Robin Brabham

Supporting Information

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1. Materials and Reagents

1.1. Solvents and Starting Materials. All solvents were dried prior to use according to standard methods,[1] with the exception of solvents used for flash chromatography purposes, where GPR-grade solvents were used. All commercially-available reagents were used as received.

1.2. General Procedures. All solution-phase reactions were carried out under a dry nitrogen atmosphere using oven-dried glassware unless otherwise stated. All concentrations were performed *in vacuo* unless stated otherwise. Analytical TLC was performed on silica gel 60-F^{254} with detection by fluorescence and/or charring following immersion in a solution of ninhydrin (1.5 g in 100 mL *n*-butanol and 3 mL acetic acid). Flash column chromatography was carried out using Sigma silica (pore size 60 Å, 200-400 mesh).

1.3. Plasmids. The vectors pEVOL, harbouring pylT and pylRS (*M*. *mazei*, wild type) genes, and pBAD-GFP(Y39TAG) genes have been previously described.[2] The pBAD vector harbouring the sfGFP(N150TAG) gene was a gift from Ryan Mehl (Addgene plasmid #85483).

2. Buffers

A1: H_2O (HPLC grade), 0.1% (v/v) FA. B1: MeCN (HPLC grade), 0.1% (v/v) FA.

A2: 4 × PBS, pH 8.0, 10 mM imidazole. B2: 4 × PBS, pH 8.0, 500 mM imidazole.

D1: 1 × PBS, pH 7.4. D2: 10 × PBS, pH 7.4. D3: 100 mM sodium phosphate buffer (PB), pH 7.0.

M1: 50:50:1 (v/v) MeCN (HPLC grade): H_2O (HPLC grade): formic acid.

3. Mass Spectrometry and NMR

Protein ESI mass spectra were obtained on a Bruker Solarix XR 9.4 T instrument. Samples of GFP were desalted and analysed at a final concentration of 0.4-10 µM in buffer M1. Small-molecule HRMS data were obtained at room temperature on a Bruker Daltonics micrOTOF.

Analytical HPLC of peptides was performed on a Shimadzu Prominence HPLC equipped with a Shimadzu photodiode array using an Accucore C18 2.6 µm column, 2.1 × 150 mm**.** All samples were run using solvents A1 and B1.

^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 101 MHz respectively on a JEOL ECS 400 instrument using an internal deuterium lock at room temperature. Chemical shifts are reported according to the following references:

$CDCl_3$: δ_H 7.27 ($CHCl_3$), δ_C 77.0, centre of triplet ($CDCl_3$); D_2O: δ_H 4.79 (HOD); DMSO-d_6: δ_H 2.50 (DMSO-d_5), δ_C 39.52, centre of septet (DMSO-d_6); TFA-d: δ_H 11.5 (CF_3CO_2H), δ_C 116.6, centre of quartet (CF_3CO_2D).

4. Introduction of Aldehydes into Proteins

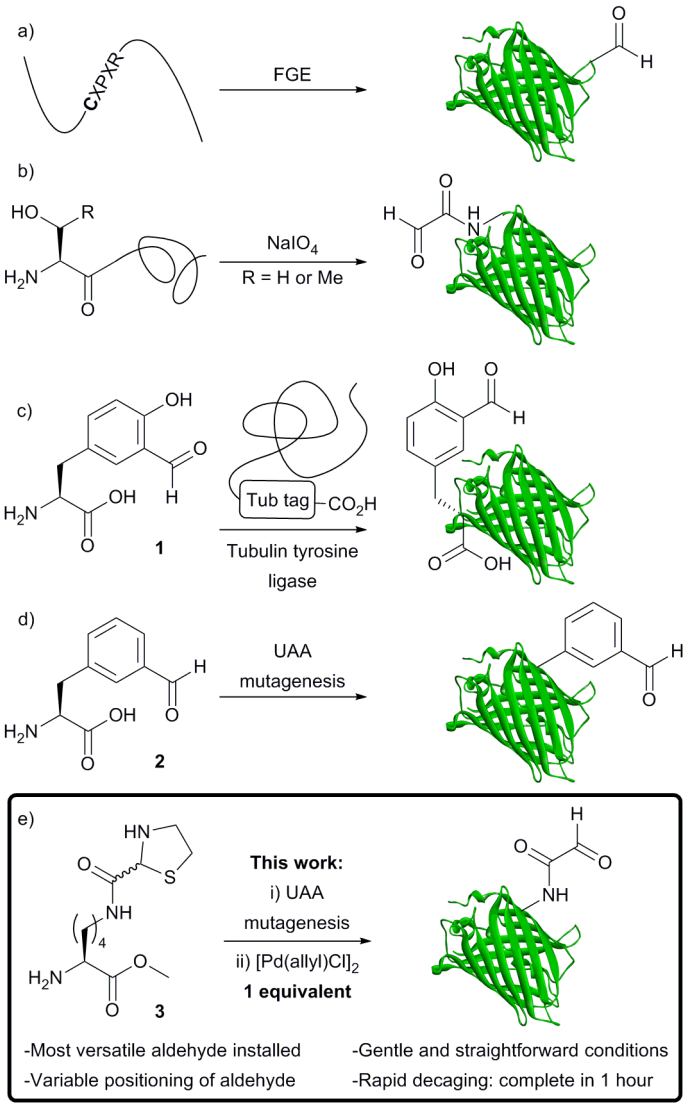
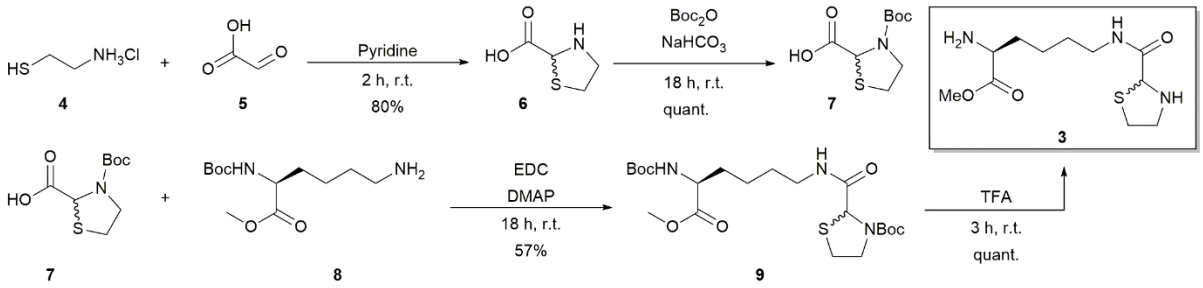


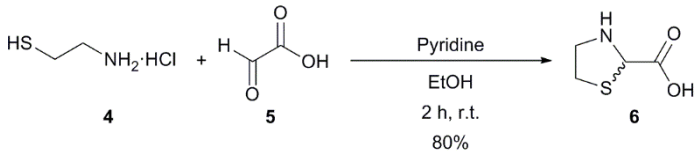
Figure S1: Selected methods for the installation of aldehydes in proteins, including chemical, enzymatic, and genetic incorporation methods.

5. Solution-phase synthesis (adapted)[3]



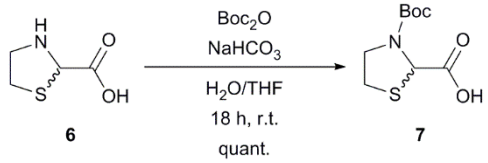
Scheme S1: Synthesis of **3**.

5.1. Thiazolidine 2-carboxylic acid (racemate) 6



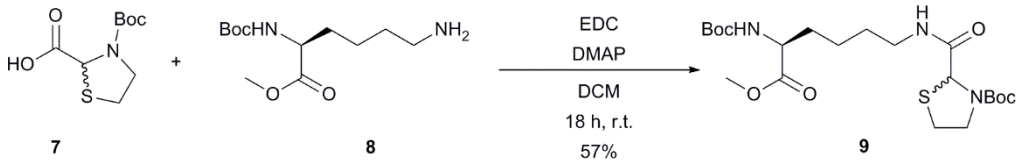
A solution of 2-aminoethane thiol hydrochloride **4** (2.84 g, 25 mmol) in 5:2 (v/v) ethanol:pyridine (14 mL) was added to a stirred solution of glyoxylic acid **5** (50% solution in water, 3.7 g, 25 mmol, 1 eq.) in ethanol (5 mL). The reaction was left to stir for 2 h at rt, after which the off-white precipitate was isolated by filtration and washed with ethanol, yielding racemic thiazolidine-2-carboxylic acid **6** as a white powder (2.67 g, 80%), used without further purification; δ_H (400 MHz, D_2O): 5.10 (s, 1H, CH), 3.68-3.74 (m, 1H), 3.57-3.63 (m, 1H), 3.16-3.20 (m, 2H); δ_C (101 MHz, D_2O): 171.7 (RCO_2H), 62.3 (CH), 49.3 (CH_2), 29.5 (CH_2); IR (ATR): 3110, 1622, 1590, 1376, 1354, 1323, 1300, 1277, 998, 881, 866, 721, 643; HRMS: Found $[M+H]^+$ 134.0270; $C_4H_8NO_2S$ requires 134.0270 (Δ = 0.2 ppm).

