Chemistry–A European Journal

Supporting Information

Interactive Bioconjugation at *N*-Terminal Cysteines by Using *O*-Salicylaldehyde Esters towards Dual Site-Selective Functionalization

Maria J. S. A. Silva,* Rafaela A. N. Cavadas, Hélio Faustino, Luís F. Veiros, and Pedro M. P. Gois*

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1. General Remarks

NMR spectra were recorded in a Bruker Fourier 300 and 400 using $CDCl_3$, D_2O or $(CD_3)_2SO$ as deuterated solvents. All coupling constants are expressed in Hz and chemical shifts (δ) in ppm. Multiplicities are given as: s (singlet), d (doublet), dd (double doublet), dt (double triplet), t (triplet), td (triple triplet), tt (triple triplet), q (quartet), quint (quintuplet) and m (multiplet). IR spectra were recorded with a Bruker ALPHA II FT-IR spectrometer .UV spectra were traced in Thermo Scientific Evolution 201 UV-visible spectrophotometer. Fluorescence measurements were taken on a SHIMADZU spectrofluorophotometer RF-6000 instrument. Low Resolution Mass spectra were recorded in LCQ Fleet Ion Trap Mass Spectrometer, Thermo Fisher Scientific, Germany. High Resolution Mass spectra were recorded in a Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Scientific™ Q Exactive™ Plus). The exactive plus mass spectrometer was operated in positive ion mode with alternating MS scans of the precursor ions and AIF (all ion fragmentation) scans in which the conjugates were fragmented by SID (Surface-Induced Dissociation) with 50 eV. Both scan types were performed with 70,000 resolution (at *m/z* 200) with each scan taking 0.05 s, the maximal fill time was set to 0.2 s and target value was 3 x 10^6 ions.

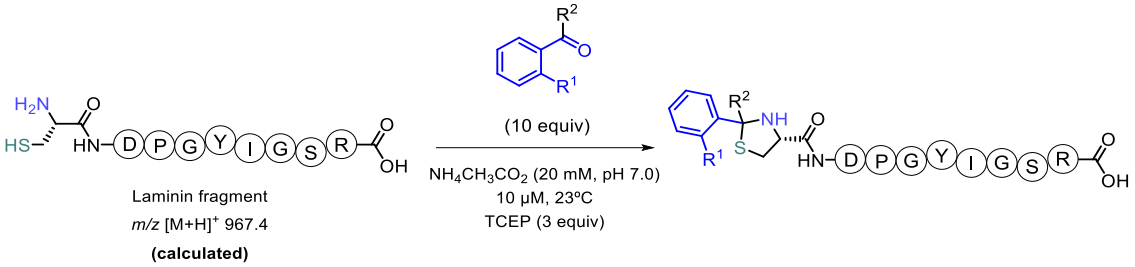
The Liquid chromatography–mass spectrometry (LC-MS) runs were realized using a Dionex Ultimate 3000 UHPLC+ system equipped with a Multiple-Wavelength detector, an imChem Surf C18 TriF 100Å 3 µm 100x2.1mm column connected to Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Scientific™ Q Exactive™ Plus). The HPLC runs were carried out with a gradient of A (Milli Q water containing 0.1% v/v FA, TCI LC-MS grade) and B (acetonitrile containing 0.1% v/v FA). Method A: mobile phase was t = 0 min, 1% B; t = 10 min, 75%; t= 10.1 min, 95% B; t = 12 min, 95% B; t = 12.5 min, 1%, t = 15 min, 1% stop, at a flow rate of 0.3 mL/min. Method B: mobile phase was t = 0 min, 10% B; t = 5 min, 10%; t= 15 min, 95% B; t = 17 min, 95% B; t = 18 min, 10%, t = 20 min, 10% stop, at a flow rate of 0.2 mL/min.

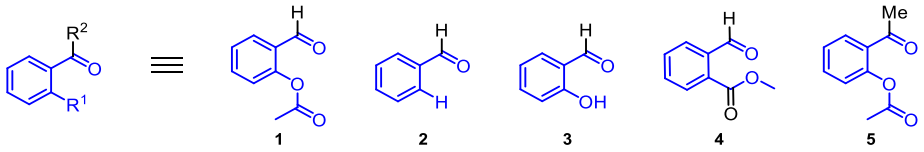
Reaction mixtures were analysed by thin layer chromatography using Merck silica gel $60F_{254}$ aluminium plates and visualized by UV light. Column chromatography was performed with silica gel Geduran® Si 60 (0.040-0.063 mm) purchased from Merck.

All solvents were of analytical reagent grade and were purchased from Merck, Fluorochem, Alfa Aesar, TCI, Carlo Erba or Sigma-Aldrich. Salicylaldehyde was purchased from Aldrich. 2-formylphenyl acetate, 2-formylphenyl isobutyrate,[1] 2-formylphenyl pivalate,[2] 2-formylphenyl benzoate,[3] (*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid[4] are known compounds, the spectral data is according to the reported and were prepared from the corresponding acyl chlorides following a reported procedure.[2] 7-hydroxy-8-formyl-coumarin,[5] and methyl 2-formylbenzoate,[6] are known compounds and were prepared according to their reported procedures. Acetic acid was purchase from Carlo Erba. TCEP and *N,N*-dimethylpyridin-4-amine (DMAP) were purchased from Aldrich. *C*-Ovalbumin, Laminin fragment, Calcitonin, Cys-Bombesin, Cys-Cys-Bombesin and Cys-Ala-OMe peptides were purchased from GeneCust.

2. Assays with Laminin fragment

2.1 General procedure for the ESI-MS assays with Laminin fragment





To a solution of Laminin Fragment (1.0 mg/mL in water, 0.925 mM) (5.41 µL, 5.00 nmol) in ammonium acetate solution 20 mM, pH 7.0 (500 µL) was added a tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (1.0 mg/mL in water, 3.5 mM) (4.29 µL, 15.0 nmol) and the solution mixed for 1-2h at 23 ºC. Then, the aldehyde or ketone **1**-**5** (10.0 mM in ACN) (5 µL, 50.0 nmol) was added. After 1h the reaction was monitored in Positive Mode of ESI-MS.

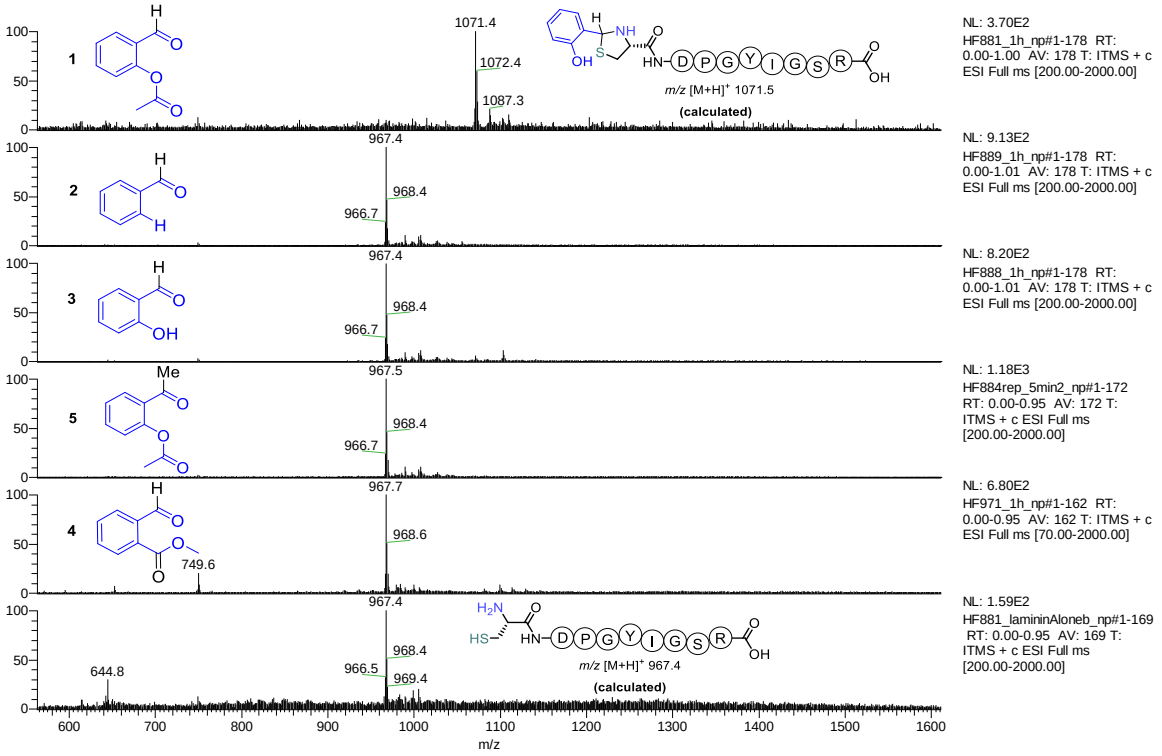


Figure S 1 – ESI+-MS spectra of laminin reaction with aldehydes or ketone after 1h. (**A**) Laminin + TCEP; (**B**) Laminin + TCEP + methyl 2-formylbenzoate **4**; (**C**) Laminin + TCEP + 2-acetylphenyl acetate **5**; (**D**) Laminin + TCEP + salicylaldehyde **3**; (**E**) Laminin + TCEP + benzaldehyde **2**; (**F**) Laminin + TCEP + O-Salicylaldehyde methylester **1**. Only reaction with *O*-Salicylaldehyde methylester **1** show appreciable conversion.

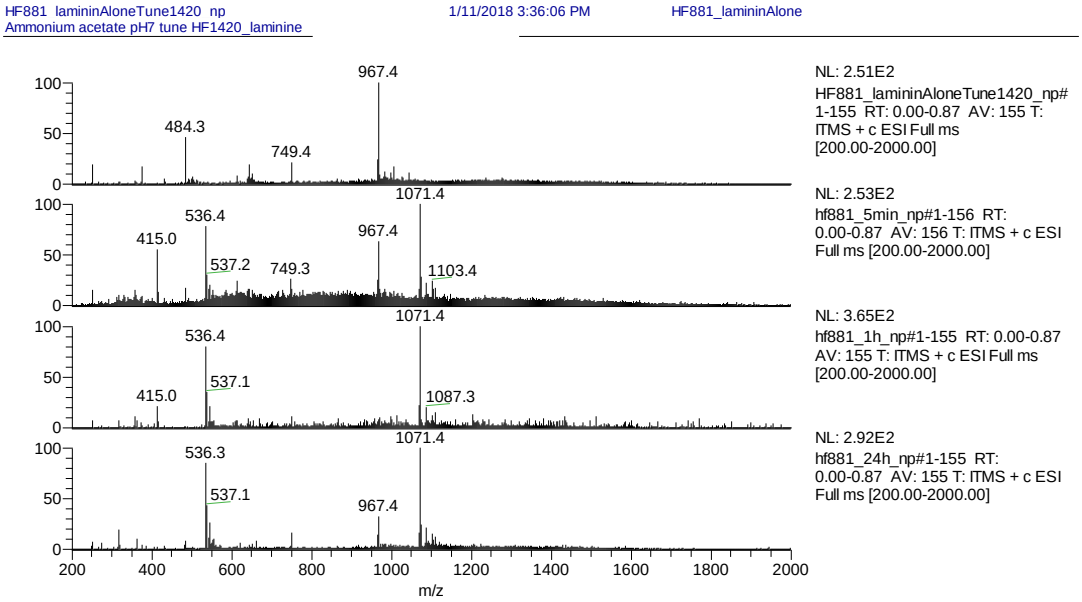


Figure S 2 – ESI+-MS spectra of Laminin fragment reaction progress with 2-formylphenyl acetate **1** in ammonium acetate 20 mM, pH 7.0. (**A**) Laminin + TCEP (3 equiv), 1 h; (**B**) reaction mixture 5 min after addition of 2-formylphenyl acetate **1** (10 equiv); (**C**) reaction after 1 h; (**D**) reaction after 24 h, showing the low intense peak of reverted *N*-terminal Cys protection into the starting Laminin peptide.

