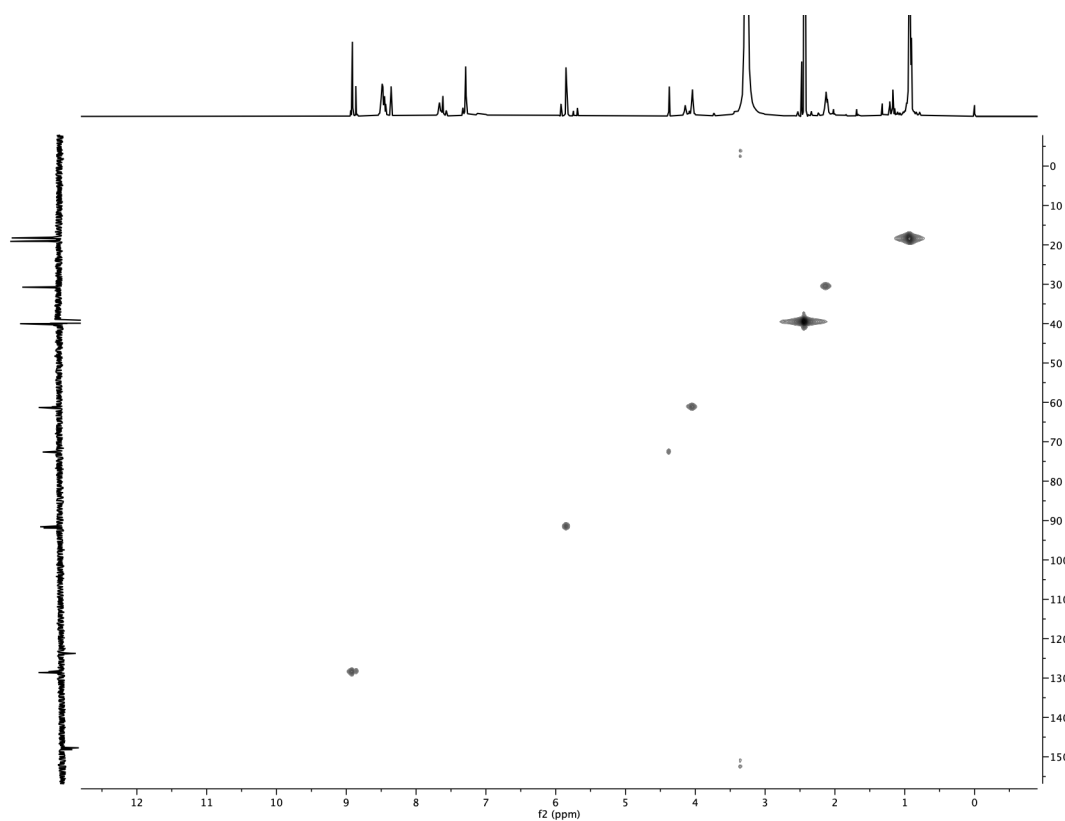
Atom #	¹ H (D- β -serine)	¹³ C (D- β -serine)	Atom #	¹ H (β -3''-D ₂ -serine)	¹³ C (D- β -serine)
1	-	147.6	1	-	147.7
2	-	123.7	2	-	123.7
3	8.97 (s, 1H)	128.3	3	8.98 (s, 1H)	128.6
4	-	123.6	4	-	123.7
5	-	148.2	5	-	148.2
6	6.10 (s, 1H)	91.8	6	5.91 (s, 1H)	91.9
L-Valinamide					
2'	4.16 (dd, J = 7.8, 5.5 Hz, 1H)	61.2	2'	4.11 (m, 1H)	61.4
3'	2.21 (dq, J = 13.5, 6.7 Hz, 1H)	30.7	3'	2.19 (m, 1H)	30.7
4'	0.99 (dd, J = 6.8, 2.3 Hz, 6H)	19.1 18.2	CH ₃	1.01 - 0.97 (m, 6H)	19.1 18.2
NH	8.48 (s, 1H)	-	NH	8.52 (d, J = 7.8 Hz, 1H)	-
CONH ₂	7.77 (d, J = 2.1 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H)	171.6	C(1')ONH ₂	7.68 (br. s, 1H), 7.35 (br. s, 1H)	171.7
D- β -serine					
2''	4.21 (m, 1H)	72.5	2''	4.21 (s, 1H)	67.6
3''	3.59 (m, 1H) 3.22 (m, 1H)	45.9	3''	-	40.0
NH	8.59 (t, J = 4.8 Hz, 1H)		NH	8.93 (s, 1H)	-
CO ₂ H	-	176.3	CO ₂ H	-	176.4



SUPPLEMENTARY FIGURE 32. ^1H -NMR (700 MHz, DMSO) of Marfey's labelled β -3'' D_2 -serine.