5.2. 3-(*tert*-butoxycarbonyl)thiazolidine-2-carboxylic acid (racemate) 7



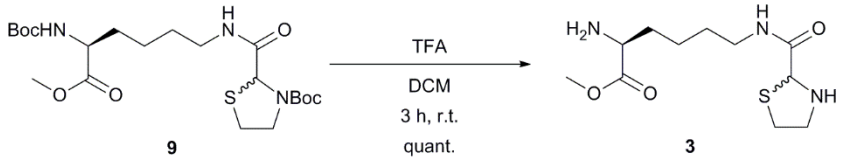
A stirred solution of thiazolidine-2-carboxylic acid **6** (2.7 g, 20 mmol, 1 eq.) in THF (70 mL) was cooled to 0 °C with stirring and di-*tert*-butyl dicarbonate (11.0 g, 50 mmol, 2.5 eq.) added, followed immediately by a solution of sodium hydrogencarbonate (10.0 g, 120 mmol, 12 eq.) in water (70 mL). The mixture was allowed to warm to rt and left stirring for 18 h. The reaction mixture was concentrated to remove organic solvent, diluted with water (70 mL), and washed with Et_2O (2 × 30 mL). The aqueous layer was acidified with 6 M HCl to pH 1 and washed with DCM (3 × 30 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated to yield the crude product **7** as a thick pale yellow oil in quantitative yield used without further purification; δ_H (400 MHz, DMSO-d_6): 5.07 (s, 1H, CH, rotamers present), 3.71 (m, 2H, CH_2), 3.05 (br s, 2H, CH_2), 1.46 (s, 9H, CH_3); δ_C (101 MHz, $CDCl_3$): 169.9 (CO_2H), 152.9 ($NHCO_2$), 82.0 and 81.1 ($\underline{C}(CH_3)_3$), 60.9 and 60.3 (CH), 49.9 and 49.6 (CH_2), 30.7 and 29.6 (CH_2), 28.3 and 27.9 (CH_3); IR (ATR): 2982, 1804, 1756, 1371, 1211, 1113, 1062, 843, 774; HRMS: found $[M+Na]^+$ 256.0610; $C_9H_{15}NO_4SNa$ requires 256.0614 (Δ = 1.9 ppm).

5.3. Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-6-(thiazolidine-2-carboxamido-3-(*tert*-butoxycarbonyl)) hexanoate (mixture of diastereomers) 9



3-(*tert*-butoxycarbonyl)thiazolidine-2-carboxylic acid **7** (2.0 g, 8.6 mmol, 1.0 eq.) in dry DCM (2 mL) was added to a stirred solution of methyl 2-(*tert*-butoxycarbonylamino) hexanoate **8** (2.7 g, 10.4 mmol, 1.2 eq.) in dry DCM (5 mL) under nitrogen atmosphere. The reaction vessel was cooled to 0 °C and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.5 g, 7.9 mmol, 1.0 eq.) and 4-dimethylaminopyridine (400 mg, 3.3 mmol, 0.4 eq.) were added sequentially in one portion. The reaction vessel was left to warm to r.t. and stirred under nitrogen atmosphere for 18 h. Concentration of the reaction material afforded a pale yellow oil, which was purified by flash chromatography (silica, DCM:MeOH (v/v) 20:0 → 19:1) to afford the product **9** as a colourless oil (2.34 g, 57%); δ_H (400 MHz, $CDCl_3$): 6.09 and 5.67 (2 × s, total 1 H, $N_\varepsilon H$), 5.23 (s, 1H, Thz CH), 5.09 (d, 1H, $^3J_{H\text{-}H}$ 7.0 Hz, $N_\alpha H$), 4.26 (m, 1H, H_α), 3.85-3.95 (br m, 1 H, Thz CH_2), 3.74-3.79 (m, 1H, Thz CH_2), 3.73 (s, 3H, OCH_3), 3.06-3.34 (br m, 3H, H_ε and Thz CH_2), 2.93 (ddd, 1H, $^2J_{H\text{-}H}$ 11.0 Hz, $^3J_{H\text{-}H}$ 6.0 Hz, $^3J_{H\text{-}H}$ 5.0 Hz, Thz CH_2), 1.71-1.87 (m, 2H, H_β), 1.49-1.57 (m, 2H, H_δ), 1.44 (s, 18H, Boc), 1.30-1.40 (m, 2H, H_γ); δ_C (101 MHz, $CDCl_3$): 173.4 (CO_2R), 170.3 (CONHR), 155.6 ($RHNCO_2R$), 81.7 ($OCMe_3$), 55.3 (Thz CH), 52.5 (C_α), 52.44 (OCH_3), 50.3 (Thz CH_2), 39.4, 32.4 (Lys CH_2), 29.2 (Thz CH_2), 28.4 (Boc CH_3), 23.4, 22.5 (Lys CH_2); IR (ATR): 3346, 2976, 1689, 1516, 1365, 1248, 1160, 785; HRMS: found $[M+Na]^+$ 498.2251; $C_{21}H_{37}N_3O_7SNa$ requires 498.2244 (Δ = -0.8 ppm).

5.4. Methyl 2-amino-6-(thiazolidine-2-carboxamido) hexanoate (mixture of diastereomers) 3



Trifluoroacetic acid (0.5 mL) was added dropwise to a stirred solution of methyl 2-(*tert*-butoxycarbonylamino)-6-(thiazolidine-2-carboxamido-3-(*tert*-butoxycarbonyl)) hexanoate **9** (210 mg, 0.44 mmol) in DCM (1 mL) at 0 °C and left to reach rt. Once the reaction was determined complete by TLC, usually within 3 h, the solvent was removed *in vacuo*. The resulting yellow oil was redissolved in 10% (v/v) aqueous acetic acid and lyophilised to afford the product **3** as a thick yellow oil in quantitative yield and in purity sufficient for further manipulations; δ_H (400 MHz, TFA-d): 5.58 and 5.54 (2 × s, total 1 H, Thz CH, mixture of diastereomers), 4.17-4.28 (m, 1H, H_α), 3.99-4.05 (m, 1H, Thz CH_2), 3.83 and 3.87 (s, 3H, OCH_3), 3.69-3.78 (m, 1H, Thz CH_2), 3.28-3.36 (m, 2H, H_ε), 3.15-3.27 (m, 2H, Thz CH_2), 1.96-2.12 (m, 2H, H_β), 1.62-1.54 (m, 2H, H_δ) 1.43-1.53 (m, 2H, H_γ); δ_C (101 MHz, TFA-d): 171.5 (CO_2R), 168.8 (CONHR), 63.2 (Thz CH), 55.1 (C_α), 52.1 (OCH_3), 41.1 (Thz CH_2), 30.5 (Lys CH_2), 30.2 (Thz CH_2), 28.3, 22.6 (Lys CH_2); IR (ATR): 2960, 1667, 1179, 1130, 837, 798, 722; HRMS: found $[M+H]^+$ 276.1387; $C_{11}H_{22}N_3O_3S$ requires 276.1376 (Δ = -3.2 ppm).

6. Protein Expression and Characterisation

6.1. Expression of GFP/sfGFP containing 3

The pBAD vector containing ampicillin resistance and either Ser-GFP(Y39TAG) or sfGFP(N10TAG) genes, together with the pEVOL vector containing $tRNA^{Pyl}$, pylRS (*M. mazei*, wild type) and chloramphenicol resistance genes, were co-transformed into electrocompetent *E. coli* Top10 cells and selected on LB agar plates containing ampicillin (100 µg/ml) and chloramphenicol (35 µg/ml).