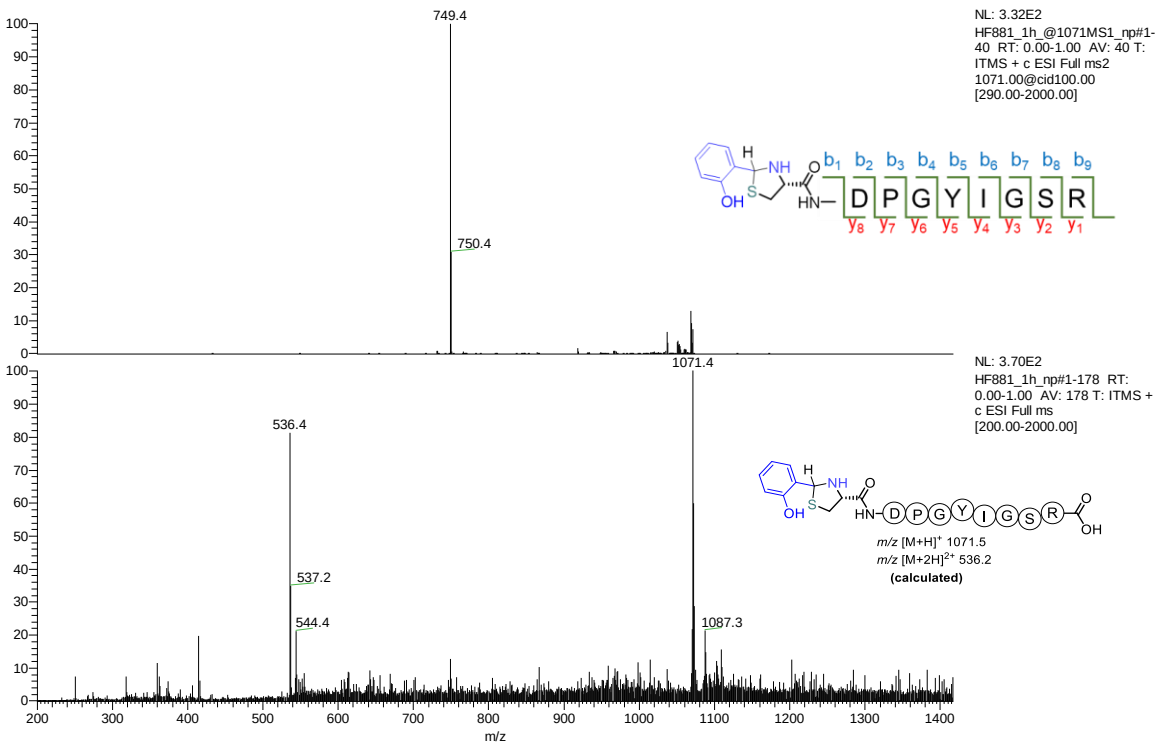


Figure S 3 – ESI$^+$-MS spectrum of conjugate obtained in the reaction of laminin peptide with 2-formylphenyl acetate **1** (below); MS fragmentation spectrum of *m/z* $[M+H]^+$ 1071.4 (above). MS fragmentation of the peaks correspondent to the expected product m/z resulted in the same profile to afford a daughter peak of *m/z* 749.4. This fragment confirm that the laminin modification occurred in the *N*-terminus of the peptide.

2.2 General procedure for the aldehyne or ketone 1-5 screening with Laminin fragment by LC-MS

To a solution of Laminin Fragment (1.0 mg/mL in water, 0.925 mM) (5.00 µL, 4.60 nmol) in ammonium acetate solution 20 mM, pH 7.0 (200 µL) was added a tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (1.0 mg/mL in water, 3.5 mM) (3.96 µL, 14.0 nmol) and the solution mixed for 1 h at 25 °C. Then, the aldehyde or ketone **1**-**5** (10.0 mM in ACN) (5 µL, 50.0 nmol) was added. After 1 h the reaction was monitored by LC-HRMS using HPLC method A.

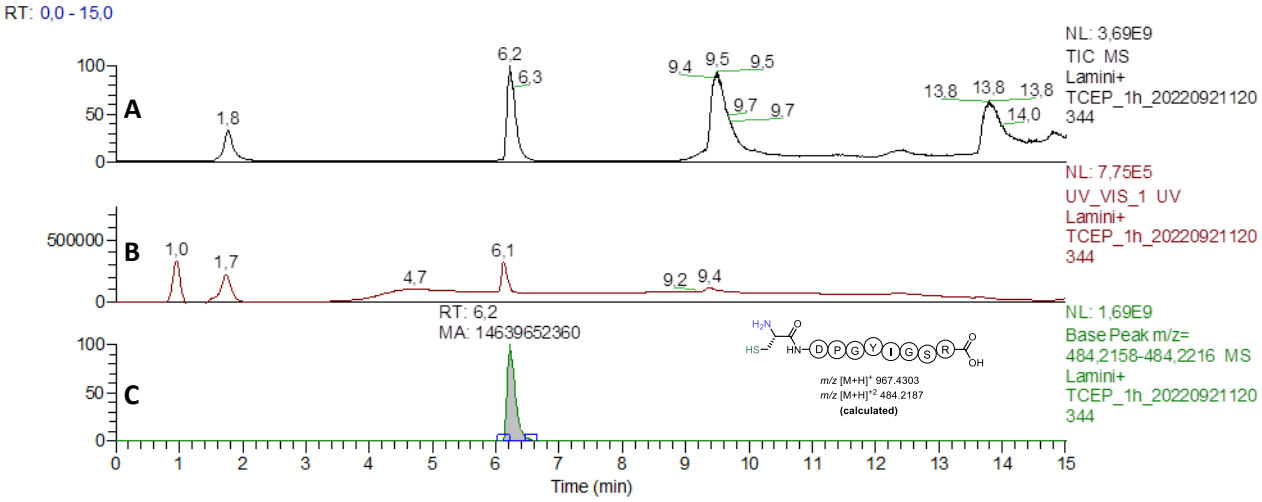


Figure S 4 – Reduced Laminin peptide with TCEP (3 equiv) after 60 min. (**A**) Total ion current (TIC) chromatogram; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Laminin peptide (base peak *m/z* 484.2187, within δ 6 ppm range).

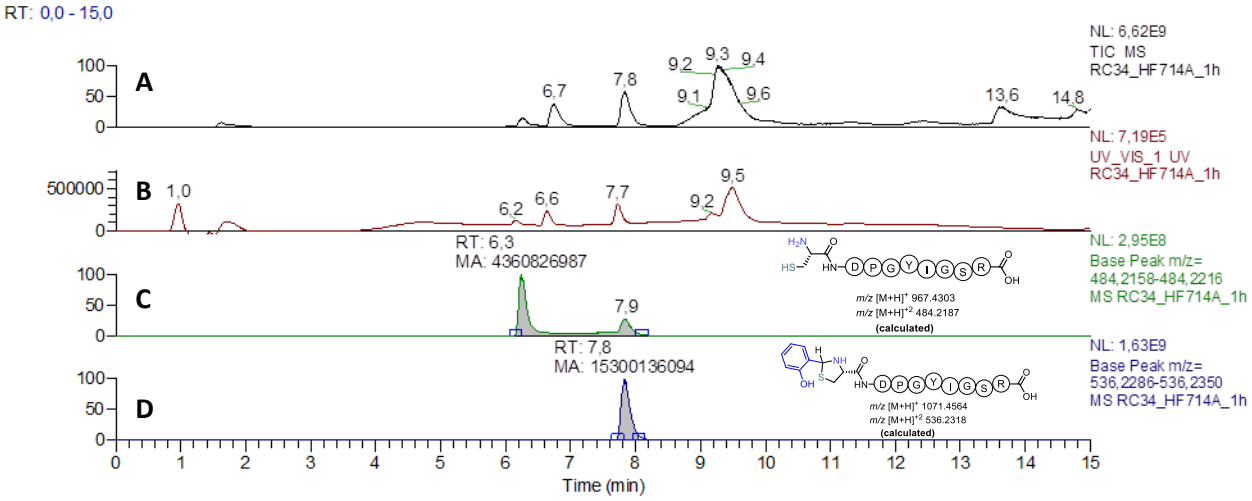


Figure S 5 – Reaction between Laminin peptide and 2-formylphenyl acetate **1** after 60 min at 25 µM. (**A**) Total ion current (TIC) chromatogram; *****RT 6.2 and 7.9 min reduced Laminin; RT 6.6 min TCEP adduct with 2-formylphenyl acetate **1**; RT 7.8 min Phenolic thiazolidine **6**; RT 9.3 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Laminin peptide (base peak *m/z* 484.2187, within δ 6 ppm range), giving a conversion of 71% of starting Laminin into the conjugate **6**; (**D**) EIC chromatogram of modified Laminin peptide (base peak *m/z* 536.2318, within δ 6 ppm range).

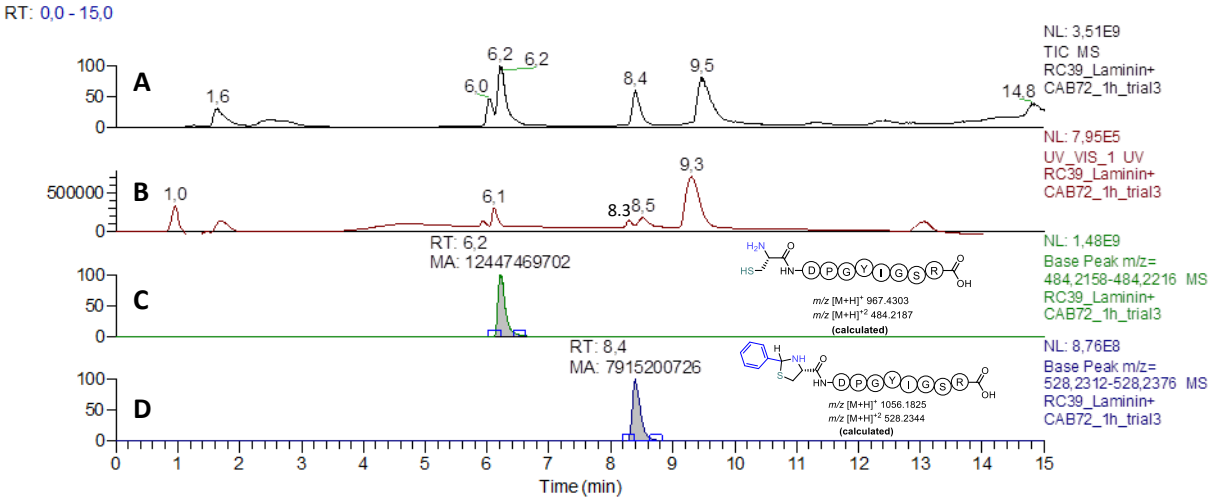


Figure S 6 – Reaction between Laminin peptide and benzaldehyde **2** after 60 min at 25 µM. (**A**) Total ion current (TIC) chromatogram; *****RT 6.0 min oxiized Laminin; RT 6.2 reduced Laminin; RT 8.4 min thiazolidine; RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Laminin peptide (base peak *m/z* 484.2187, within δ 6 ppm range), giving a conversion of 15% of starting Laminin into the conjugate **6**; (**D**) EIC chromatogram of modified Laminin peptide (base peak *m/z* 528.2344,within δ 6 ppm range).

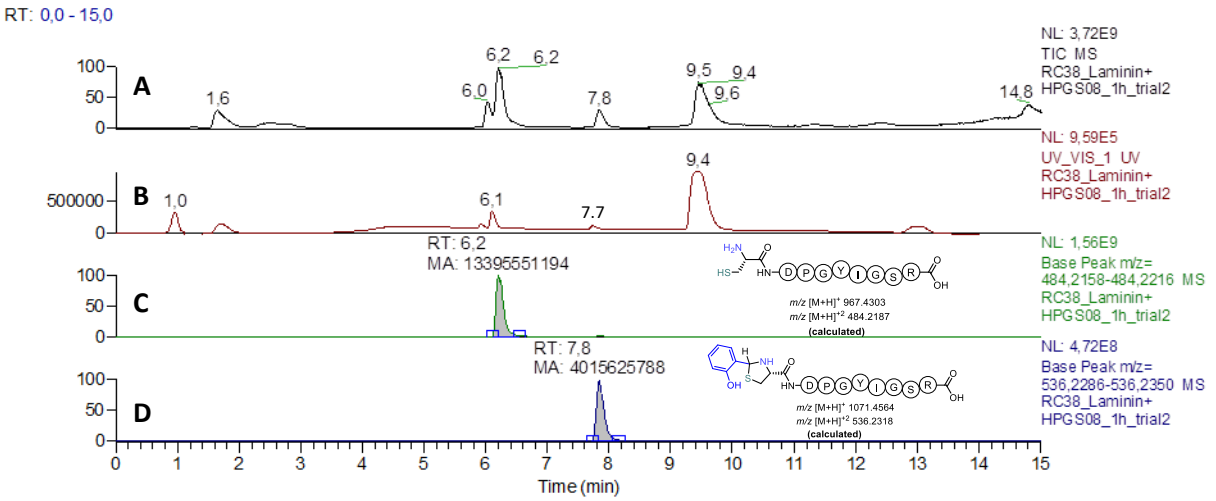


Figure S 7 – Reaction between Laminin peptide and salicylaldehyde **3** after 60 min at 25 µM. (**A**) Total ion current (TIC) chromatogram; *****RT 6.0 min oxiized Laminin; RT 6.2 min reduced Laminin; RT 7.8 min Phenolic thiazolidine **6**; RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Laminin peptide (base peak *m/z* 484.2187, within δ 6 ppm range), giving a conversion of 8% of starting Laminin into the conjugate **6**; (**D**) EIC chromatogram of modified Laminin peptide (base peak *m/z* 536.2318, within δ 6 ppm range).