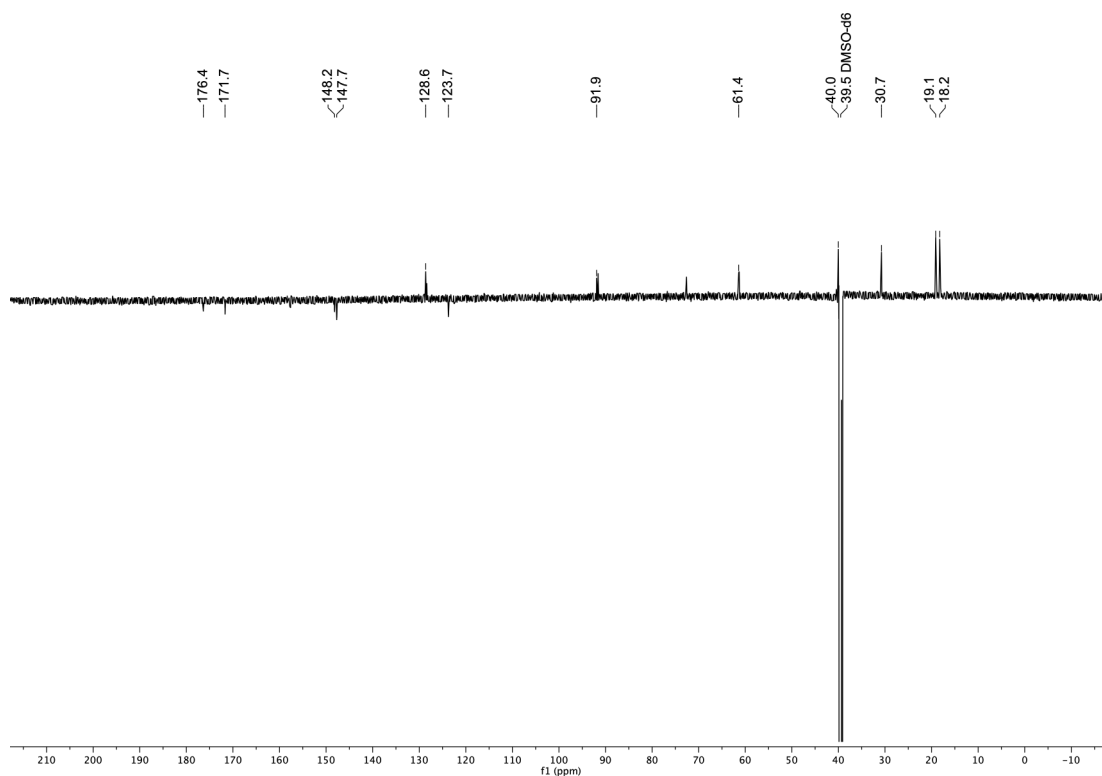


SUPPLEMENTARY FIGURE 33. ^1H - ^1H -COSY (700 MHz, DMSO) spectra of Marfey's labelled β -3'' D_2 -serine.

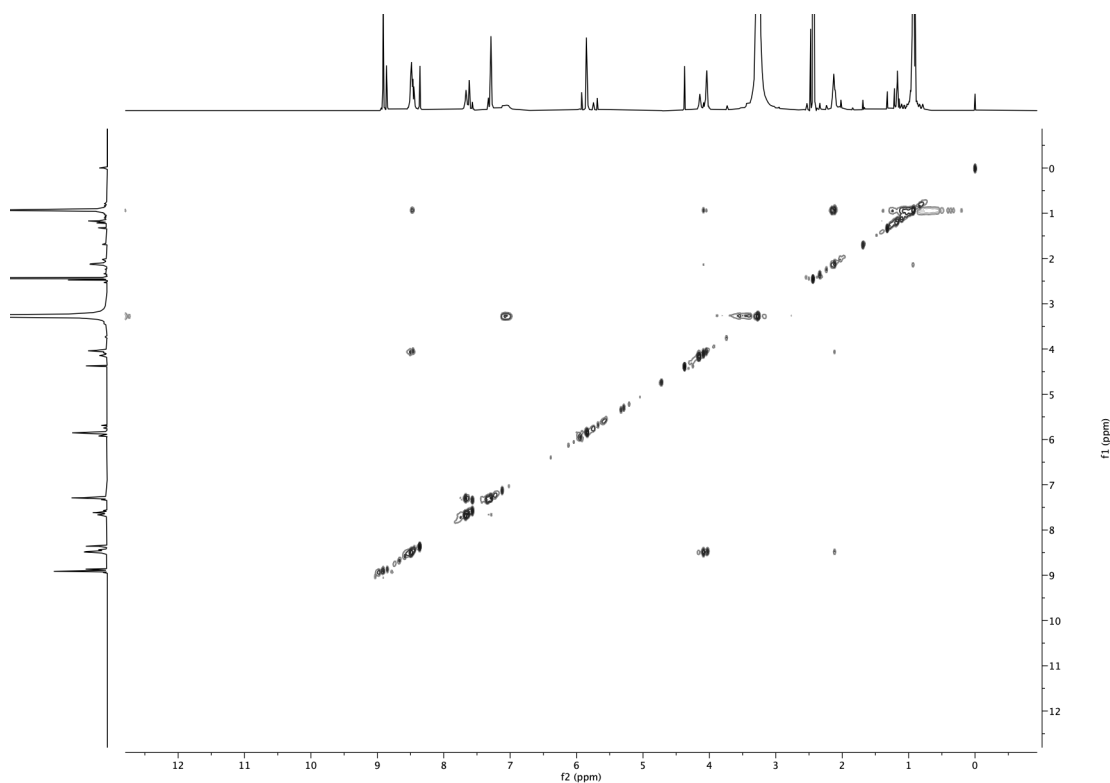


4

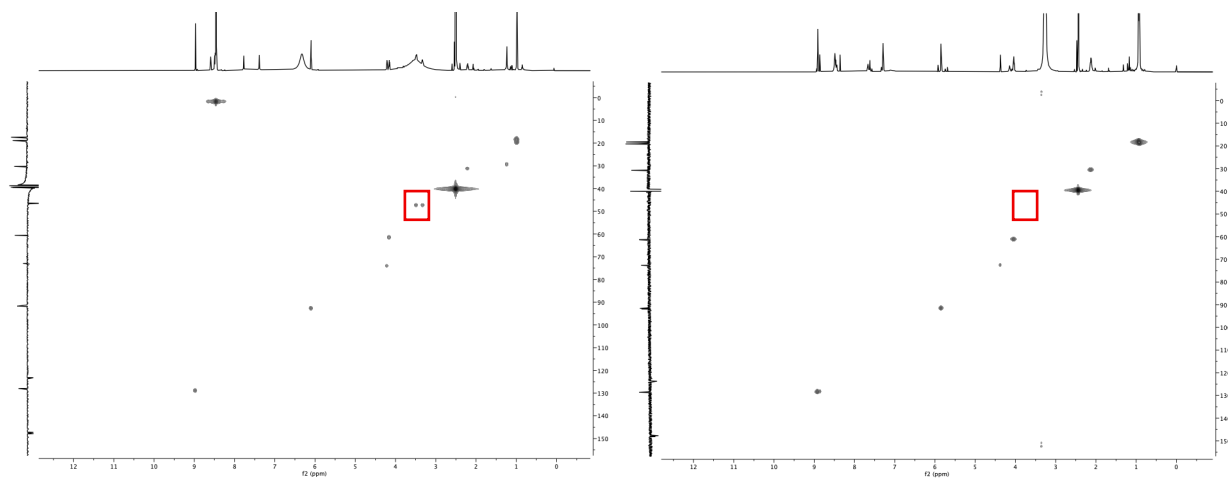
SUPPLEMENTARY FIGURE 34. ^1H - ^{13}C HSQC (700 MHz, DMSO) spectra of Marfey's labelled β - $3''\text{D}_2$ -serine.