For small-scale expression, 0.5 mL of an overnight culture grown from a single colony was inoculated into 50 mL Terrific Broth Medium containing ampicillin (100 µg/ml) and chloramphenicol (35 µg/ml) in a 250 mL baffled conical flask. At 37 °C with shaking (220 rpm), cells typically grew within 3 h to an OD_{600} of 0.2-0.3, at which point **3** (stock solution 80 mM in 0.1 M NaOH (aq.)) was added to a final concentration of 1.6 mM. The cultures were allowed to grow until an OD_{600} of 0.4-0.6, at which point protein expression was induced by addition of L-arabinose (stock solution 20% (w/w)) at a final concentration of 0.02% (w/w). After further growth for 16-18 h (37 °C, 220 rpm), the cultures were harvested by centrifugation (6 000 × g, 4 °C, 20 min). Pellets were resuspended in buffer A2 with a Pierce Protease Inhibitor (EDTA-free) tablet and then lysed by sonication on ice for 6 × 30 s with 30 s intervals. The lysate was clarified by centrifugation (20 000 × g, 4 °C, 20 min) and loaded onto a Ni HiTrap Chelating HP column (1 ml, GE Healthcare) pre-equilibrated in buffer A2. The column was washed with 10 column volumes of A2 and then eluted using a gradient of 0-100% B2 over 7.5 column volumes, taking 0.5 mL fractions, and the column washed with 7.5 column volumes of B2, taking 0.5 mL fractions. Fractions containing full-length protein (as determined by SDS-PAGE) were pooled, dialysed into C1 and concentrated (Vivaspin centrifugal concentrator, 10000 MWCO) to a final concentration of 330 µM (as determined by UV-visible spectroscopy, $\varepsilon_{280} = 2.0 \times 10^4$ dm^3 mol^{-1} cm^{-1}) and stored at -80 °C.

6.2. Sequence of GFP(Y39ThzK) 10

Highlighting: FLAG tag (with *N*-terminal Asp, present in the original plasmid, mutated to Ser). Amber stop codon (Y39TAG). His_6 tag.

```
        10         20         30         40         50         60
[M]SYKDDDDKV SKGEELFTGV VPILVELDGD VNGHKFSVSG EGEGDATXGK LTLKFICTTG
        70         80         90        100        110        120
KLPVPWPTLV TTLTYGVQCF SRYPDHMKQH DFFKSAMPEG YVQERTIFFK DDGNYKTRAE
       130        140        150        160        170        180
VKFEGDTLVN RIELKGIDFK EDGNILGHKL EYNYNSHNVY IMADKQKNGI KANFKIRHNI
       190        200        210        220        230        240
EDGSVQLADH YQQNTPIGDG PVLLPDNHYL STQSALSKDP NEKRDHMVLL EFVTAAGITL
       250
GMDELYKHHH HHH*
```

Mutation of the *N*-terminal residue to serine (from aspartate as part of a FLAG tag originally encoded in pBAD-GFP(Y39TAG)) was performed using an Agilent Quikchange Multi Site-Directed Mutagenesis Kit according to the provided protocol using the following primers (Sigma):

5'-CACTTTATCATCATCATCTTTGTAAGACATGGTTAATTCCTCCTGTTAGCCC-3'
5'-GGGCTAACAGGAGGAATTAACCATGTCTTACAAAGATGATGATGATAAAGTG-3'

6.3. Characterisation of GFP(Y39ThzK) 10

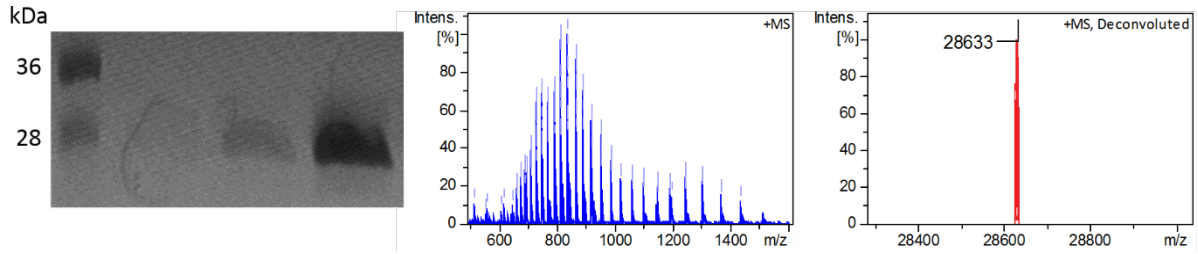


Figure S2: (left) Coomassie-stained SDS-PAGE of **10** after Ni affinity purification; (centre) ESI-FTICR-MS of **10** raw data; (right) deconvoluted spectrum of **10**, calc. 28634, found 28633.

6.4. Sequence of sfGFP(N150ThzK) 11

Highlighting: Amber stop codon (N150TAG). His_6 tag.

```
        10         20         30         40         50         60
[M]VSKGEELFT GVVPILVELD GDVNGHKFSV RGEGEGDATN GKLTLKFICT TGKLPVPWPT
        70         80         90        100        110        120
LVTTLTYGVQ CFSRYPDHMK RHDFFKSAMP EGYVQERTIS FKDDGTYKTR AEVKFEGDTL
       130        140        150        160        170        180
VNRIELKGID FKEDGNILGH KLEYNFNSHX VYITADKQKN GIKANFKIRH NVEDGSVQLA
       190        200        210        220        230        240
DHYQQNTPIG DGPVLLPDNH YLSTQSVLSK DPNEKRDHMV LLEFVTAAGI THGMDELYKG

SHHHHHH*
```

6.5. Characterisation of sfGFP(N150ThzK) 11

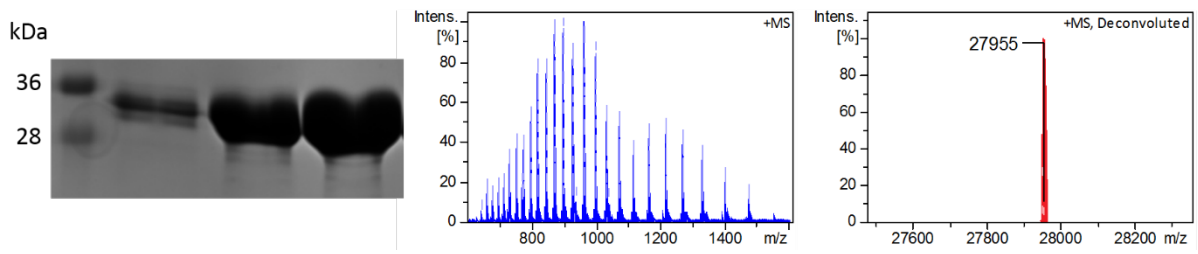


Figure S3: (left) Coomassie-stained SDS-PAGE of **11** after Ni affinity purification; (centre) ESI-FTICR-MS of **11** raw data; (right) deconvoluted spectrum of **11**, calc. 27959, found 27955.

6.6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis and staining

All SDS-PAGE analysis was performed using handcast 12% polyacrylamide gels. Samples were reduced by boiling for 10 min (2% SDS, 2 mM 2-mercaptoethanol, 4% glycerol, 40 mM Tris-HCl pH 6.8, 0.01% bromophenol blue). Molecular weight markers used were PageRuler Plus Prestained Protein Ladder (Thermo Scientific). Each gel was run at 200 volts for 45-80 min. For Coomassie stain experiments, the gel was washed with fixing solution (40% (v/v) MeOH, 10% AcOH), stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 (50% (v/v) MeOH, 10% AcOH), and finally repeatedly washed with destain solution (50% (v/v) MeOH, 10% AcOH). Images of the resulting gels were captured and analysed using a Syngene G:BOX Chemi XRQ equipped with a Synoptics 4.0 MP camera, with GeneSys software (Version 1.5.7.0).

6.7. Fluorescent imaging

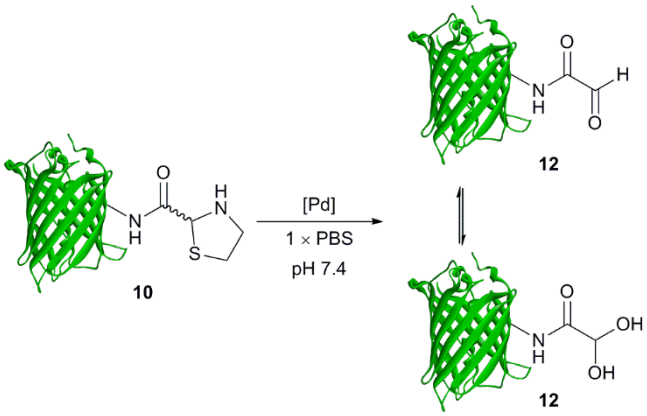
For fluorescent imaging of fluorescently modified proteins, the SDS PAGE gel was washed with fixing solution (40% MeOH, 10% AcOH). Visualisation of protein fluorescence, and images of the resulting gels, were captured by excitation at 302 nm and analysed using a Syngene G:BOX Chemi XRQ equipped with a Synoptics 4.0 MP camera in line with GeneSys software (Version 1.5.7.0).