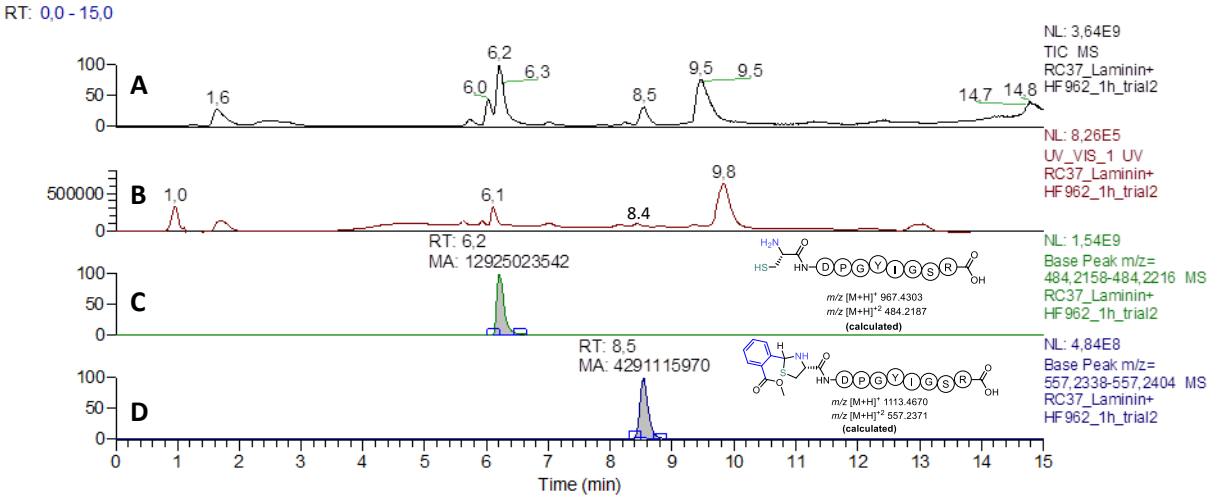


Figure S 8 – Reaction between Laminin peptide and methyl 2-formylbenzoate **4** after 60 min at 25 µM. (**A**) Total ion current (TIC) chromatogram; *RT 6.0 min oxiized Laminin; RT 6.2 reduced Laminin; RT 8.5 min thiazolidine; RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Laminin peptide (base peak *m/z* 484.2187, within δ 6 ppm range), giving a conversion of 12% of starting Laminin into the conjugate **6**; (**D**) EIC chromatogram of modified Laminin peptide (base peak *m/z* 557.2371, within δ 6 ppm range).

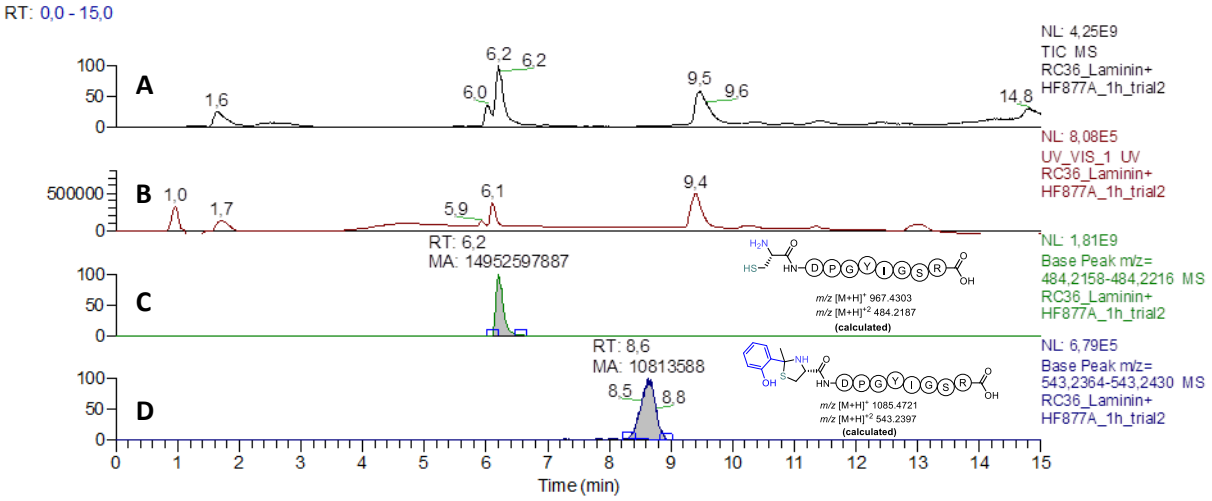


Figure S 9 – Reaction between Laminin peptide and 2-acetylphenyl acetate **5** after 60 min at 25 µM. (**A**) Total ion current (TIC) chromatogram; *RT 6.0 min oxized Laminin; RT 6.2 reduced Laminin; RT 8.5 min phenolic thiazolidine; RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Laminin peptide (base peak *m/z* 484.2187, within δ 6 ppm range), giving negligible conversion of starting Laminin into the conjugate **6**; (**D**) EIC chromatogram of modified Laminin peptide (base peak *m/z* 543.2397, within δ 6 ppm range).

3. Mechanistical studies of the reaction

3.1 DFT

Computational Details

All calculations were performed using the Gaussian 09 software package,[7] and the M06-2X functional, without symmetry constraints. That is a hybrid meta-GGA functional developed by Truhlar and Zhao,[8] and it was shown to perform very well for main-group kinetics, providing a good description of long range effects such as van der Waals interactions or π-π stacking.[9] The optimized geometries were obtained with a standard 6-31+G(d,p) basis set.[10] Transition state optimizations were performed with the Synchronous Transit-Guided Quasi-Newton Method (STQN) developed by Schlegel *et al.*,[11] after a thorough search of the Potential Energy Surfaces (PES). Frequency calculations were performed to confirm the nature of the stationary points, yielding one imaginary frequency for the transition states and none for the minima. Each transition state was further confirmed by following its vibrational mode downhill on both sides, and obtaining the minima presented on the energy profiles. The electronic energies obtained at that level (Eb1) were converted to free energy at 298.15 K and 1 atm (Gb1) by using zero point energy and thermal energy corrections based on structural and vibration frequency data calculated at the same level. Solvent effects (water) were accounted for in all calculations by means of the Polarisable Continuum Model (PCM) initially devised by Tomasi and coworkers[12] with radii and non-electrostatic terms of the SMD solvation model, developed by Truhlar et al.[13]

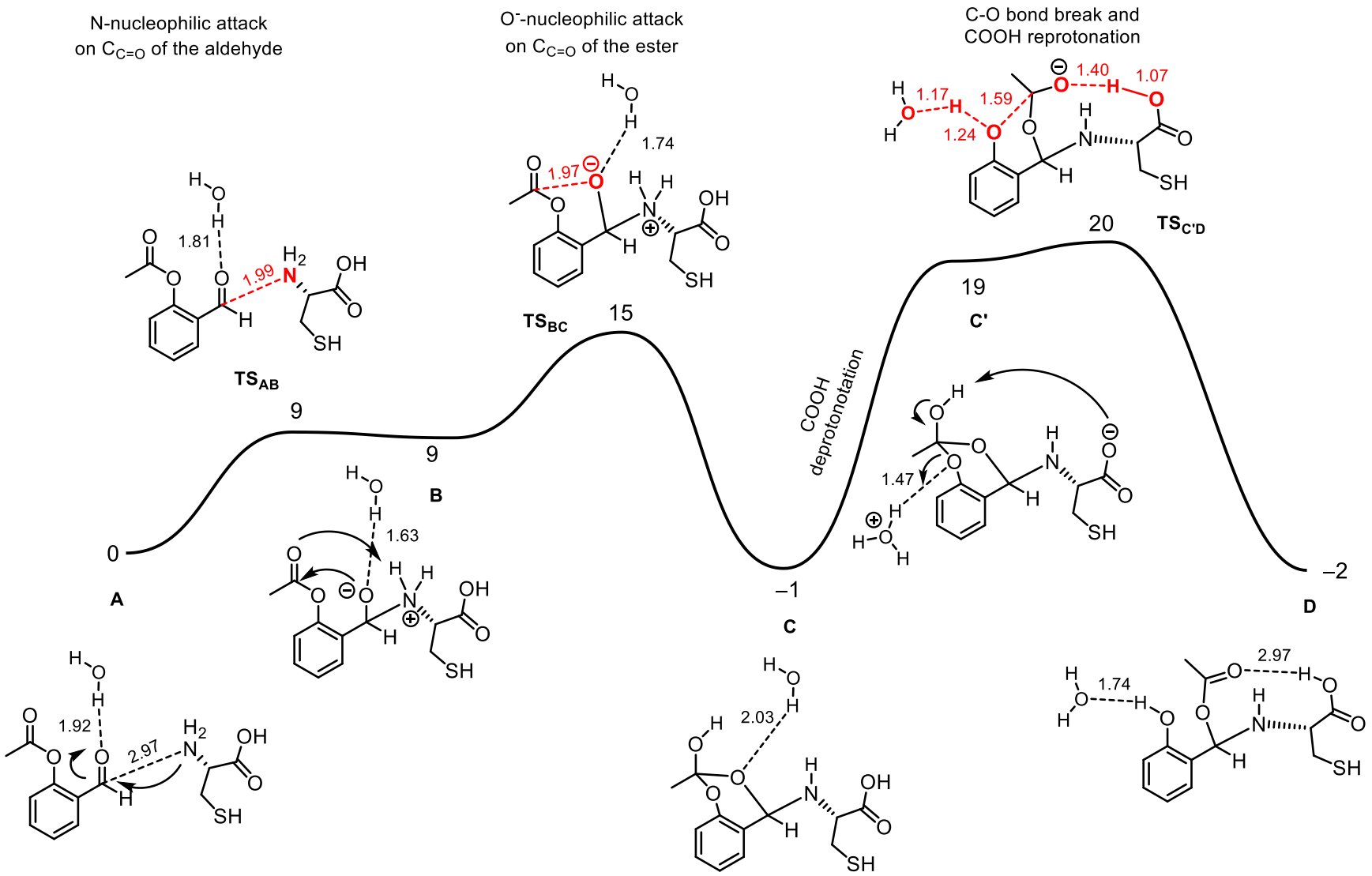


Figure S 10 – Free energy profile calculated for the formation of thiazolidine from Cys and 2-formylphenyl acetate (first part). Free energy values (kcal/mol) relative to the initial reactants (**A**) and distances in Å.

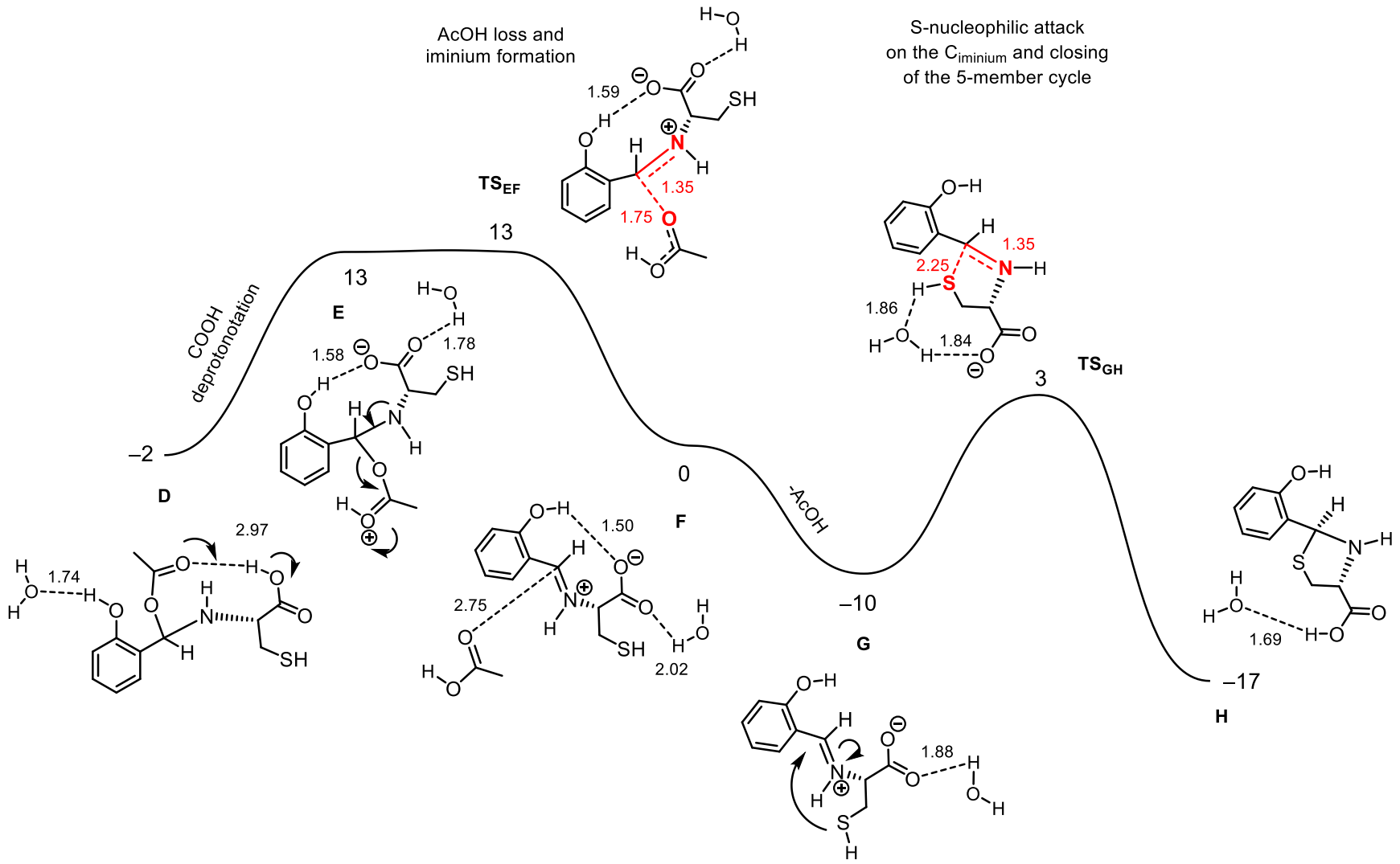


Figure S 11 – Free energy profile calculated for the formation of thiazolidine from Cys and 2-formylphenyl acetate (second part). Free energy values (kcal/mol) relative to the initial reactants (**A**) and distances in Å.