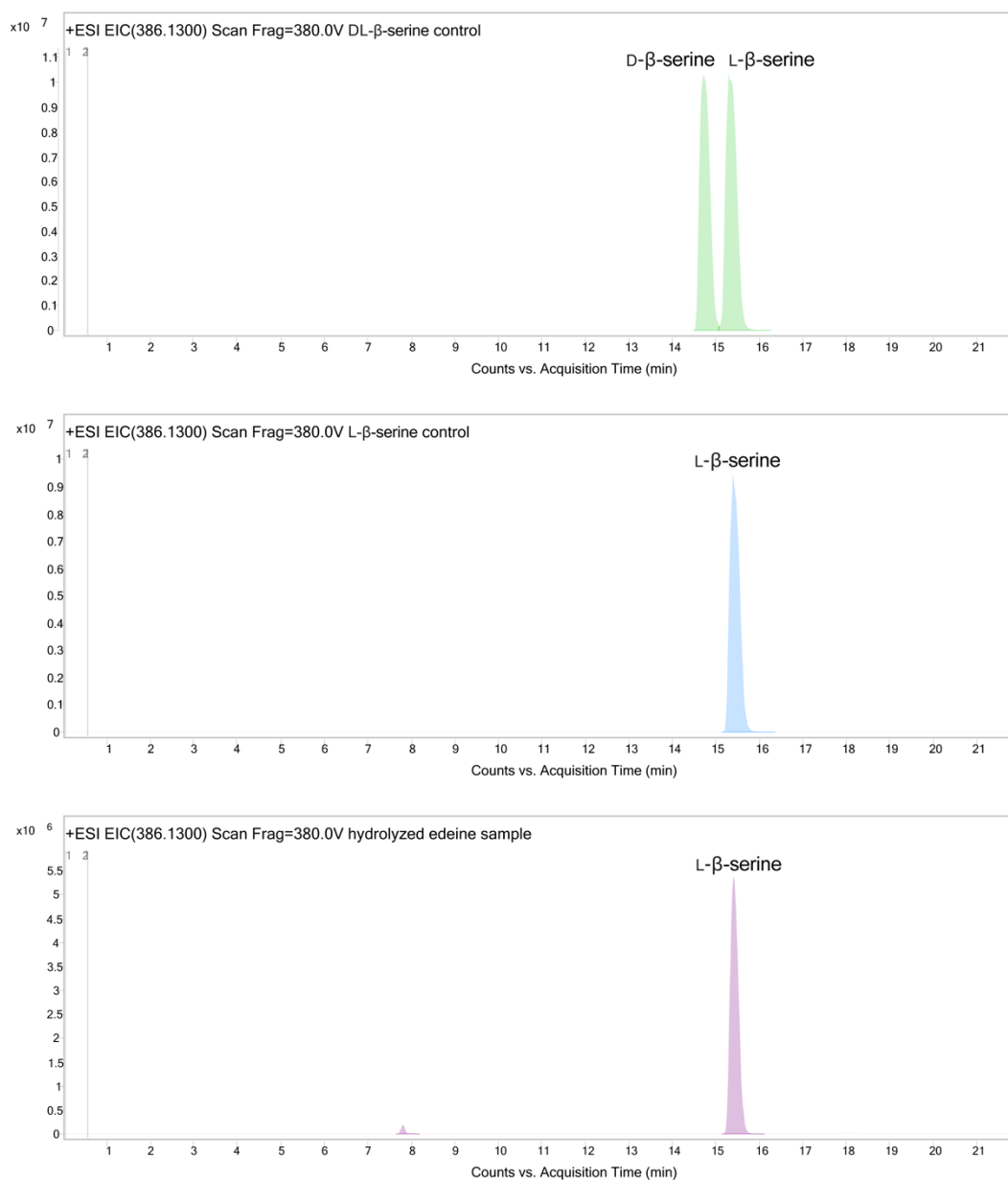
SUPPLEMENTARY FIGURE 35. ^{13}C NMR DEPTQ-135 (700 MHz, DMSO) spectra of Marfey's labelled β -3''D₂-serine.



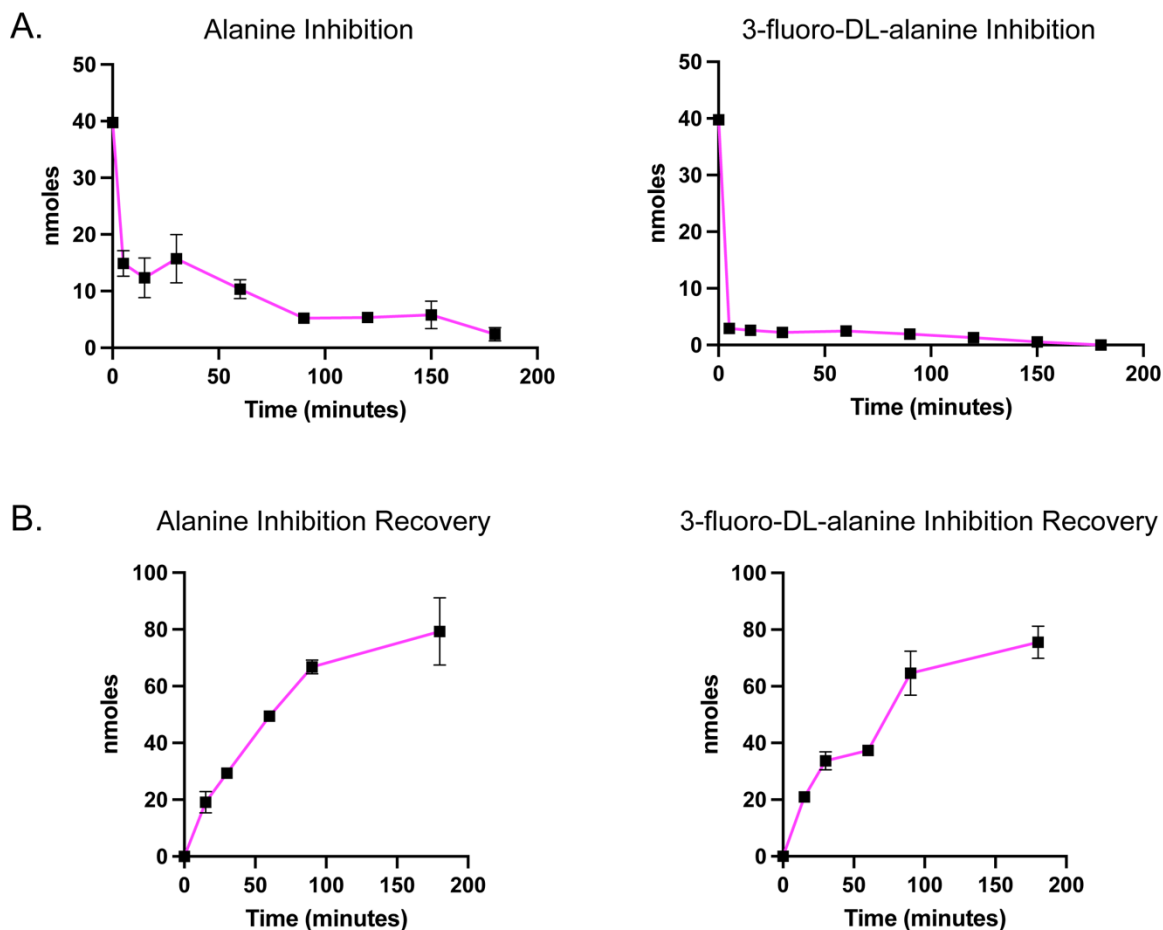
SUPPLEMENTARY FIGURE 36. ^1H - ^1H HMBC (700 MHz, DMSO) spectra of Marfey's labelled β -3''D₂-serine.