6.8. Western blot analysis

For Western blot analysis, 2.5 µg of biotinylated protein samples or negative controls were run on 12% SDS–PAGE and transferred onto a nitrocellulose membrane filter (0.45 µm, Amersham Protran Sandwich, GE Healthcare) using an electroblot apparatus (Bio-Rad, Hercules, CA) at 100 V, 350 mA for 1 h in cooled transfer buffer (25 mM Tris–HCl pH 8.3, 192 mM glycine, 0.1% SDS, 20% (v/v) methanol). The membrane was incubated in blocking solution (Phosphate-buffered saline (PBS) tablets, Sigma)) containing 5% (w/v) non-fat dry milk powder for 16 h at 4 °C. The membrane was processed through sequential incubations with primary antibody, alkaline phosphatase anti-biotin (goat, Vector Labs, CA) 1:1000 dilution in PBS for 1 hour at room temperature, followed by washing in PBS, 0.01% Tween-20, and then incubation with visualising substrate BCIP/NBT Alkaline Phosphatase Substrate Kit (Vector Labs, CA) until immunoreactive proteins on the membrane were visible (*ca*. 20 min). The reaction was stopped by washing the membrane in distilled water. The membranes were imaged using a Syngene G:BOX Chemi XRQ equipped with a Synoptics 4.0 MP camera, with GeneSys software (Version 1.5.7.0).

7. Protein Modification

7.1. Pd reagent screening conditions



For a typical 100 µL scale reaction, palladium reagent **14-17** (stock 0.55 M in DMSO, 6 µL, final concentration 33 mM) was added to **10** (stock 330 µM in buffer D1, 100 µL, final concentration 300 µM) in a 0.5 mL Eppendorf tube. The reaction mixture was vortex mixed for 1 s and incubated in a water bath at 37 °C. The reaction was quenched by the addition of DTT (*ca*. 5 mg) and centrifuged (5000 × *g*, 2 min). The supernatant was decanted and desalted using a PD SpinTrap G25 column (GE Healthcare Life Sciences) into H_2O for mass spectrometry analysis or further manipulation.

7.2. Optimised procedure for decaging using 17

For a typical 100 µL scale reaction, tris(dibenzylideneacetone)dipalladium(0) **17** (stock 0.40 M in DMSO, 9 µL, final concentration 30 mM, 8% (v/v) DMSO) was added to **10** or **11** (stock 0.33 mM in buffer D1, 100 µL, final concentration 0.30 mM) in a 0.5 mL Eppendorf, mixed by pipette tip swirling, and incubated at 37 °C for 24 h. The reaction mixture was quenched by the addition of DTT (*ca*. 5 mg) and centrifuged (5000 × *g*, 2 min). The supernatant was decanted, diluted to 500 µL and desalted using a PD Minitrap G-25 (GE Healthcare) into H_2O for analysis and further manipulation.

7.3. Optimised procedure for decaging using 16

For a typical 100 µL scale reaction, allylpalladium(II) chloride dimer **16** (stock 30 mM in DMSO, 1 µL, final concentration 0.30 mM, 1% (v/v) DMSO) was added to **10** or **11** (stock 0.30 mM in buffer D1, 99 µL, final concentration 0.30 µM) in a 0.5 mL Eppendorf, mixed by pipette tip swirling, and left at rt for 1 h. The reaction mixture was quenched by 3-mercaptopropanoic acid (stock 1% (v/v) in D2, 10 µL, final concentration 0.1% (v/v)) and left at rt for 15 min. The reaction mixture was diluted up to 500 µL and desalted using a PD Minitrap G-25 (GE Healthcare), gravity method, into H_2O for analysis and further manipulation.

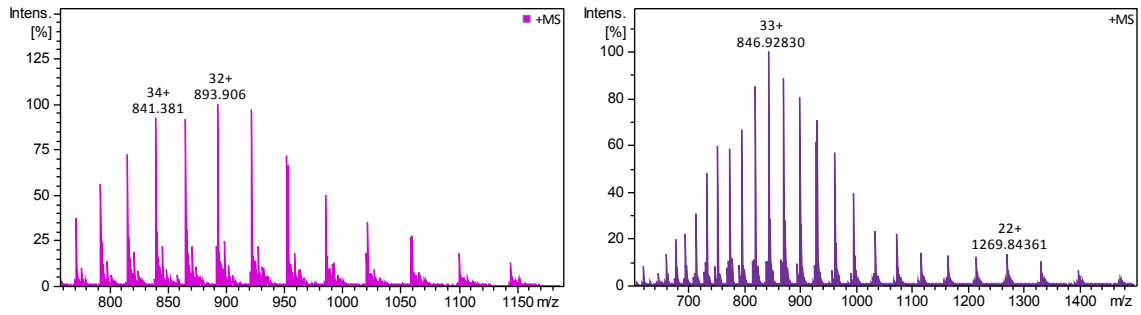
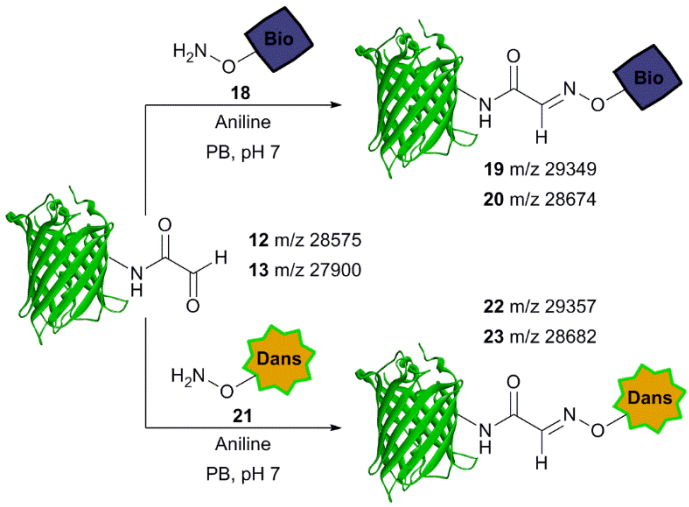


Figure S4: Raw ESI-FTICR mass spectra of **12** (left) and **13** (right).

7.4. Oxime ligation



For a typical 100 µL scale reaction, aniline (neat, 1 µL, final concentration 0.10 M) and probe **18** or **21** (stock 100 mM in H_2O, 2 µL, final concentration 2.0 mM) were added to **12** or **13** (stock 60 µM, 97 µL, final concentration 58 µM in D3) and mixed by pipette tip swirling. After incubation at 37 °C in a water bath for 24 h, the reaction mixture was diluted up to 500 µL and desalted using a PD Minitrap G-25 (GE Healthcare), gravity method, into H_2O for analysis and further manipulation.