3.2 NMR

*Experiment with ethanethiol **8***

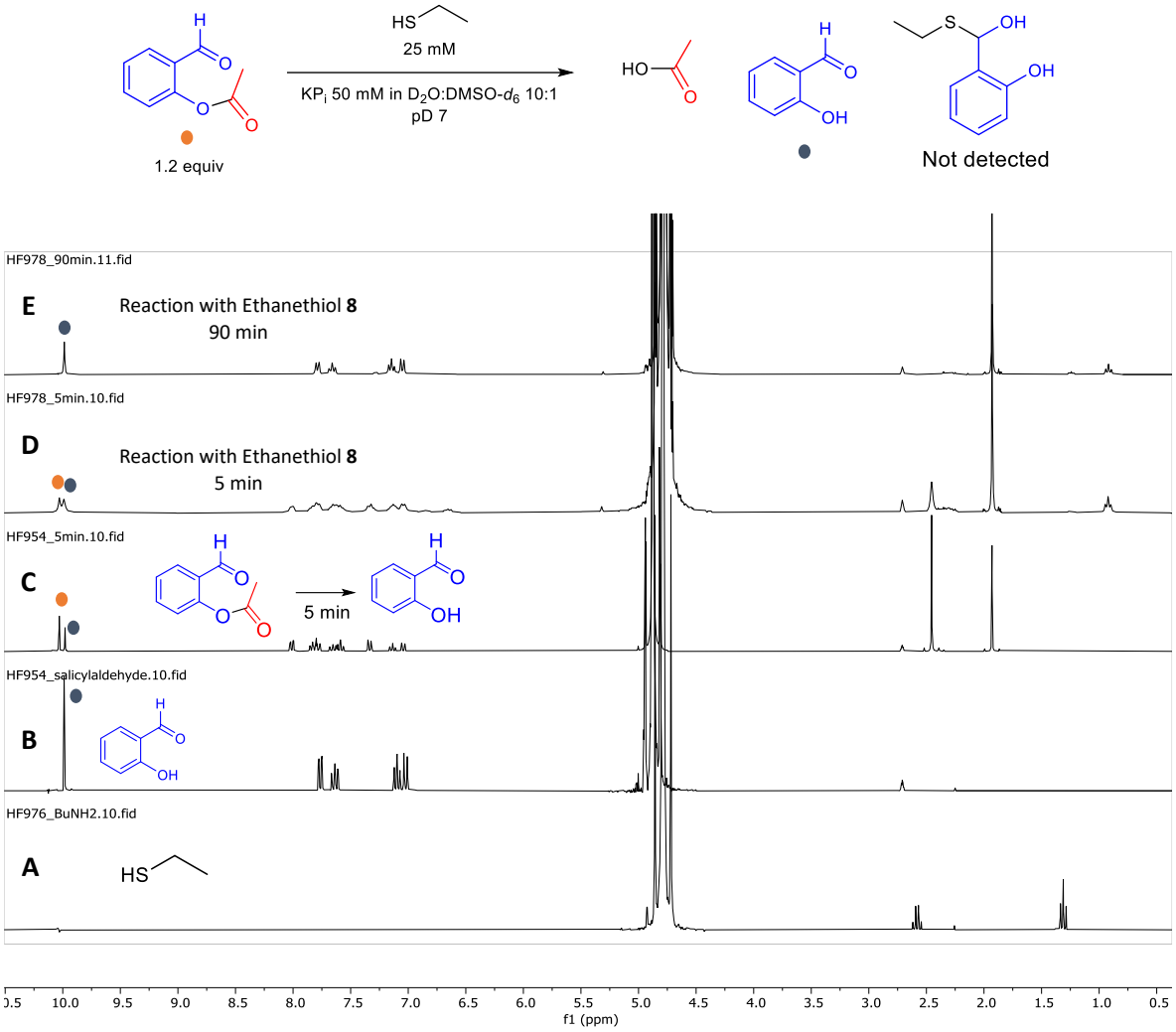
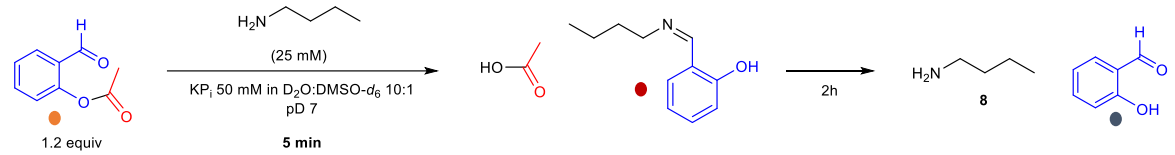


Figure S 12 – ^{1}H NMR spectra in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1: (**A**) Ethanethiol 8; (**B**) Salycilaldehyde; (**C**) 2-formylphenyl acetate **1** after 5 min in solution; (**D**) reaction of 2-formylphenyl acetate with ethanothiol after 5 min. (**E**) reaction of 2-formylphenyl acetate with ethanethiol after 90 min.

Experiment with n-butylamine



To a solution of *n*-butylamine **9** (1.4 µL, 0.014 mmol) in KP_i 50 mM in D_2O, pH 7.4 (0.5 mL) was added 2-formylphenyl acetate **1** (2.8 mg, 0.017 mmol) in DMSO-d_6 (0.05 mL). The reaction mixture immediately gained a yellow color that gradually disappeared (over 2h). The reaction was monitored by ^{1}H NMR.

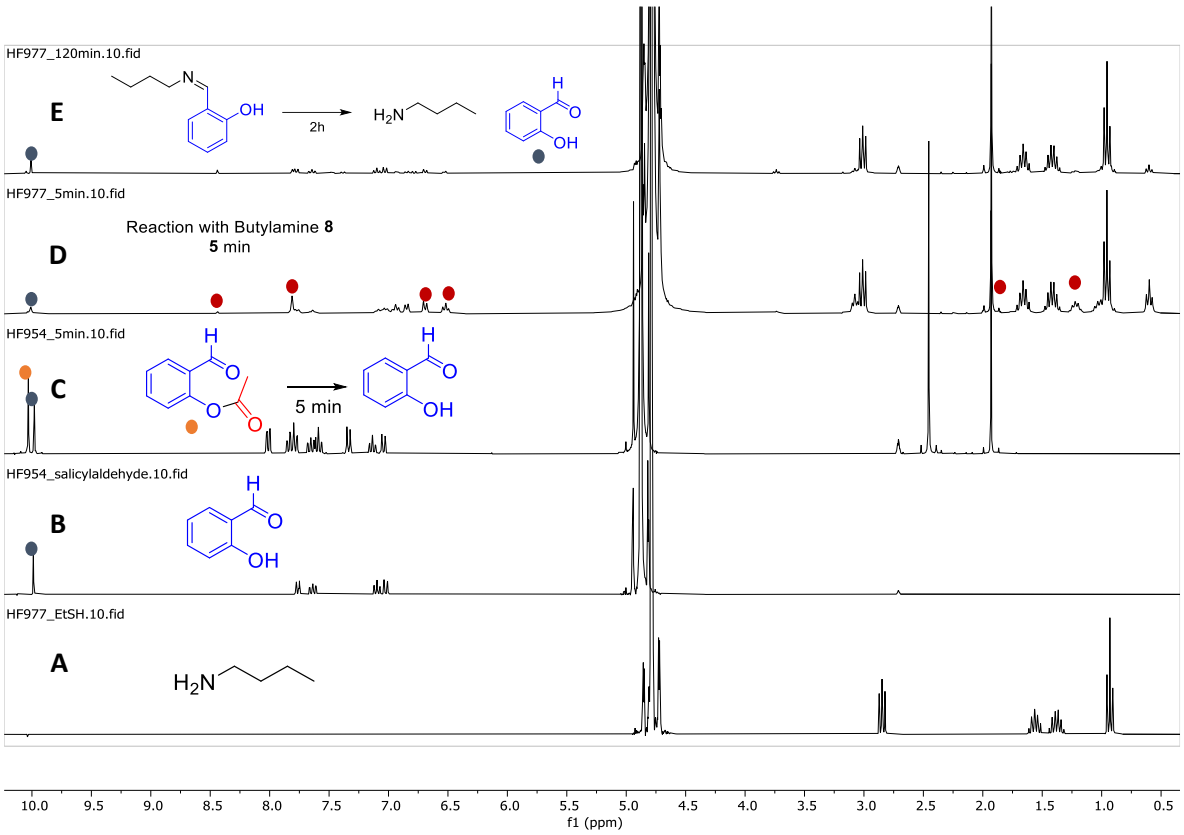
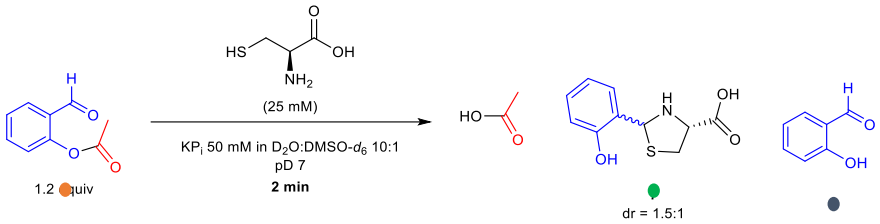


Figure S 13 – ^{1}H NMR spectra in KP_i 50 mM (pD 7)in D_2O:DMSO-d_6 10:1: (**A**) Butylamine **9**; (**B**) Salycilaldehyde; (**C**) 2-formylphenyl acetate **1** after 5 min in solution; (**D**) reaction of 2-formylphenyl acetate with butylamine after 5 min. (**E**) reaction of 2-formylphenyl acetate with butylamine after 2 h.

*Experiment with Cysteine **10***



To a solution of *L*-cysteine **10** (1.7 mg, 0.014 mmol) in KP_i 50 mM in D_2O, pD 7 (0.50 mL) was added 2-formylphenyl acetate (2.7 mg, 0.017 mmol) in DMSO-d_6 (0.05 mL). The reaction was monitored by ^{1}H-NMR.

After 2 min complete conversion was obtained. The expected thiazolidine was formed wth a dr = 1.5:1. The product is compared to an refence sample of (*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid **7** with a diastereoisomeric ratio of 2.2:1 obtained according a known procedure.[4]

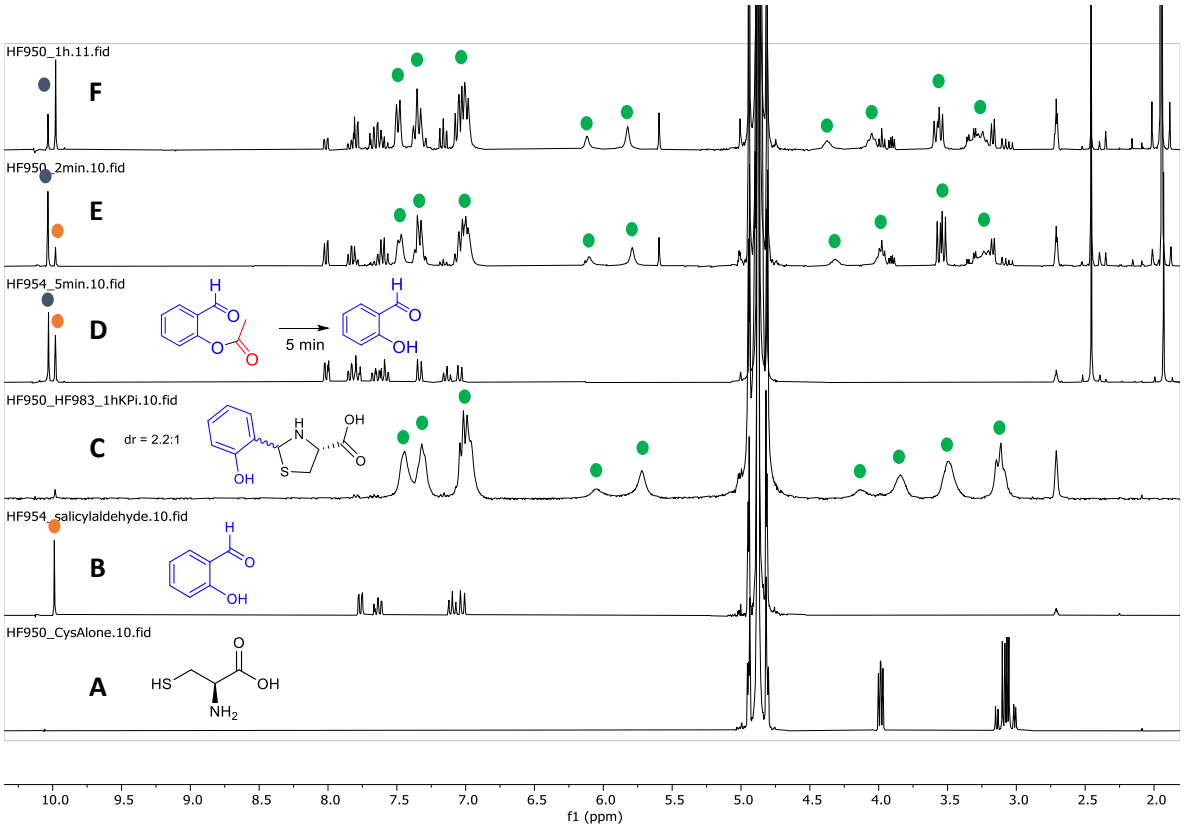
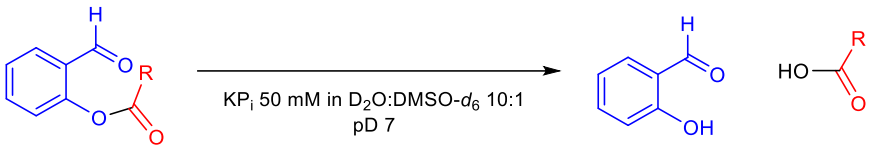


Figure S 14 – ^{1}H NMR spectra in KP_i 50 mM (pD 7)in D_2O:DMSO-d_6 10:1: (**A**) Cysteine **10**; (**B**) Salycilaldehyde; (**C**) (*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid **7** (d.r. = 2.2:1); (**D**) 2-formylphenyl acetate **1** after 5 min in solution; (**E**) reaction of 2-formylphenyl acetate with cysteine after 5 min. (**F**) reaction of 2-formylphenyl acetate with cysteine after 60 min.