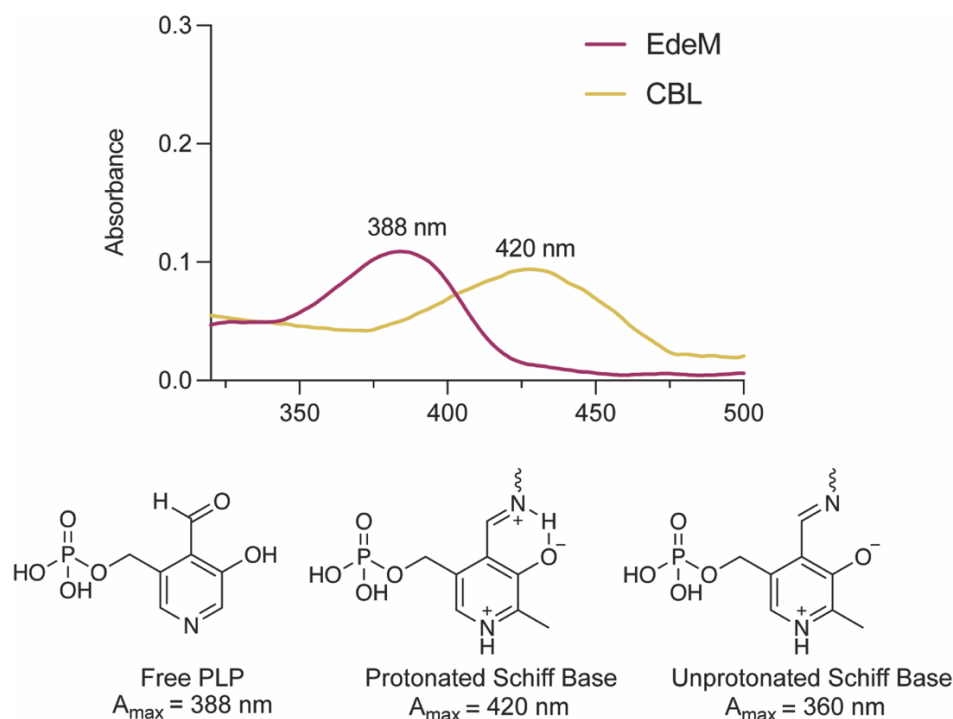
SUPPLEMENTARY FIGURE 37. ^1H - ^{13}C HSQC (700 MHz, DMSO) spectra of Marfey's derivatized D- β -serine-1- ^{13}C reaction product (left) and β -3'' D_2 -serine reaction product from an reaction in D_2O (right). Red square indicates the crosspeaks for the protons on the left whereas they are missing in the deuterated sample on the right.



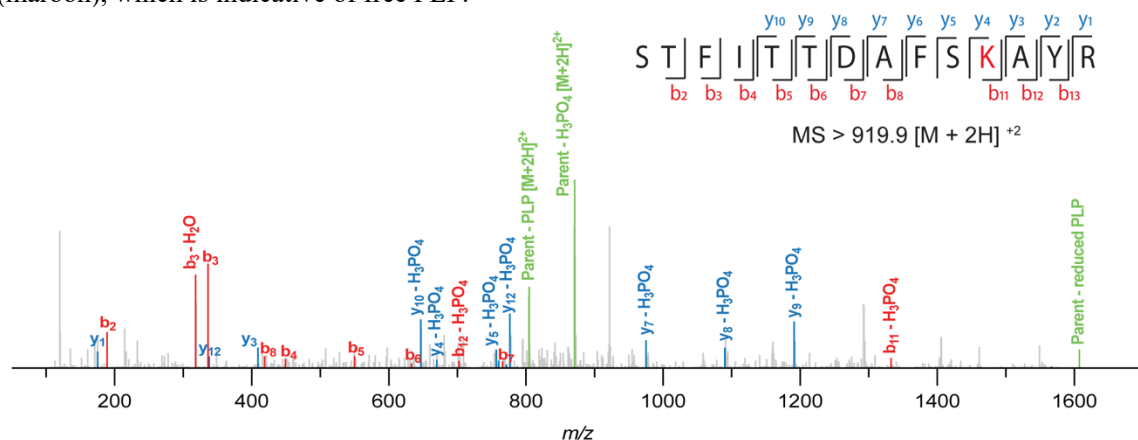
SUPPLEMENTARY FIGURE 39. Extracted ion chromatogram in positive mode (+EIC) at 386.13 m/z for standards containing a D-/L-β-serine mixture (top) and L-β-serine (middle), and hydrolyzed edeine sample (bottom). D-β-serine has a retention time of ~14.5 minutes, and L-β-serine has a retention time of ~15 minutes. Results demonstrate that L-β-serine is present in the hydrolyzed edeine sample.