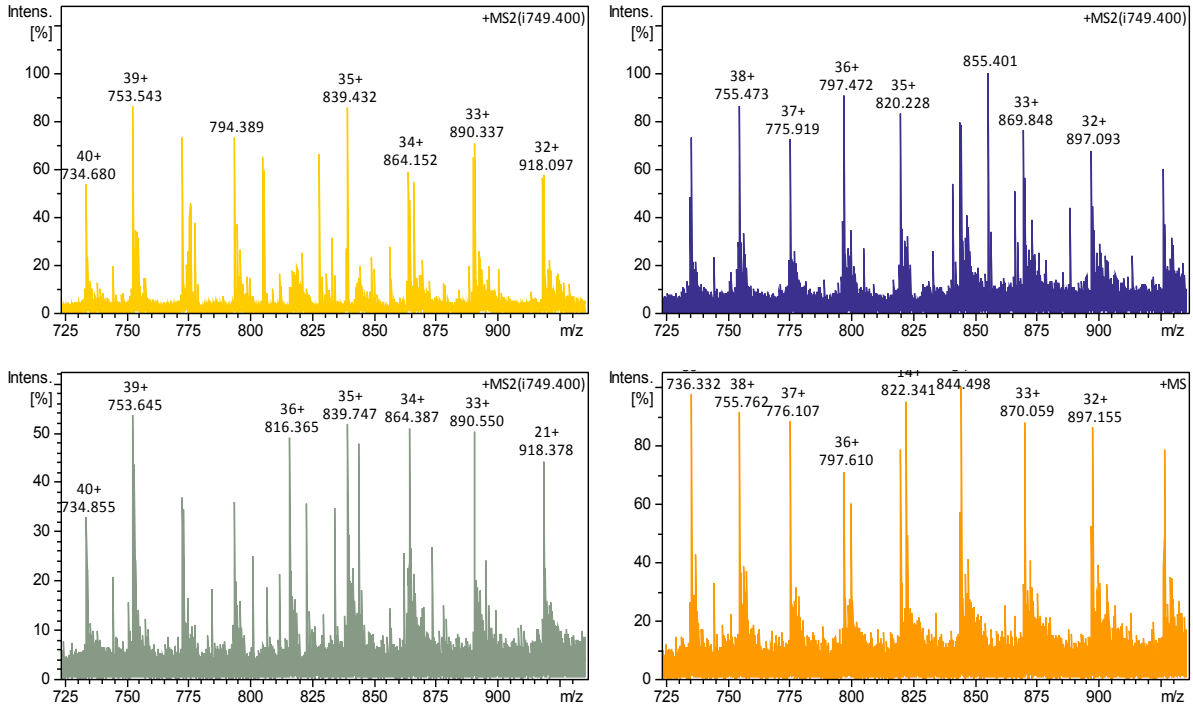


Figure S5: (clockwise, starting top left) Raw ESI-FTICR-MS of oxime ligation products **19**, **20, 22** and **23**. Small molecule contamination may be seen from the probes **18** (793 $[M+H]^+$, 815 $[M+Na]^+$) and **21** (801 $[M+H]^+$, 823 $[M+Na]^+$) and plasticisers, and efforts to suppress signals arising from these ions have been made using sweep + shot excitation.

8. Solid-phase Peptide Synthesis

8.1. General Procedures

Preloaded resin preparation. The preloaded 2-chlorotrityl resin was weighed out into a 2 mL SPPS cartridge fitted with a PTFE stopcock, swollen in DCM for 30 min and then filtered.

Fmoc deprotection. A solution of 20% piperidine in DMF was added to the resin and gently agitated by rotation for 2 minutes. The resin was filtered off and repeated four more times, followed by washes with DMF (5 × 2 min with rotation).

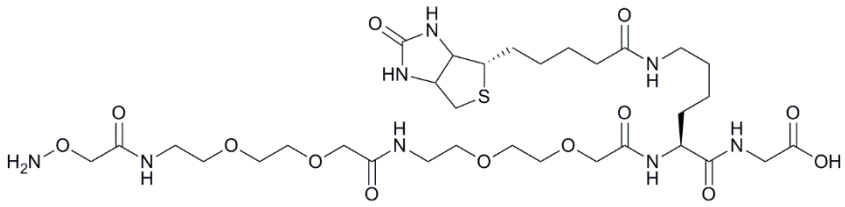
Amino acid coupling. DIPEA (11 eq.) was added to a solution of amino acid (5 eq.) and HCTU (5 eq.) dissolved in the minimum volume of DMF and the solution added to the resin. The reaction mixture was gently agitated by rotation for 1 h, and the resin filtered off and washed with DMF (3 × 2 min with rotation).

Cleavage Cocktails: Deprotection and resin cleavage: 95:2.5:2.5 (v/v) TFA:H_2O:triisopropylsilane.

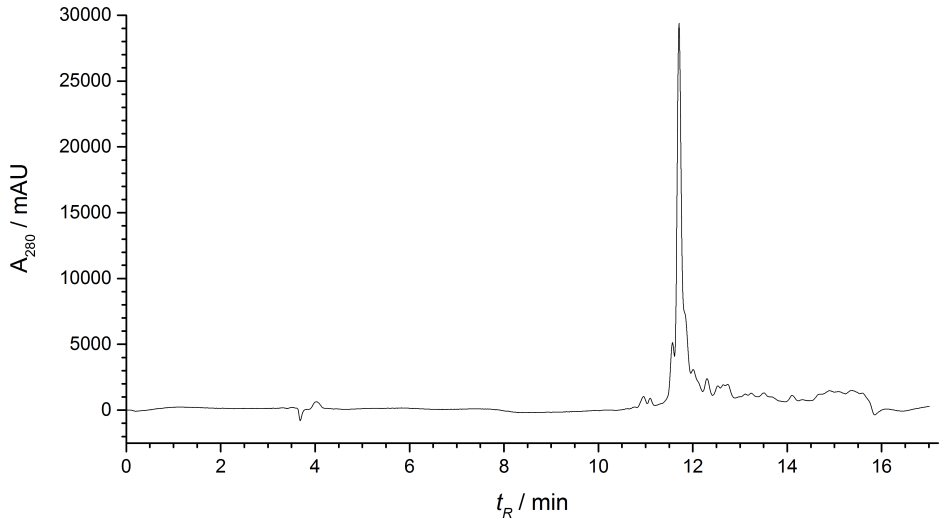
Cleavage and Isolation: The resin was washed with DCM (3 × 2 min with rotation) and MeOH (3 × 2 min with rotation). The resin was dried on a vacuum manifold and further dried on a high vacuum line overnight. A solution of cleavage cocktail was added to the resin and gently agitated by rotation for 60 min.

For peptides cleaved and deprotected: the reaction mixture was drained into ice-cold Et_2O and centrifuged at 4000 rpm at 4 °C until pelleted (*ca*. 5-10 min). The supernatant was carefully decanted and subsequently resuspended, centrifuged and supernatant decanted three more times. The precipitated peptide pellet was dissolved in 10% aq. AcOH and lyophilised.

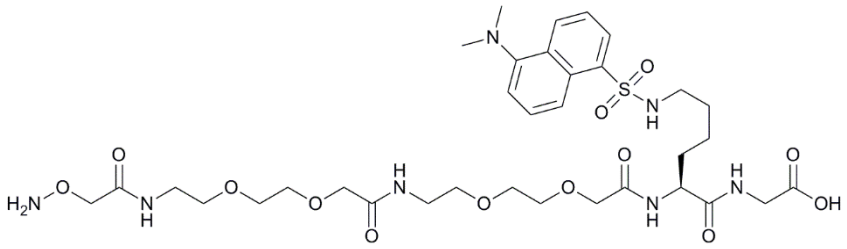
8.2. Aminooxyacetyl-P2-P2-Lys(biotin)-Gly-OH 18



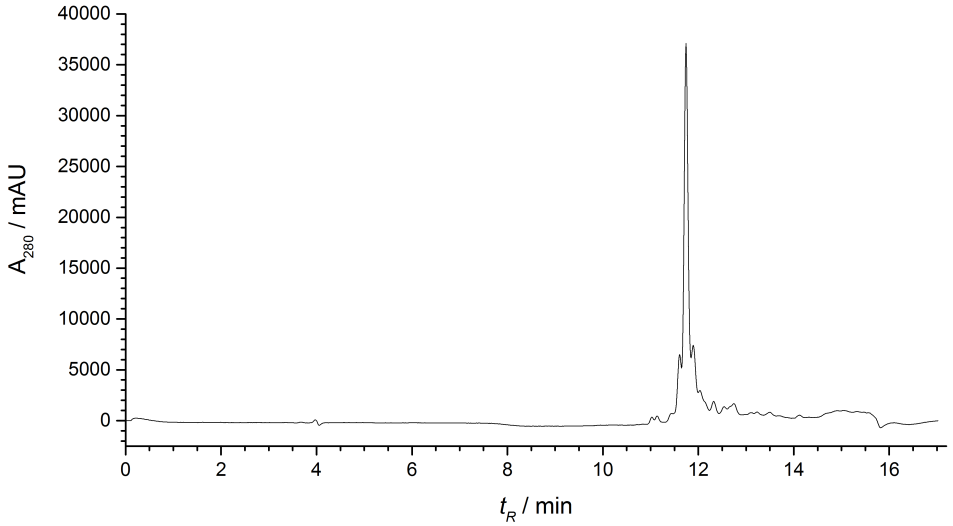
Peptide **18** was synthesised and purified according to the above procedure using preloaded H-Gly-2ClTrt resin (168 mg, loading 0.53 mmol g^{-1}, 0.089 mmol), Fmoc-Lys(biotin)-OH and (2-(2-(Fmoc-amino)ethoxy)ethoxy)acetic acid and capped with 3 eq. *N*'-Boc-aminooxyacetyl *N*-hydroxysuccinimide ester, no HCTU.[4] Resin cleavage and deprotection, purification, and lyophilisation afforded the peptide as a fluffy white powder (10.7 mg, 0.014 mmol, 16%); HRMS: Found $[M+H]^+$ 793.3784; $C_{32}H_{57}N_8O_{13}S$ requires 793.3760 (Δ = -2.4 ppm); HPLC: t_R 11.70 min.