4. Ester stability

4.1 General procedure the measurement of the ester stability



R	$t_{1/2}$ (min)
Me	15
*t*Bu*	58
Ph*	290
*i*Pr	53

* partially insoluble

To a solution of KP_i 50 mM in D_2O, pD 7 (0.50 mL) was added the ester (0.017 mmol) in DMSO-d_6 (0.05 mL). The hydrolysis of the ester to salicylaldehyde was monitored by ^{1}H-NMR. The conversion was calculated based on the peaks of the aldehyde. The ester by plotting the conversion of 2-formylphenyl acetate of the ester *vs* time and adjust to a exponential equation to obtain k_{obs}, then $t_{1/2}$ was calculated according to the formula $t_{1/2} = \ln(2) / k_{obs}$.

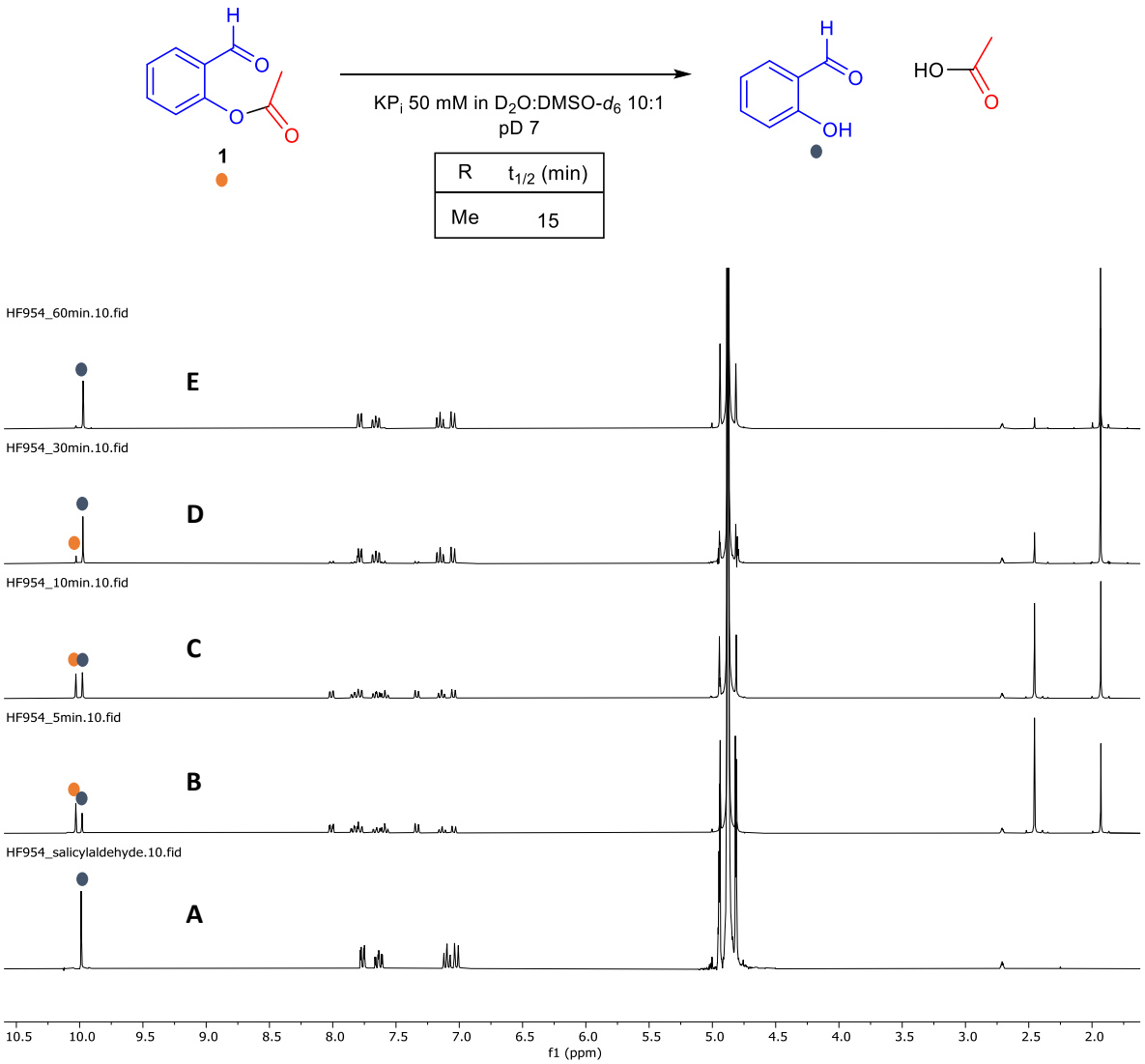


Figure S 15 – ^{1}H NMR spectra in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1: (**A**) Salycilaldehyde; (**B**) Hydrolysis of 2-formylphenyl acetate **1** at 5 min; (**C**) Hydrolysis of 2-formylphenyl acetate at 10 min; (**D**) Hydrolysis of 2-formylphenyl acetate at 30 min; (**E**) Hydrolysis of 2-formylphenyl acetate 30 min.

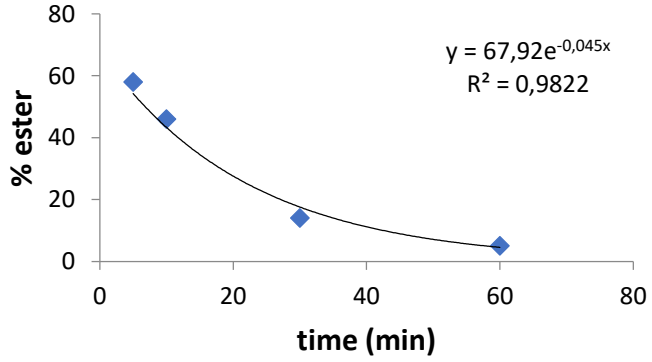


Figure S 16 – Hydrolysis kinetics of 2-formylphenyl acetate **1** over time in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1.

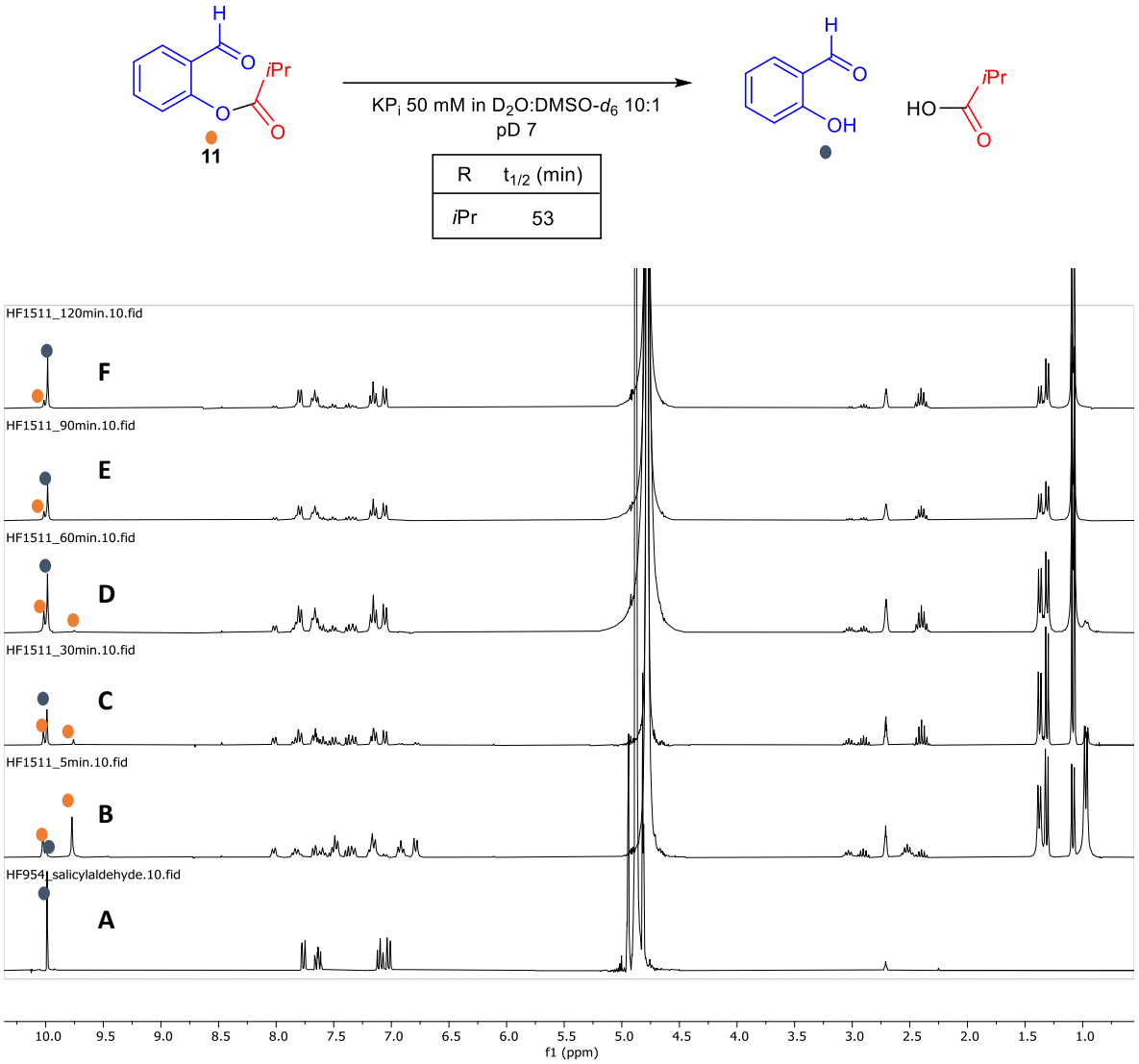


Figure S 17 – ^{1}H NMR spectra in KP$_i$ 50 mM (pD 7) in D$_2$O:DMSO-d_6 10:1: (**A**) Salycilaldehyde; (**B**) Hydrolysis of 2-formylphenyl isobutyrate **11** 5 min; (**C**) Hydrolysis of 2-formylphenyl isobutyrate at 30 min; (**D**) Hydrolysis of 2-formylphenyl isobutyrate at 60 min; (**E**) Hydrolysis of 2-formylphenyl isobutyrate at 90 min; (**F**) Hydrolysis of 2-formylphenyl isobutyrate at 120 min.

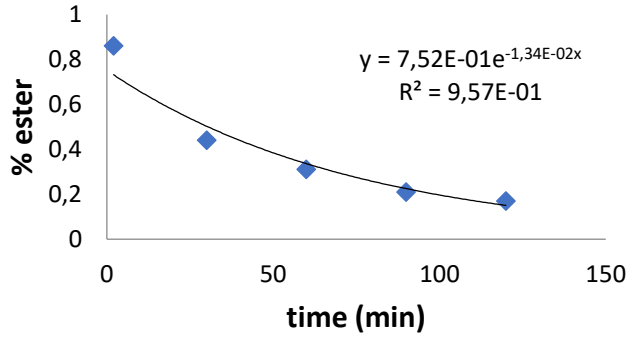


Figure S 18 – Hydrolysis kinetics of 2-formylphenyl isobutyrate **11** over time in KP$_i$ 50 mM (pD 7) in D$_2$O:DMSO-d_6 10:1.