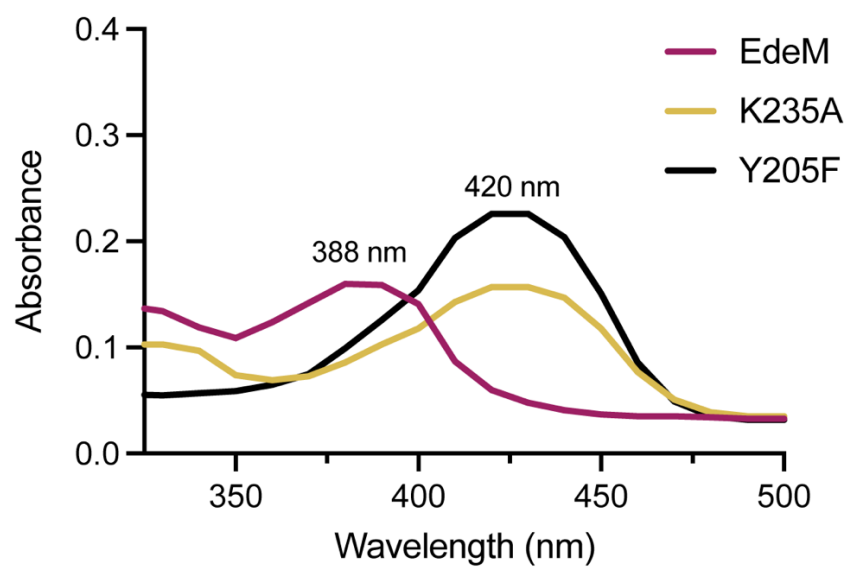
SUPPLEMENTARY FIGURE 40. EdeM was incubated with 50 mM of each inhibitor. At each time point, samples of the enzyme were taken and added to a reaction containing L-serine, which was left to react for 15 minutes. The samples were then derivatized with Marfey's reagent and analyzed by HPLC. The amount of D- β -serine produced by EdeM at each time point incubation with the inhibitor is shown in pink. **B.** EdeM was incubated with 50 mM of each inhibitor for 2 hours at room temperature to allow for complete enzyme inhibition. The samples were then processed through a Bio-Gel spin column to remove any unbound inhibitor from the enzyme before being added to a reaction containing L-serine. Time points were taken throughout the incubation to determine the duration required for EdeM to regain its activity by monitoring D- β -serine production (pink).



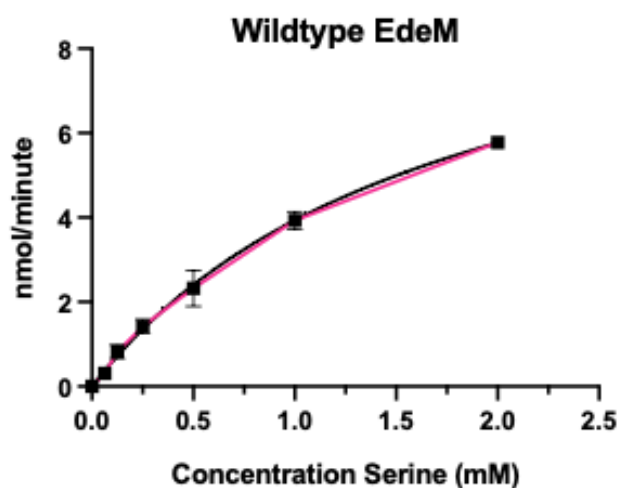
SUPPLEMENTARY FIGURE 41. UV-Vis spectra of EdeM. In PLP-DE, the Schiff base of internal aldimines is conjugated with the PLP π system and has absorption maxima of 420-430 nm when protonated and 360 nm when unprotonated. UV absorbance at 420 nm is detected for the control PLP-DE, cystathionine β -lyase (CBL) (yellow), indicating the formation of protonated Schiff base. When purified, EdeM samples are colourless and have a UV-Vis spectrum with absorbance at 388 nm (maroon), which is indicative of free PLP.



SUPPLEMENTARY FIGURE 42. EdeM Trypsin Digest. To identify the active site lysine residue in EdeM, a trypsin digest was performed on an enzyme sample reduced in the presence of NaBH₃CN. Bound PLP could only be detected for the peptide fragment STFITTDAFSKAYR, which contains K235, thereby suggesting that this is the active site lysine residue. Intact protein MS was repeated using a K235A EdeM mutant to verify this finding, and no internal aldimine formation could be detected (data not shown). These results demonstrate that EdeM is a PLP-DE and K235 is the active site lysine residue.



SUPPLEMENTARY FIGURE 43. UV-Vis spectra of EdeM, EdeM K235A, and EdeM Y205F. Wildtype EdeM has an absorbance of 388 nm, which is the same absorbance seen for free PLP. K235A and Y205F have absorbance at 420 nm, indicating the formation of a Schiff base.



K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}s^{-1}$)
1.75 ± 0.54	0.06	3.43×10^1

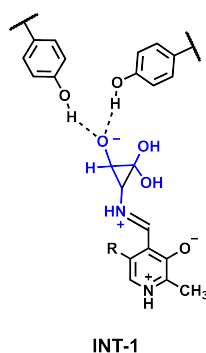
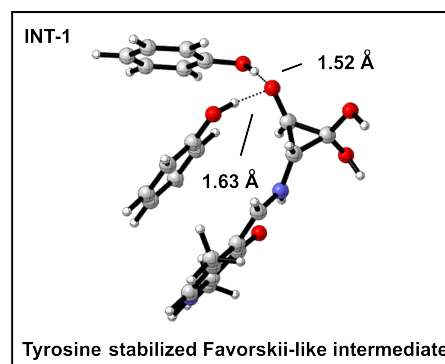
SUPPLEMENTARY FIGURE 44. Steady-state kinetic parameters of EdeM at 25 °C

Computational Chemistry:

SUPPLEMENTARY TABLE 7. Computed energies for Fig. 6.