8.3. Aminooxyacetyl-P2-P2-Lys(dansyl)-Gly-OH 21



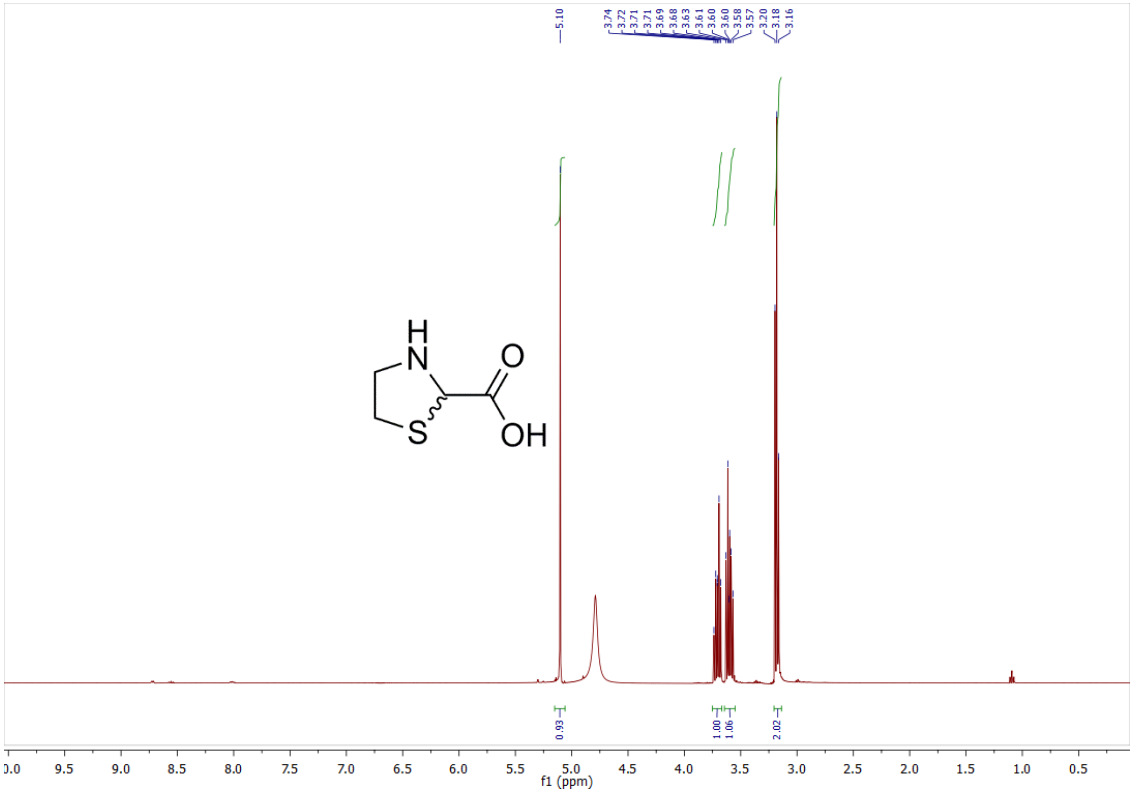
Peptide **21** was synthesised and purified according to the above procedure using preloaded H-Gly-2ClTrt resin (100 mg, loading 0.53 mmol g^{-1}, 0.053 mmol), Fmoc-Lys(dansyl)-OH and (2-(2-(Fmoc-amino)ethoxy)ethoxy)acetic acid and capped with 3 eq. *N'*-Boc-aminooxyacetyl *N*-hydroxysuccinimide ester, no HCTU.[4] Resin cleavage and deprotection, purification, and lyophilisation afforded the peptide as a fluffy yellow powder (6.8 mg, 0.009 mmol, 15%); HRMS: Found $[M+H]^+$ 800.3504; $C_{34}H_{54}N_7O_{13}S$ requires 800.3495 (Δ = -1.4 ppm); HPLC: t_R 11.74 min.



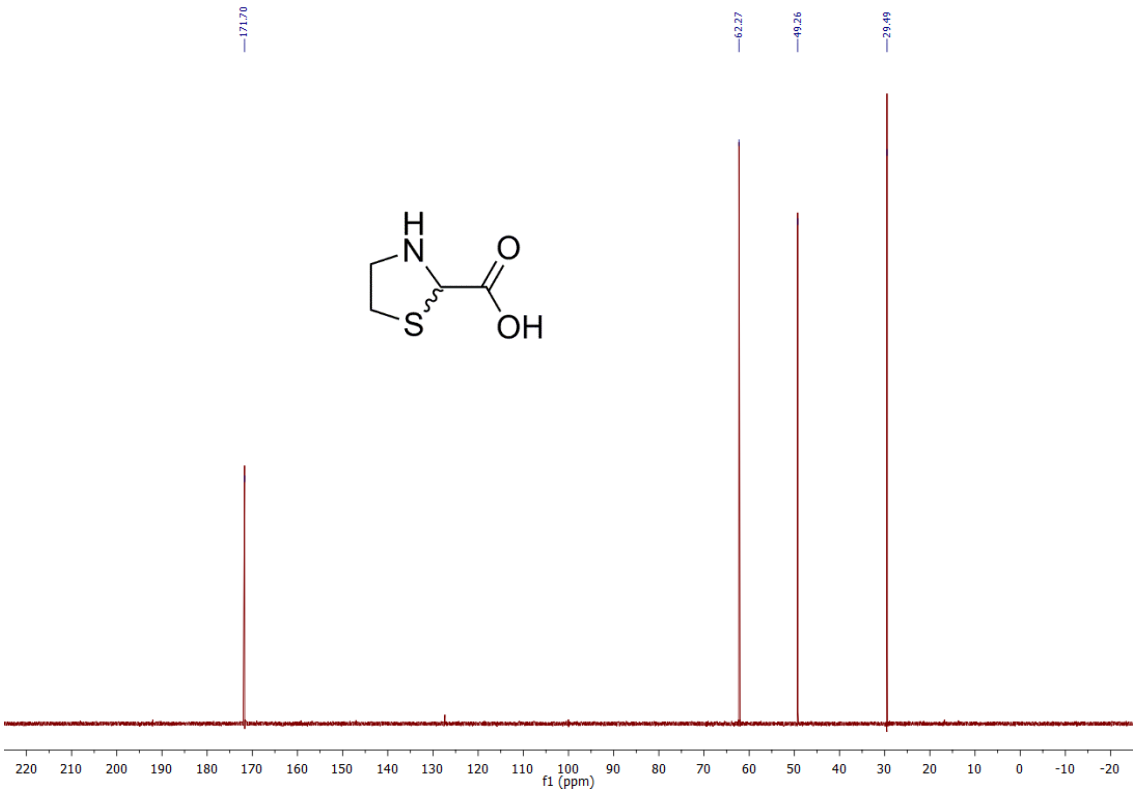
9. NMR Spectra

9.1. Thiazolidine 2-carboxylic acid (racemate) 6

^{1}H NMR (D_2O):