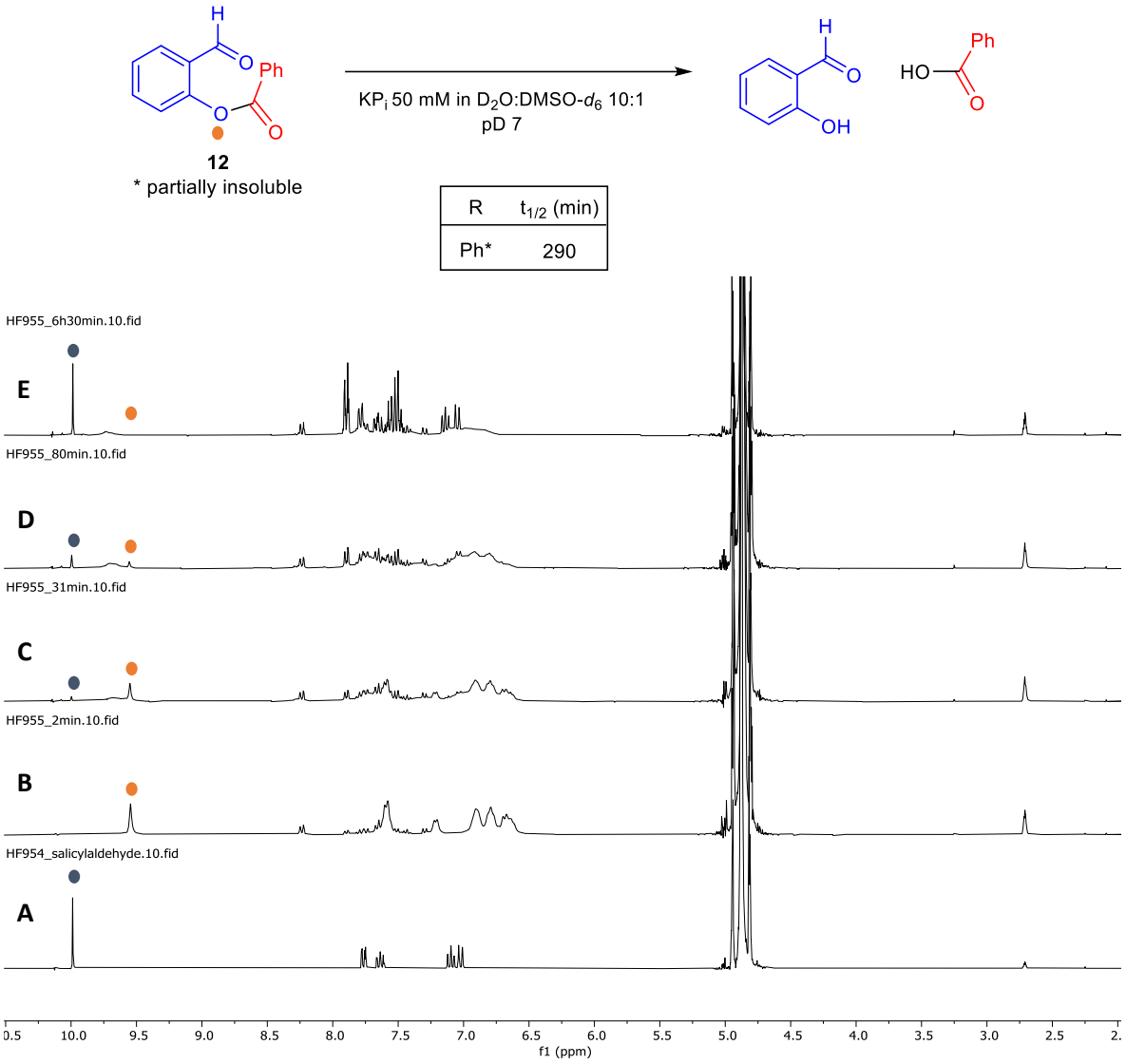


Figure S 19 – ^{1}H NMR spectra in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1: (**A**) Salycilaldehyde; (**B**) Hydrolysis of 2-formylphenyl benzoate **12** at 2 min; (**C**) Hydrolysis of 2-formylphenyl benzoate at 31 min; (**D**) Hydrolysis of 2-formylphenyl benzoate at 60 min; (**E**) Hydrolysis of 2-formylphenyl benzoate at 6.5 h.

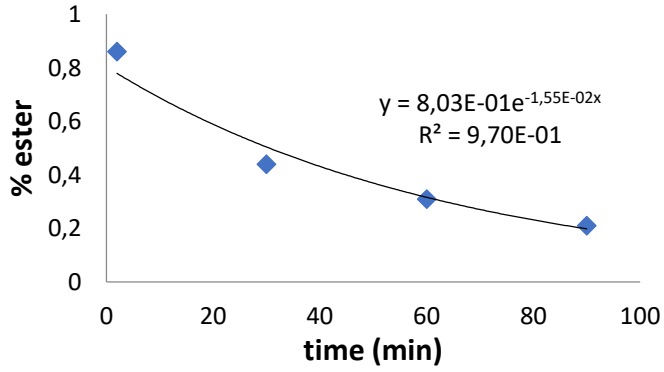


Figure S 20 – Hydrolysis kinetics of 2-formylphenyl benzoate **12** over time in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1.

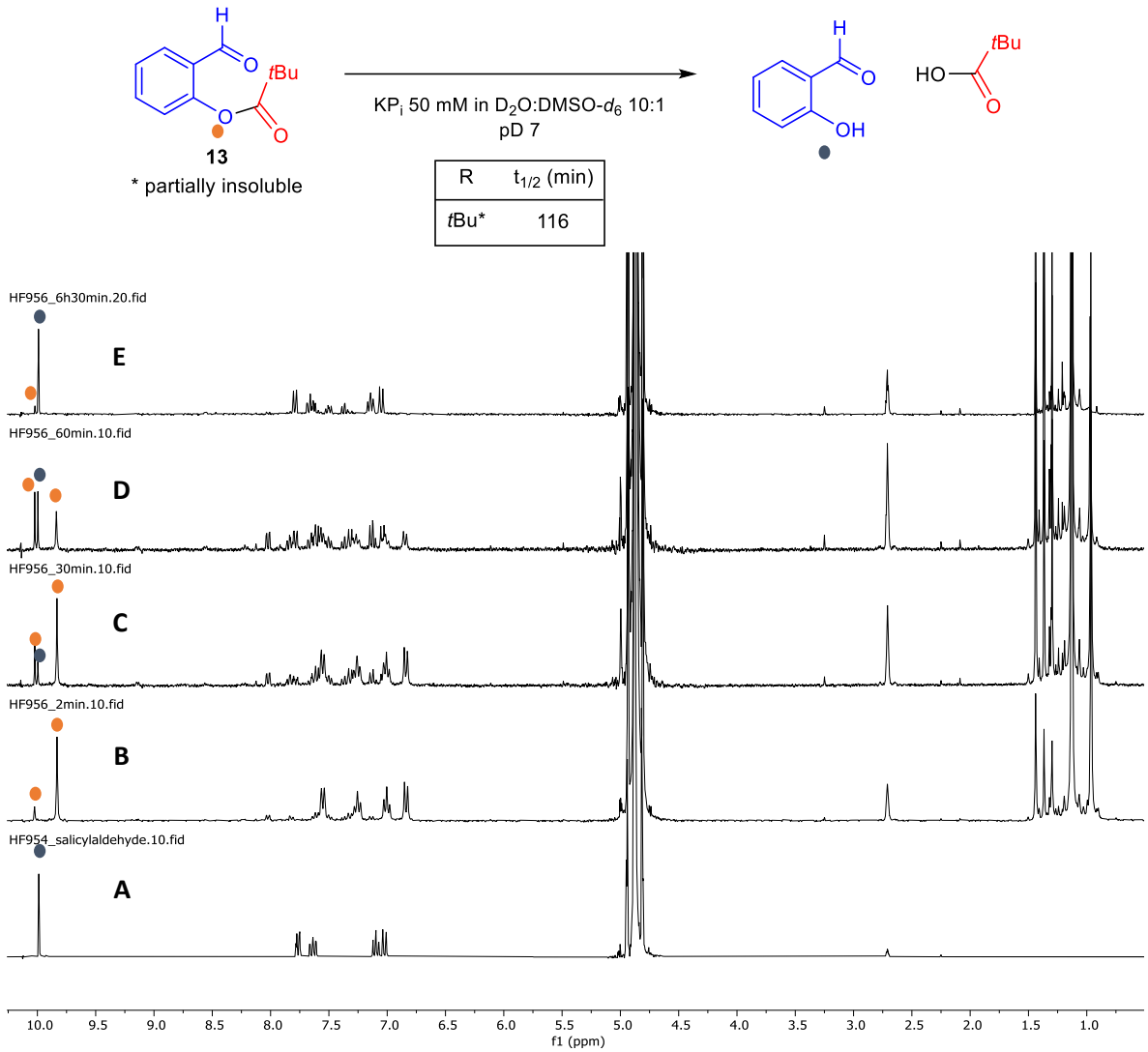


Figure S 21 – ^{1}H NMR spectra in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1: (**A**) Salycilaldehyde; (**B**) Hydrolysis of 2-formylphenyl pivalate **13** at 2 min; (**C**) Hydrolysis of 2-formylphenyl pivalate at 30 min; (**D**) Hydrolysis of 2-formylphenyl pivalate at 60 min; (**E**) Hydrolysis of 2-formylphenyl pivalate at 6.5 h.

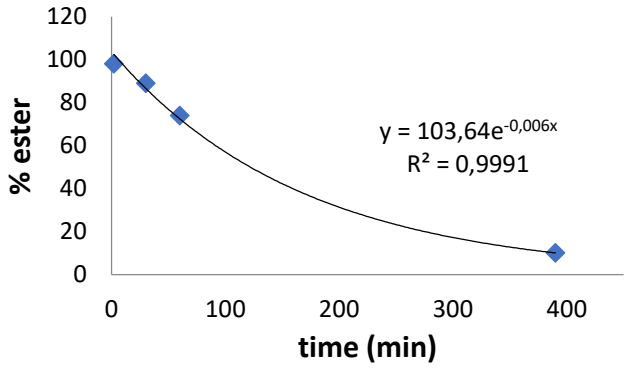
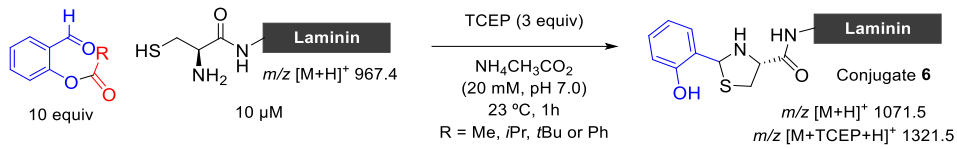


Figure S 22 – Hydrolysis kinetics of 2-formylphenyl pivalate **13** over time in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1.

5. Scope of the ester moiety

5.1 General procedure for the ESI-MS assays with Laminin fragment



To a solution of Laminin Fragment (1.0 mg/mL in water, 0.925 mM) (5.41 µL, 5.00 nmol) in ammonium acetate solution 20 mM, pH 7.0 (500 µL) was added a tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (1.0 mg/mL in water, 3.5 mM) (4.29 µL, 15.0 nmol) and the solution mixed for 1-2h at 23 °C. Then, the aldehyde (10.0 mM in ACN) (5 µL, 50.0 nmol) was added. After 1h the reaction was monitored in Positive Mode of ESI-MS.

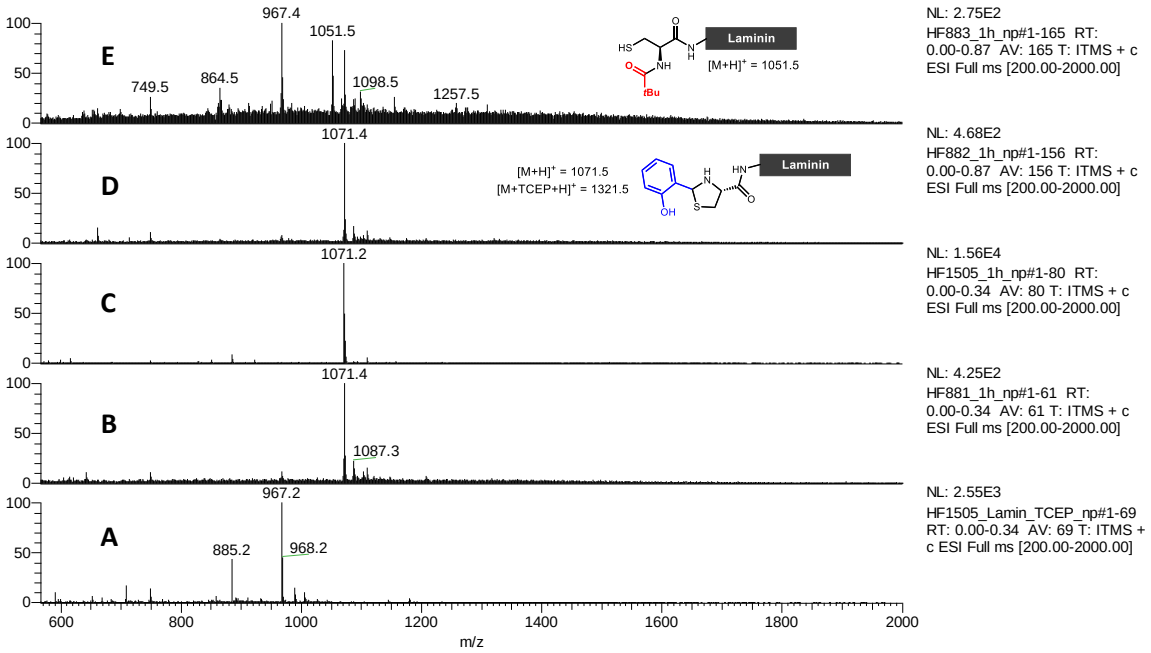
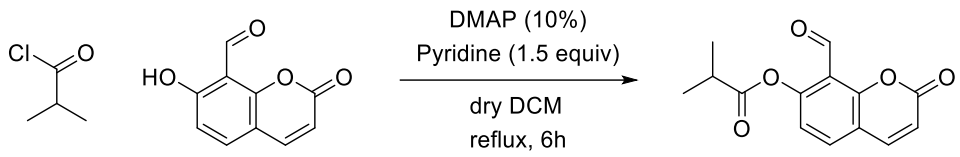