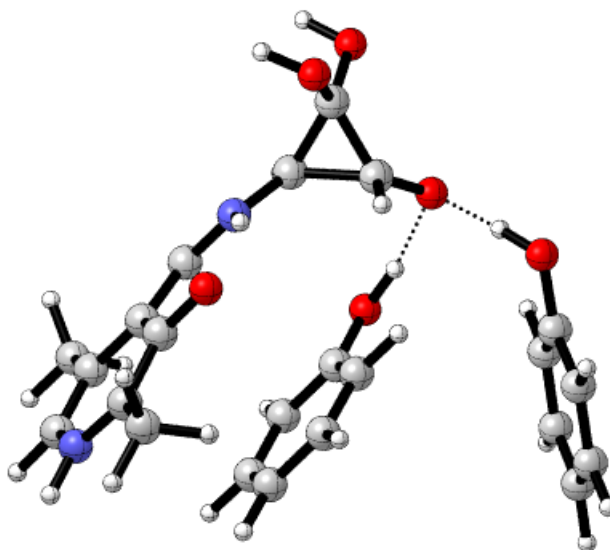
Structure	Correction	Energy
22	0.399245	-1452.184173
TS-1	0.399354	-1452.173953
23	0.403160	-1452.232981
20 Favorski-like Intermediate (INT-1)	0.398538	-1452.193983

All energies reported as Hartree/Particle, all energies computed at the (SMD = H₂O)ωB97XD/6-31+G(d,p) level of theory.



SUPPLEMENTARY FIGURE 45. Computed and optimized structure of the tyrosine-stabilized Favorskii-like intermediate **20** (INT-1).

Cartesian Coordinates for computed structures:



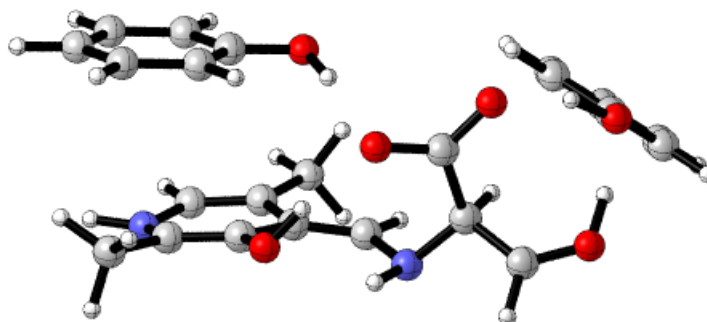
Structure of **INT-1** optimized at the SMD_{(H₂O}) ω B97XD/6-31+G(d,p) level of theory.

Zero-point correction=	0.462851 (Hartree/Particle)
Thermal correction to Energy=	0.492818
Thermal correction to Enthalpy=	0.493762
Thermal correction to Gibbs Free Energy=	0.398538
Sum of electronic and zero-point Energies=	-1452.129670
Sum of electronic and thermal Energies=	-1452.099703
Sum of electronic and thermal Enthalpies=	-1452.098759
Sum of electronic and thermal Free Energies=	-1452.193983

Charge = 0 Multiplicity = 1

C	0.71268800	3.74661900	0.24084500
C	-0.00081600	2.65485700	-0.52223900
C	1.34253500	2.38950800	0.20918000
O	2.44127200	2.04443800	-0.50895000
O	1.30688500	4.75073400	-0.51329700
N	-1.15512900	1.99186800	-0.02792800
H	3.35939600	1.26395300	0.41914400
H	1.18371700	1.86229500	1.15731200
O	0.19865400	4.15098000	1.47122200
H	0.04545500	2.71403000	-1.60444900
H	-1.25949400	1.81507000	0.98805200
C	-1.96111900	1.30148900	-0.77610200
H	-1.85317000	1.41423900	-1.85098900
C	-2.90381700	0.36588400	-0.23567000

C	-2.87213400	0.07695900	1.16452000
C	-3.74945300	-0.35744200	-1.11827700
C	-3.67860300	-1.03097100	1.61477800
C	-4.52201900	-1.36203600	-0.59683700
H	-5.20063900	-1.97113200	-1.17682800
H	-5.01933700	-2.42221400	1.06835600
N	-4.44607600	-1.65128400	0.73143700
C	-3.65371400	-1.47185600	3.03204900
H	-4.29170900	-2.34269000	3.19193100
H	-2.62655700	-1.72109300	3.31651700
H	-3.98412000	-0.65735700	3.68353800
C	-3.80058800	-0.07761200	-2.59282800
H	-2.83512000	-0.29736200	-3.06093700
H	-4.55882900	-0.70024600	-3.07085100
H	-4.03948600	0.97138700	-2.78918300
O	-2.16223900	0.71892900	2.00720400
H	-0.62875400	4.62892800	1.32110800
O	3.87689000	0.72567800	1.12217600
C	4.05066500	-0.56092000	0.71179500
C	4.11502700	-1.57095300	1.67743100
C	4.16421900	-0.89137700	-0.64362600
C	4.26780800	-2.89943100	1.28655000
H	4.02730500	-1.30598600	2.72694600
C	4.30125100	-2.22465700	-1.02404300
H	4.12753400	-0.10497400	-1.39075800
C	4.35317500	-3.23709300	-0.06523100
H	4.31046000	-3.67602300	2.04479800
H	4.37221500	-2.47073000	-2.07965000
H	4.46185000	-4.27404100	-0.36666700
H	1.83901600	0.83027900	-1.40991700
O	1.36889100	0.20056500	-2.04184400
C	0.60419800	-0.72318100	-1.40121200
C	0.66348200	-0.93000700	-0.01717900
C	-0.27278400	-1.49289700	-2.17480300
C	-0.15243800	-1.89098300	0.57646700
H	1.34162500	-0.34212800	0.59065400
C	-1.07764800	-2.45504900	-1.57017900
H	-0.31905000	-1.32270500	-3.24636200
C	-1.02605800	-2.66093300	-0.19105200
H	-0.10433300	-2.03201500	1.65247500
H	-1.76028600	-3.03721200	-2.18241400
H	-1.66138200	-3.40452600	0.27942300
H	0.61619200	5.28007000	-0.93392100



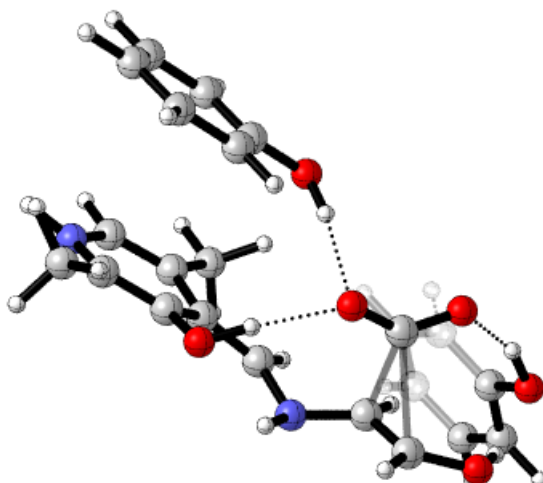
Structure of **22** optimized at the SMD_{(H₂O})ωB97XD/6-31+G(d,p) level of theory.