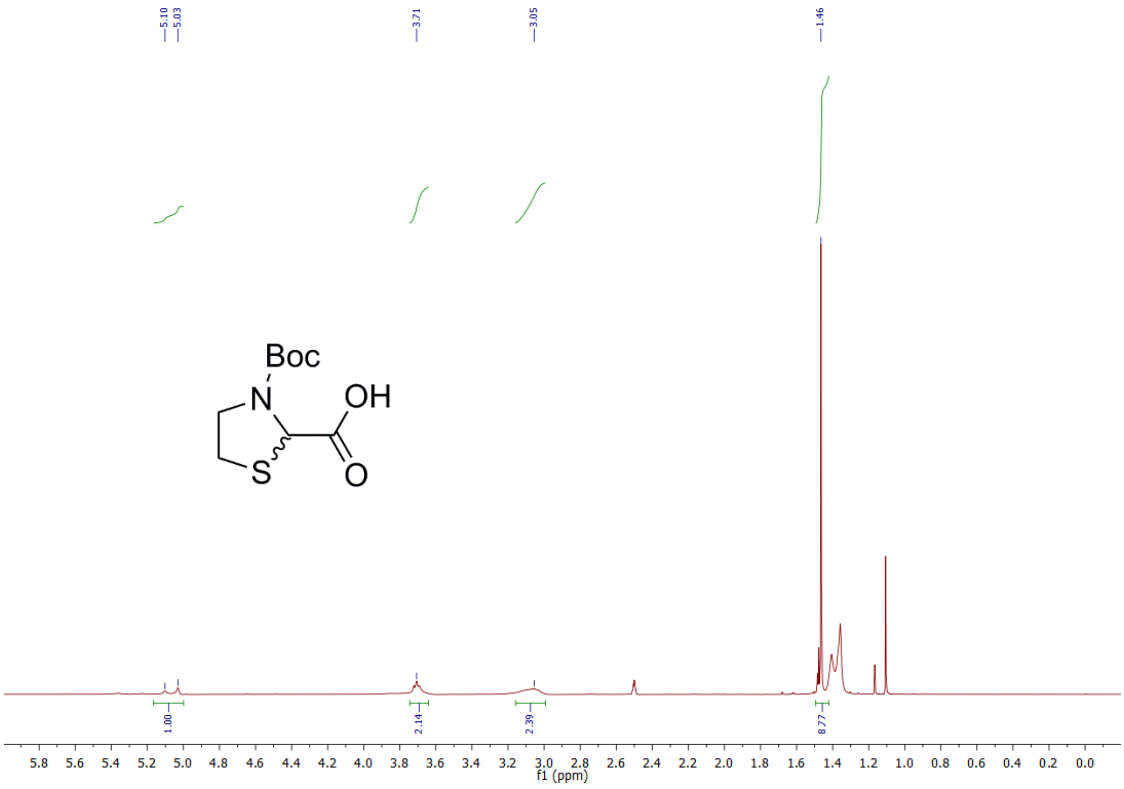


^{13}C (D_2O):

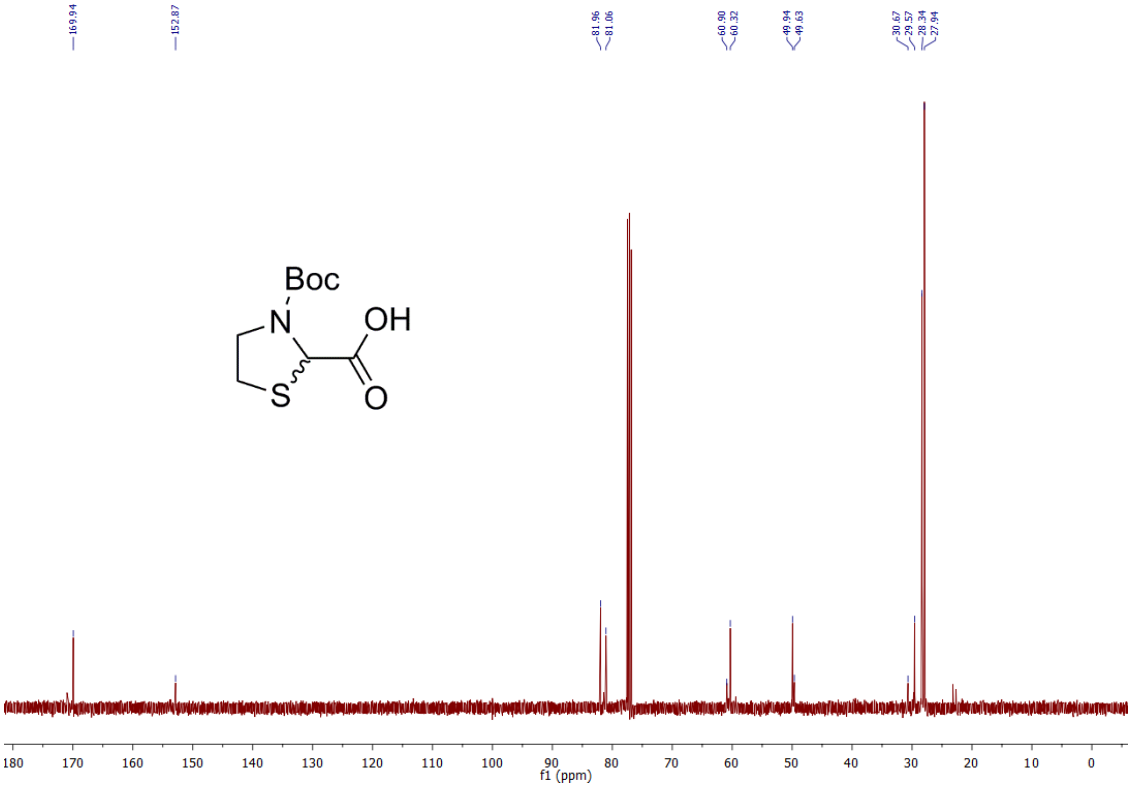


9.2. 3-(*tert*-butoxycarbonyl)thiazolidine-2-carboxylic acid (racemate) 7

^{1}H (DMSO-d_6):

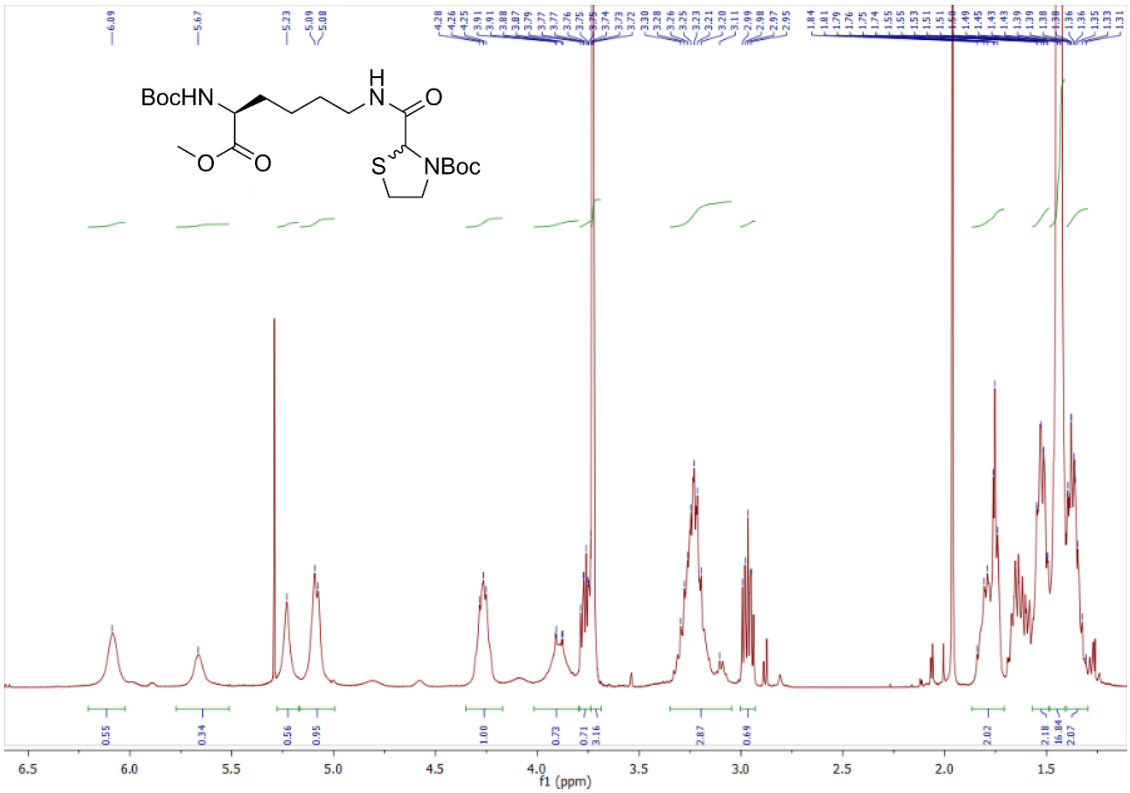


^{13}C ($CDCl_3$):