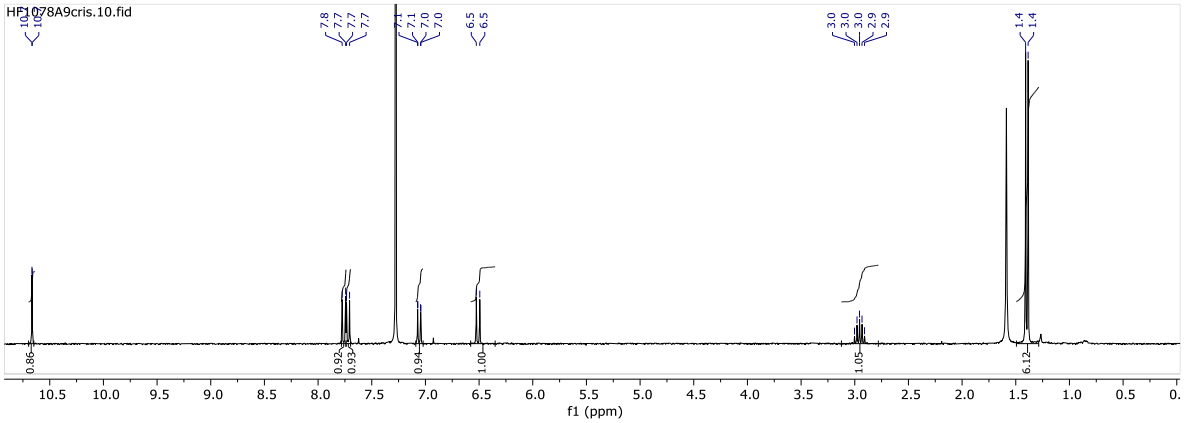
Figure S 23 – ESI+-MS spectra of laminin reaction with O-Salicylaldehyde Esters after 1h. (**A**) Laminin + TCEP; (**B**) Laminin + TCEP + 2-formylphenyl acetate **1**; (**C**) Laminin + TCEP + 2-formylphenyl isobutyrate **11**; (**D**) Laminin + TCEP + 2-formylphenyl benzoate **12**; (**E**) Laminin + TCEP + 2-formylphenyl pivalate **13**. Only reaction with 2-formylphenyl pivalate **13** show low conversion and the presence of the acetlytation of the laminin. The low conversion might be related to the low solubility of the ester.

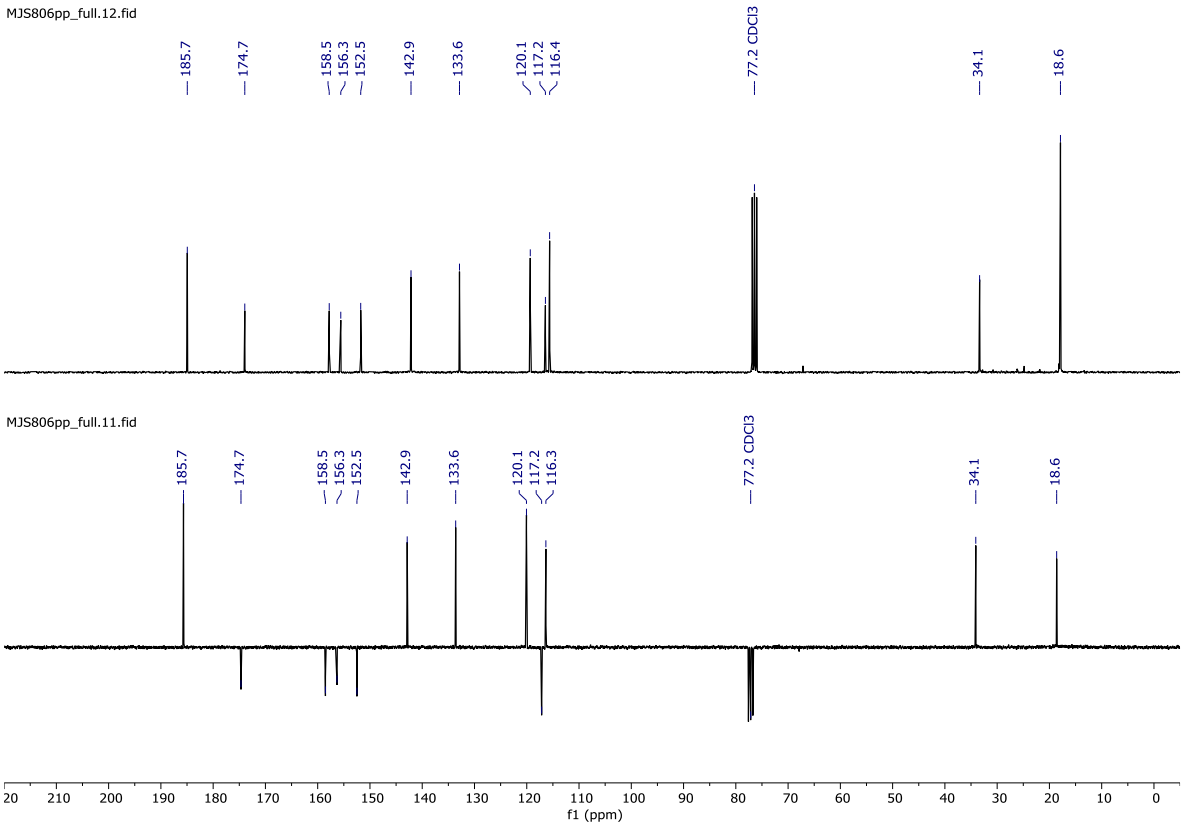
6. Fluorescence

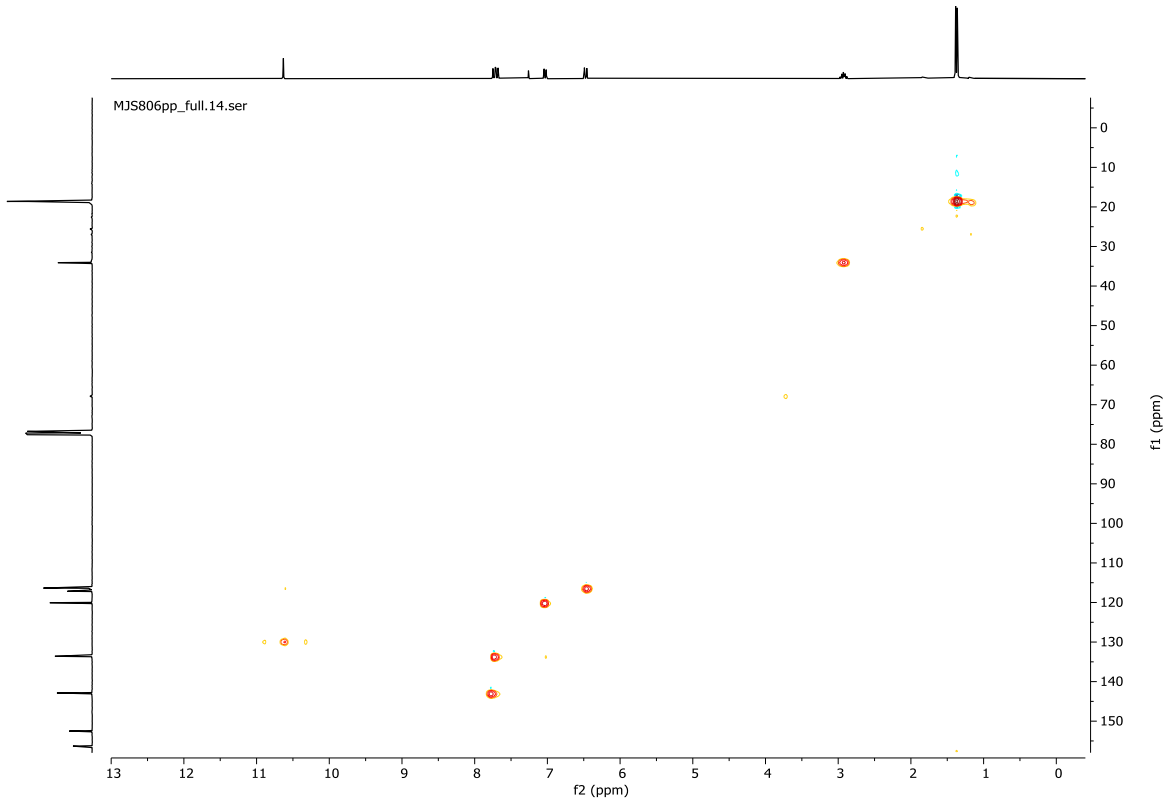
6.1 Synthesis of 7-hydroxy-8-formyl-coumarin *i*Pr ester 14

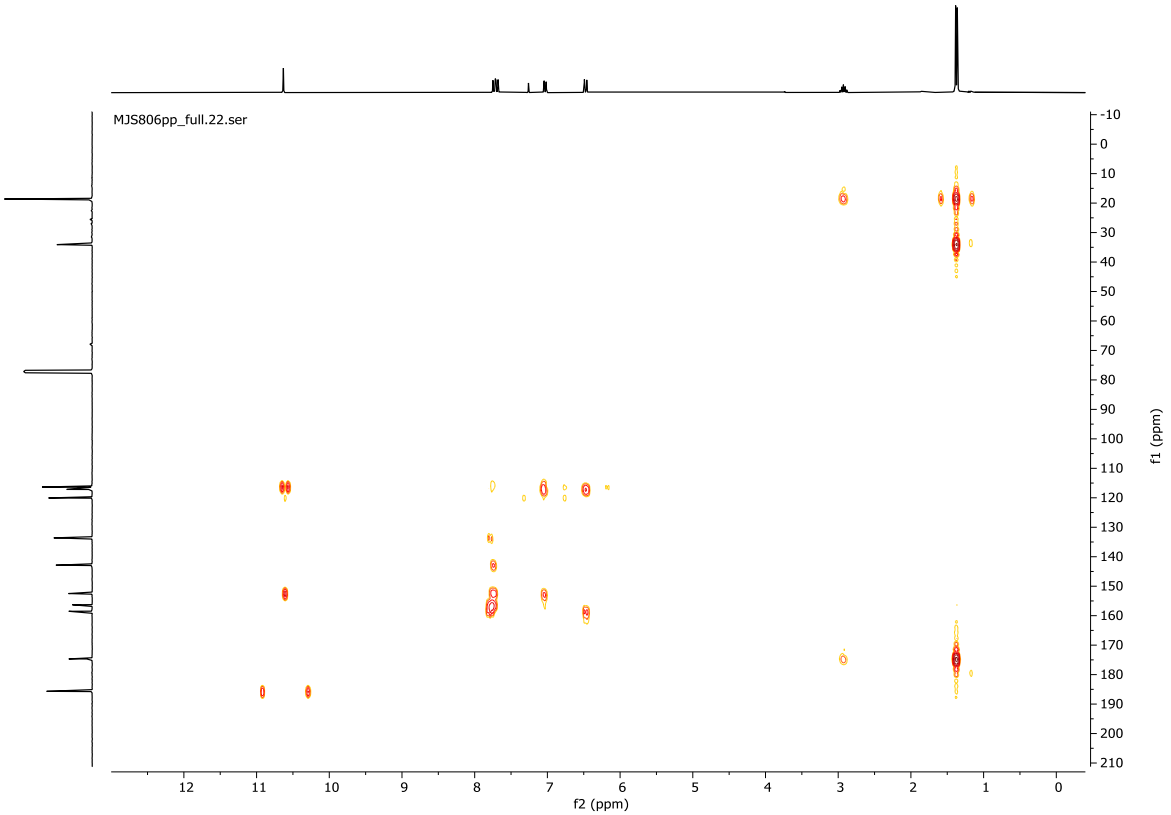


To a solution of 7-hydroxy-8-formyl-coumarin (0.2 g, 1.052 mmol) in anhydrous DCM (5 ml) under N_2 atmosphere, DMAP (0.013 g, 0.11 mmol), pyridine (0.128 ml, 1.58 mmol) and isobutyryl chloride (0.220 ml, 2.10 mmol) were added. The mixture was heated to reflux for 6 h, then quenched with HCl 5%, and extracted with DCM. The organic layer was dried over anhyd. Na_2SO_4, filtered and the solvent was removed. The residue was purified by flash chromatography (hex/MTBE 9/1) to provide 7-hydroxy-8-formyl-coumarin *i*Pr ester **14** (126 mg, 0.484 mmol, 46 % yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 10.65 (d, J = 0.7 Hz, 1H), 7.74 (d, J = 9.7 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.04 (dd, J = 8.4, 0.7 Hz, 1H), 6.49 (d, J = 9.6 Hz, 1H), 2.94 (p, J = 7.0 Hz, 1H), 1.38 (d, J = 7.0 Hz, 6H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 185.7, 174.7, 158.5, 156.3, 152.5, 142.9, 133.6, 120.1, 117.2, 116.4, 34.1, 18.6. **LRMS** (*m/z,* ESI^+): 260.8 $[M+H]^+$, 282.9 $[M+Na]^+$. **HRMS** Calculated for $C_{14}H_{13}O_5$ $[M+H]^+$: 261.0757, found 261.0754. **FTIR** (cm^{-1}): 3080 (w), 3064 (w), 2983 (w), 2936 (w), 2884 (w), 1757 (m), 1717 (s), 1682 (s), 1596 (s), 1565 (s), 1482 (s), 1402 (m), 1235 (m), 1161 (s), 1097 (s), 983 (m), 872 (m), 847 (s), 751 (s), 639 (m), 538 (m), 441 (m).