Zero-point correction=	0.463404 (Hartree/Particle)
Thermal correction to Energy=	0.493642
Thermal correction to Enthalpy=	0.494586
Thermal correction to Gibbs Free Energy=	0.399245
Sum of electronic and zero-point Energies=	-1452.120014
Sum of electronic and thermal Energies=	-1452.089776
Sum of electronic and thermal Enthalpies=	-1452.088832
Sum of electronic and thermal Free Energies=	-1452.184173

Charge = 0 Multiplicity = 1

C	1.25393100	-1.86041900	0.56210700
C	1.80965700	-1.36283900	-0.83907600
C	2.73995500	-2.37196100	-1.39949400
O	3.91018300	-2.57599400	-1.00665700
O	2.08165300	-2.01064400	1.48962500
N	0.82150200	-1.01025800	-1.83152800
H	2.41060100	-3.00534800	-2.22511200
O	0.01456400	-2.03089700	0.64258900
H	2.39919400	-0.46971900	-0.59424300
H	0.14766400	-1.75665000	-1.96356300
C	0.20663700	0.25249000	-1.59117700
H	0.93021900	1.05997500	-1.53821500
C	-1.11915900	0.53330700	-1.50148700
C	-2.20882500	-0.45149800	-1.47147000
C	-1.58035200	1.93096400	-1.41067100
C	-3.51153400	-0.10222500	-1.60242600
C	-2.89442100	2.21130700	-1.54950300

H	-3.27535800	3.22569300	-1.51205700
H	-4.81507400	1.48861800	-1.69907300
N	-3.84022300	1.23467500	-1.76200300
C	-4.65441100	-1.06854100	-1.62008700
H	-4.95652100	-1.29349200	-2.64942500
H	-4.38230500	-2.00534500	-1.13360100
H	-5.52054900	-0.64710700	-1.10078200
C	-0.58675800	3.03545400	-1.19707100
H	0.12655000	3.10537200	-2.02842000
H	-1.09247200	4.00022200	-1.10427400
H	0.00136100	2.86701000	-0.28600400
O	-1.90133000	-1.79519900	-1.31076300
H	-1.25982300	-1.88300300	-0.57215700
H	-0.73120300	-0.64616800	1.59040400
H	3.63807300	-1.40578600	1.41436900
H	4.24485800	-1.97853900	-0.21743300
O	-1.10690300	0.20203100	1.89799100
O	4.52820000	-1.19288200	1.01826300
C	-2.47354000	0.12488900	1.90994800
C	-3.19339700	1.32020300	1.93260400
C	-3.14107800	-1.10226000	1.90616900
C	-4.58568000	1.28347800	1.93826500
H	-2.65747100	2.26430100	1.92661700
C	-4.53399600	-1.12438600	1.91033300
H	-2.57040700	-2.02610500	1.88786200
C	-5.26430300	0.06399200	1.92481100
H	-5.14226300	2.21595700	1.94558700
H	-5.04966100	-2.07990900	1.89576600
H	-6.34930900	0.04041100	1.92199200
C	4.65388400	0.16949400	0.80043100
C	5.75793300	0.60846900	0.07530100
C	3.69742000	1.06518500	1.27417900
C	5.90476500	1.97081800	-0.17568300
H	6.48409600	-0.11256500	-0.28616300
C	3.85705900	2.42550300	1.01317800
H	2.84053700	0.70098700	1.83319900
C	4.95730200	2.88377000	0.28984400
H	6.76421100	2.31797800	-0.74068900
H	3.11266000	3.12622400	1.37822800
H	5.07597100	3.94358900	0.08937500



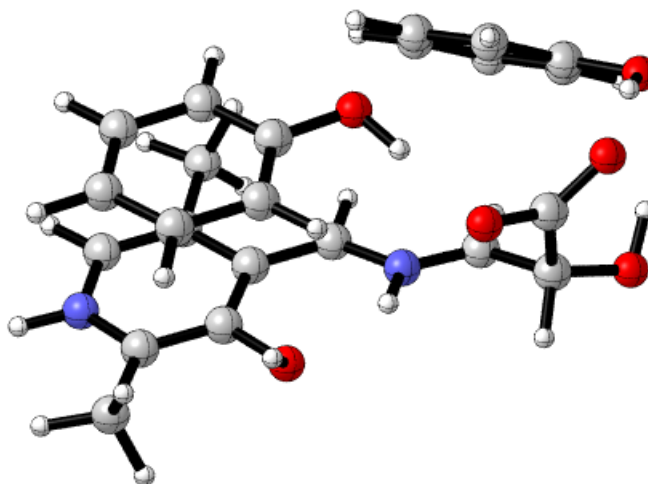
Structure of **TS-1** optimized at the SMD_{(H₂O}) ω B97XD/6-31+G(d,p) level of theory.

Zero-point correction=	0.463037 (Hartree/Particle)
Thermal correction to Energy=	0.493460
Thermal correction to Enthalpy=	0.494404
Thermal correction to Gibbs Free Energy=	0.399354
Sum of electronic and zero-point Energies=	-1452.110271
Sum of electronic and thermal Energies=	-1452.079848
Sum of electronic and thermal Enthalpies=	-1452.078904
Sum of electronic and thermal Free Energies=	-1452.173953

Charge = 0 Multiplicity = 1

C	1.20918900	-2.06063000	0.45601000
C	1.99102600	-1.26829000	-1.09246500
C	2.44476100	-2.57019000	-1.29524600
O	3.58036300	-3.03124800	-0.83010700
O	1.96615300	-2.11820000	1.41209500
N	1.01663200	-0.66735400	-1.87096900
H	1.85847000	-3.30266500	-1.84474700
O	0.00280000	-2.19692700	0.27551700
H	2.67063300	-0.56465100	-0.61790800
H	0.37991800	-1.29968000	-2.34083200
C	0.44342400	0.52122400	-1.34228800
H	1.19468400	1.22689500	-1.00452700
C	-0.87457600	0.83771800	-1.26718400
C	-1.99171200	-0.05122800	-1.59847300
C	-1.29094900	2.16945800	-0.79691900
C	-3.27010400	0.38809100	-1.69722800
C	-2.58231400	2.54658300	-0.92266700