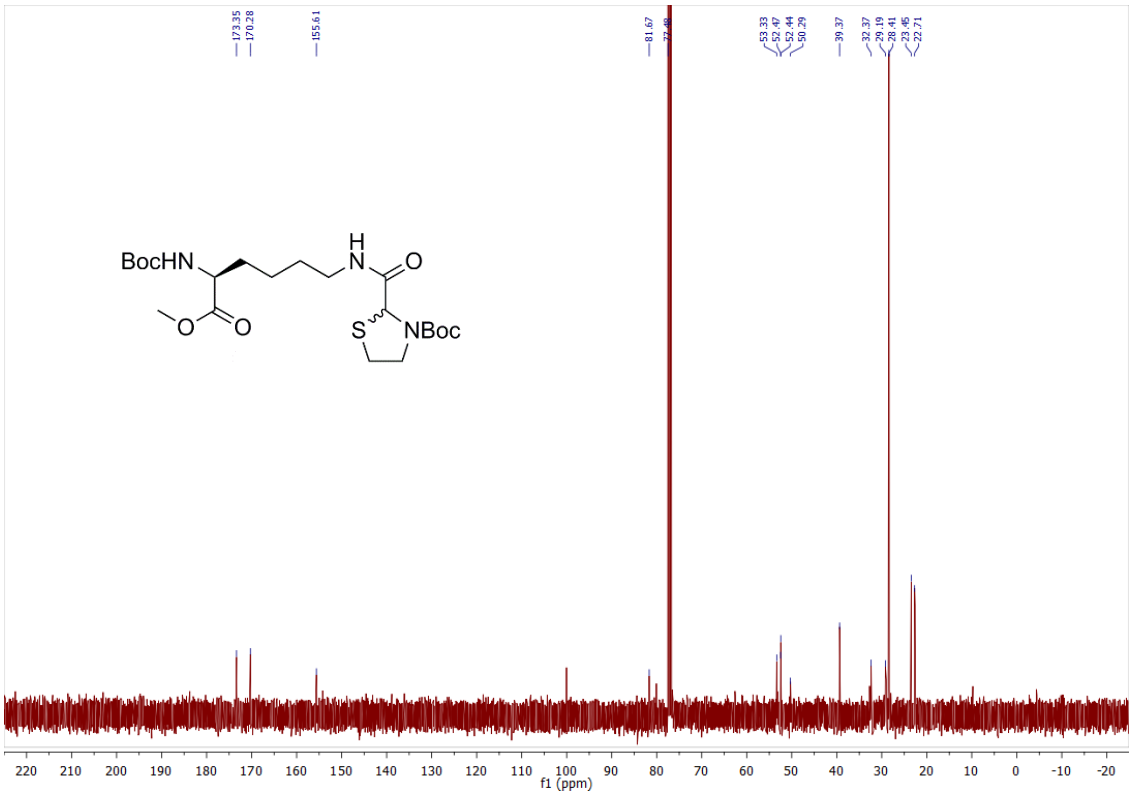


9.3. Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-6-(thiazolidine-2-carboxamido-3-(*tert*-butoxycarbonyl)) hexanoate (mixture of diastereomers) 9

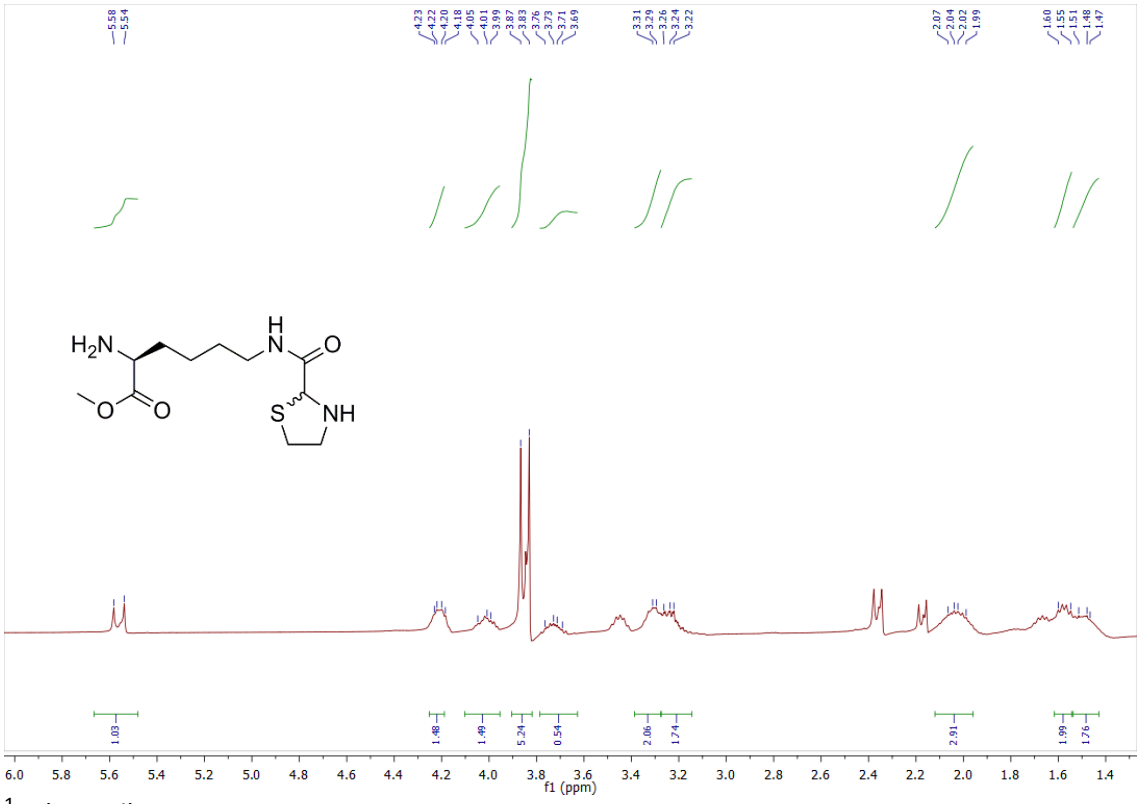
^{1}H ($CDCl_3$):



^{13}C ($CDCl_3$):

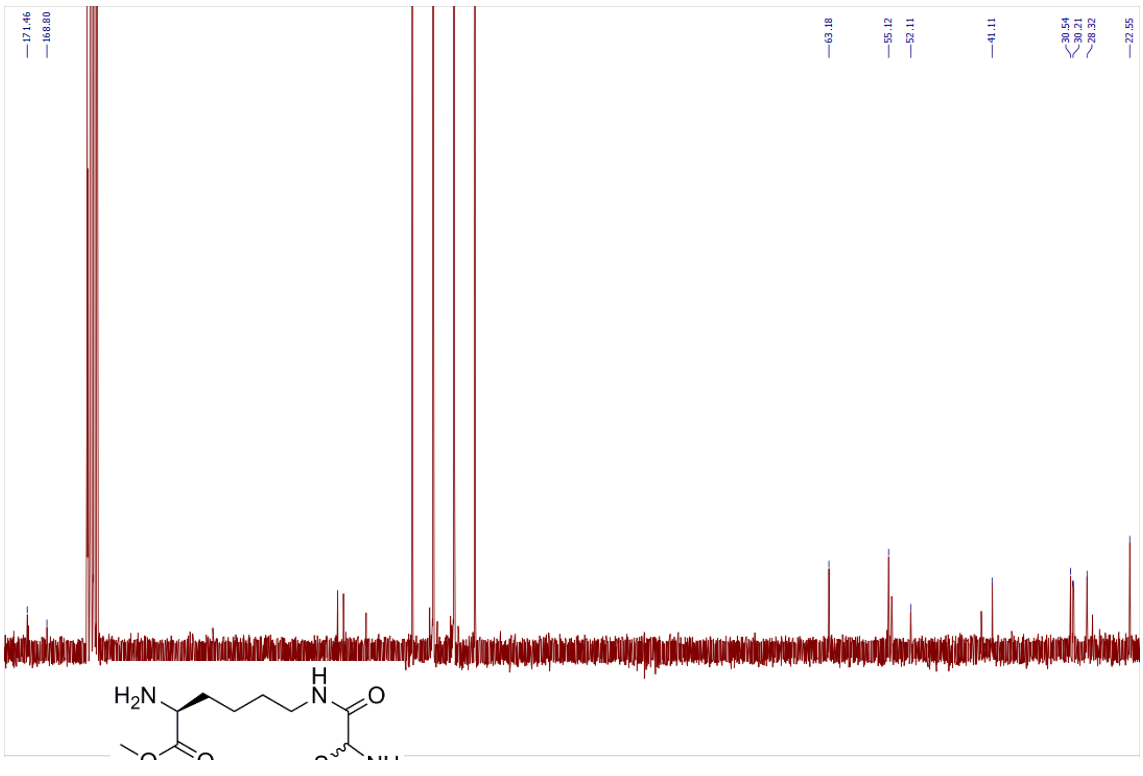


9.4. Methyl 2-amino-6-(thiazolidine-2-carboxamido) hexanoate (mixture of diastereomers) 3



^{1}H (TFA-d):

^{13}C (TFA-d):



10. References

1. Armarego, W. L. F., Perrin, D. D., *Purification of Laboratory Compounds,* Butterworth Heinemann, Oxford, 4th ed., 1996.

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3. X. Bi, K. K. Pasunooti, J. Lescar, C. F. Lui, *Bioconjugate Chem.,* 2017, **28**, 325-329.

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