H	-2.93232700	3.52312100	-0.60769900
H	-4.50868600	2.00638700	-1.40650400
N	-3.54121700	1.72462000	-1.46656100
C	-4.43765400	-0.47218100	-2.06557000
H	-4.66658100	-0.37855900	-3.13318300
H	-4.23050000	-1.52108600	-1.85268300
H	-5.32668000	-0.17198000	-1.50305900
C	-0.27736600	3.09914800	-0.19616900
H	0.50953300	3.36228600	-0.91467700
H	-0.75043900	4.02484400	0.14167000
H	0.22008500	2.63293800	0.66408000
O	-1.73742300	-1.39904900	-1.82415100
H	-1.20679500	-1.73718400	-1.07897500
H	-0.88802300	-0.99826500	1.44530900
H	3.67951700	-1.43628800	1.45258300
H	4.10248400	-2.34195600	-0.36231100
O	-1.28587300	-0.23414700	1.90031500
O	4.56065600	-1.18599400	1.10867200
C	-2.64956700	-0.29226900	1.79856900
C	-3.37290100	0.86141700	2.10368200
C	-3.31108900	-1.45971900	1.41029400
C	-4.76231600	0.84583200	2.00766000
H	-2.84090800	1.76180900	2.39496400
C	-4.70107800	-1.46063800	1.31687200
H	-2.73749200	-2.35078900	1.17178600
C	-5.43461500	-0.31172100	1.61302500
H	-5.32095100	1.74822600	2.23779300
H	-5.21113100	-2.36681900	1.00418100
H	-6.51693100	-0.31706100	1.53385500
C	4.59055100	0.16252800	0.84345500
C	5.72189600	0.67431900	0.20879100
C	3.51948200	0.99716300	1.16938400
C	5.77461700	2.03040600	-0.10407000
H	6.54316700	0.00917500	-0.03877100
C	3.58238900	2.35100800	0.84192200
H	2.64072400	0.58680200	1.65896500
C	4.70685600	2.87488500	0.20606100
H	6.65570000	2.42744200	-0.59891800
H	2.74177700	2.99402900	1.08545100
H	4.75173400	3.92921800	-0.04655900



Structure of **23** optimized at the SMD_{(H₂O})ωB97XD/6-31+G(d,p) level of theory.

Zero-point correction=	0.465199 (Hartree/Particle)
Thermal correction to Energy=	0.495598
Thermal correction to Enthalpy=	0.496542
Thermal correction to Gibbs Free Energy=	0.403160
Sum of electronic and zero-point Energies=	-1452.170942
Sum of electronic and thermal Energies=	-1452.140543
Sum of electronic and thermal Enthalpies=	-1452.139599
Sum of electronic and thermal Free Energies=	-1452.232981

Charge = 0 Multiplicity = 1

C	2.41135000	-2.44025400	-0.24070200
C	1.82199100	-0.36397800	-1.47741600
C	2.48712000	-1.70126000	-1.61210800
O	3.79579900	-1.55822600	-2.11625500
O	3.47306400	-2.61177200	0.40459500
N	0.53985900	-0.23481400	-1.38374700
H	1.93624400	-2.32118800	-2.32657800
O	1.25482800	-2.79530400	0.11126700
H	2.43375700	0.53120100	-1.41474300
H	-0.04555000	-1.07288600	-1.46845300
C	-0.15097900	0.93537300	-1.10527200
H	0.48172300	1.78655600	-0.89938600
C	-1.53138200	1.01695800	-1.05499800
C	-2.44486100	-0.04960300	-1.35984100
C	-2.14918000	2.26273400	-0.64334000
C	-3.80349700	0.10904300	-1.33111000

C	-3.50576100	2.35417800	-0.60015100
H	-4.01763500	3.25503800	-0.28474200
H	-5.31294300	1.42868400	-0.89979100
N	-4.30893500	1.31429700	-0.95120500
C	-4.76263900	-0.97746100	-1.69602400
H	-4.55703300	-1.34179000	-2.70648900
H	-4.66981400	-1.82483000	-1.00980400
H	-5.79200800	-0.61587300	-1.65661500
C	-1.30115100	3.42214200	-0.20924200
H	-0.63246500	3.75569000	-1.01119900
H	-1.92564200	4.26757500	0.08781900
H	-0.67055700	3.14221200	0.64366500
O	-1.91149200	-1.26645100	-1.75583000
H	-2.29178400	-1.98237500	-1.22805500
H	0.50211600	-1.71275800	1.26959400
H	4.51360400	-1.17126200	0.66875200
H	4.35850000	-1.16155000	-1.43299100
O	0.13698100	-0.98346100	1.81747300
O	5.00368600	-0.34401800	0.46765200
C	-1.22413400	-1.01711200	1.83274400
C	-1.89646900	0.12444600	2.27549100
C	-1.94447000	-2.14963300	1.44106300
C	-3.28772300	0.13206700	2.32042300
H	-1.32165400	1.00044000	2.56167200
C	-3.33754000	-2.12795200	1.49063400
H	-1.41438000	-3.03498900	1.10195000
C	-4.01712900	-0.99101900	1.92728700
H	-3.80416200	1.02804700	2.65207600
H	-3.89268500	-3.00845700	1.18069900
H	-5.10203800	-0.97708800	1.95233000
C	4.17495300	0.73863600	0.62146400
C	4.52056300	1.92208100	-0.03097800
C	3.00358500	0.66566900	1.37944100
C	3.67708200	3.02791200	0.06095900
H	5.43110700	1.95994400	-0.62075000
C	2.16502800	1.77613700	1.45634200
H	2.74442500	-0.26117500	1.88209300
C	2.49364800	2.96051900	0.79721100
H	3.94384300	3.94409500	-0.45723400
H	1.24369900	1.70758700	2.02718100
H	1.83149100	3.81889200	0.85118000

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