HF1078A9cris.10.fid
10.2
10.2
7.8
7.7
7.7
7.7
7.7
7.1
7.1
7.0
7.0
6.5
6.5
3.0
3.0
3.0
2.9
2.9
1.4
1.4
0.86
0.92
0.93
0.94
1.00
1.05
6.12
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)


MJS806pp_full.12.fid
185.7
174.7
158.5
156.3
152.5
142.9
133.6
120.1
117.2
116.4
77.2 CDCl3
34.1
18.6
MJS806pp_full.11.fid
185.7
174.7
158.5
156.3
152.5
142.9
133.6
120.1
117.2
116.3
77.2 CDCl3
34.1
18.6
20 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MJS806pp_full.14.ser
f2 (ppm)
f1 (ppm)


MJS806pp_full.22.ser
f2 (ppm)
f1 (ppm)

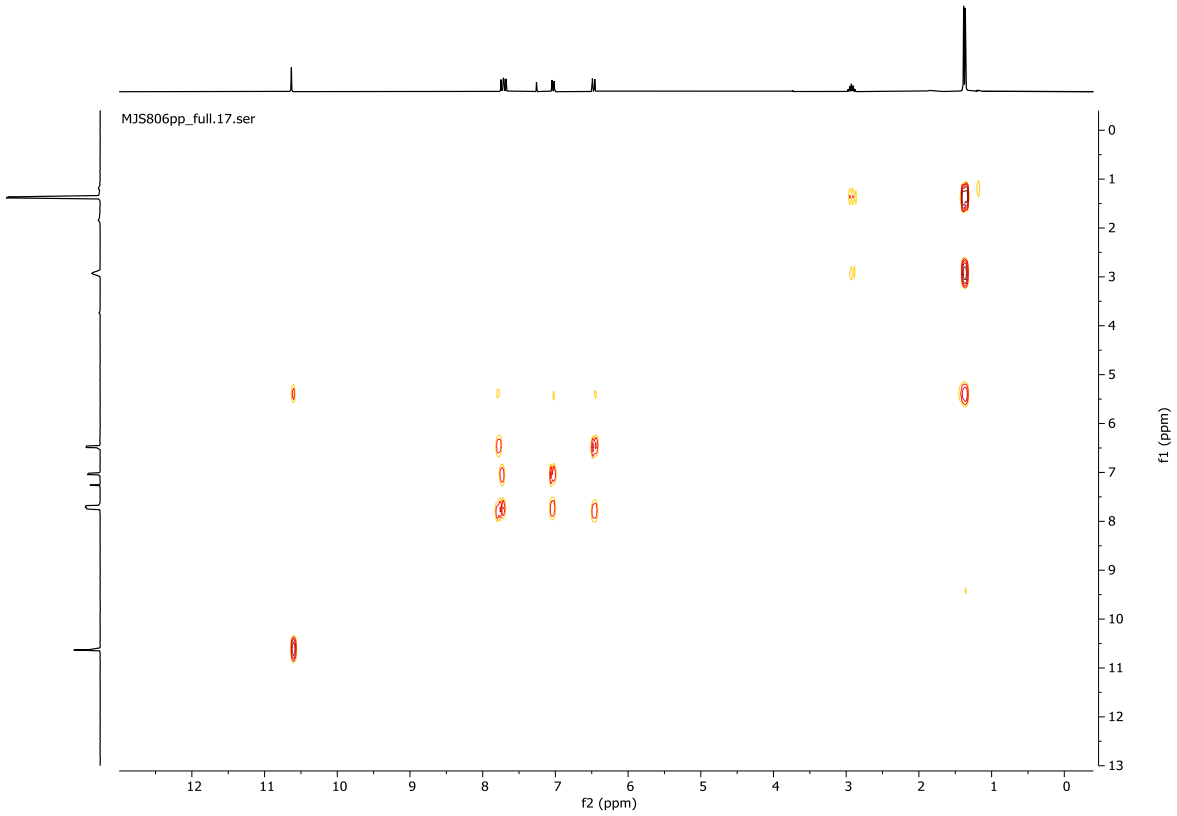


Figure S 24 – ^{1}H-NMR, ^{13}C-NMR, ^{13}C-APT, HSQC, HMBC and COSY of 7-hydroxy-8-formyl-coumarin *i*Pr ester **14** in $CDCl_3$.

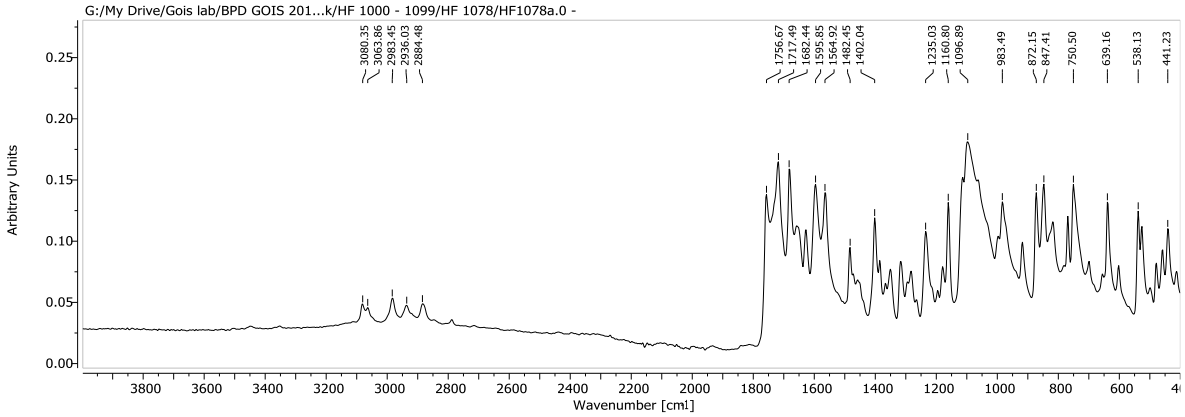


Figure S 25 – FTIR spectrum of 7-hydroxy-8-formyl-coumarin *i*Pr ester **14**.

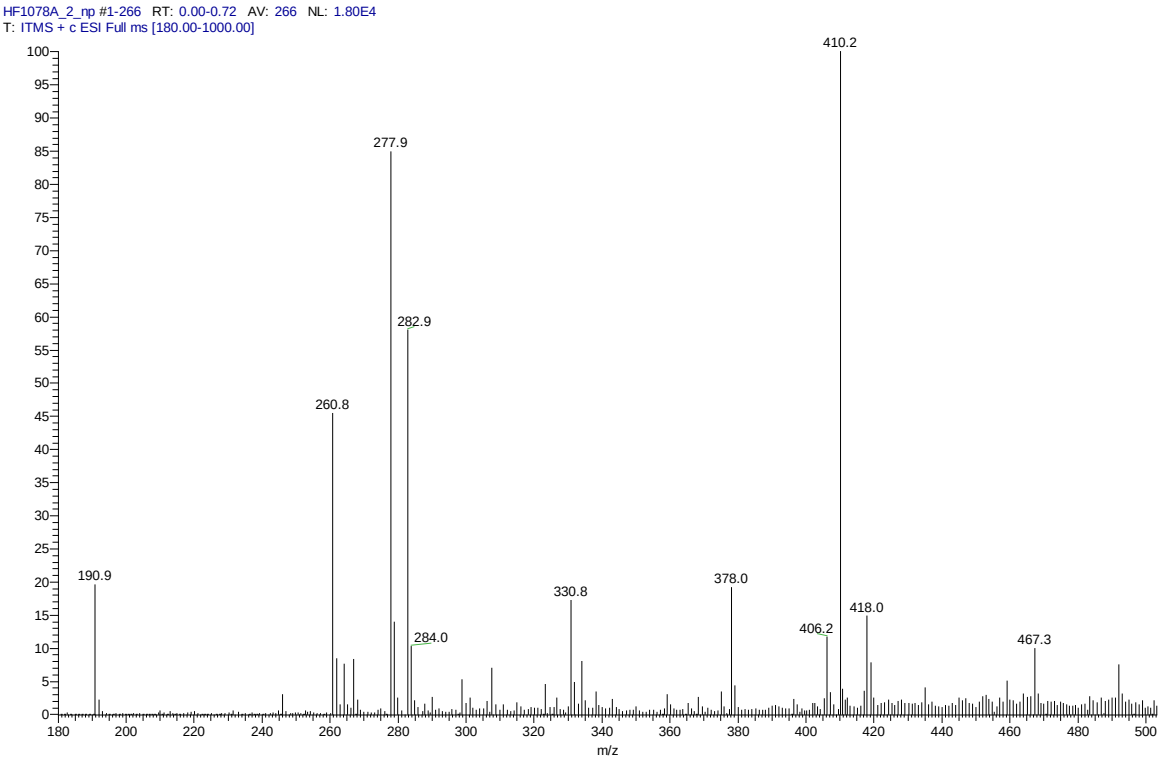


Figure S 26 – LRMS - ESI⁺ spectrum of 7-hydroxy-8-formyl-coumarin *i*Pr ester **14**.

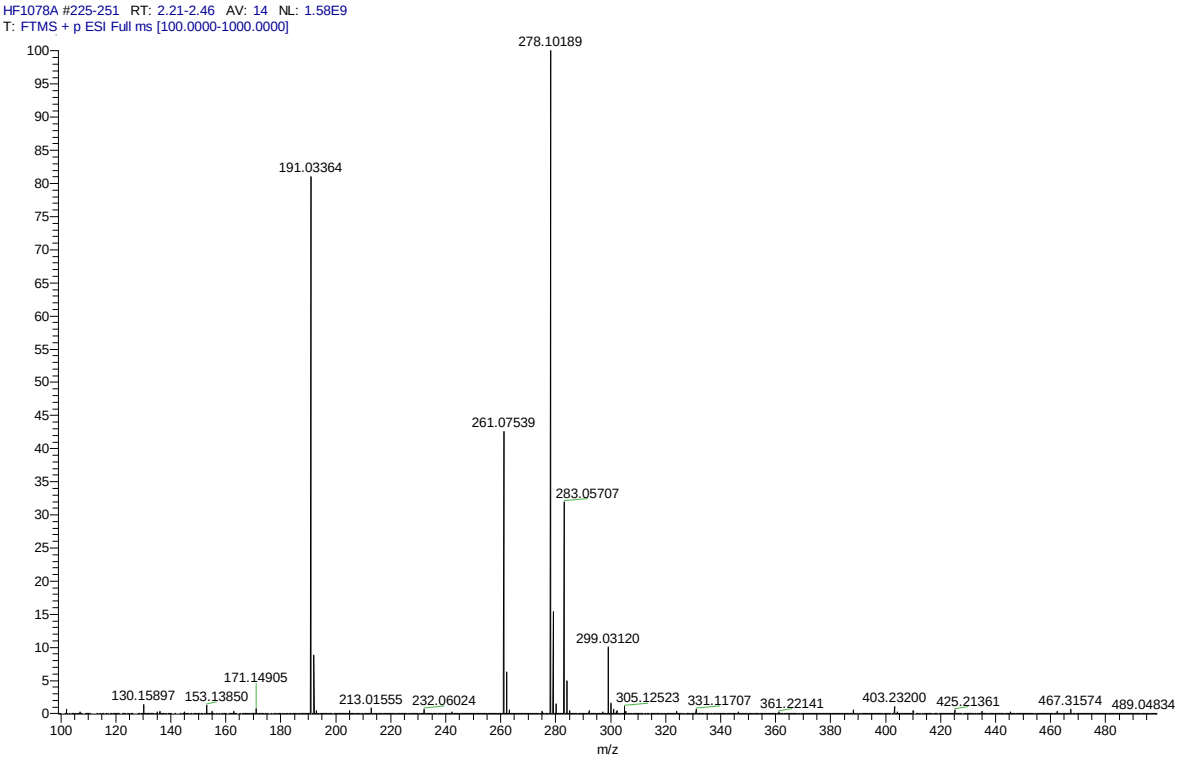
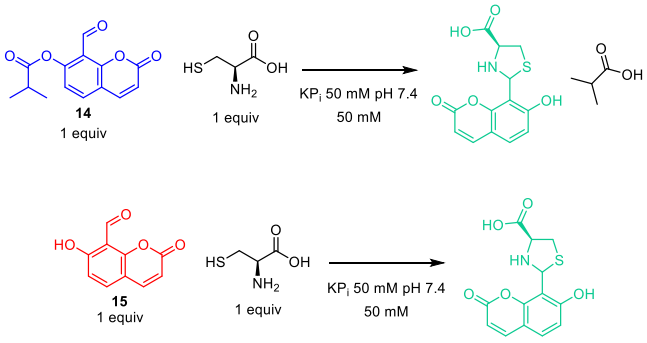


Figure S 27 – HRMS - ESI⁺ spectrum of 7-hydroxy-8-formyl-coumarin *i*Pr ester **14**.

6.2 UV-Vis and Fluorescence



To a solution of *L*-cysteine (10 mM in water) (15.0 µL, 0.15 µmol) in KP_i 50 mM pH 7.4 (3 mL) was added the aldehyde (15 µL, 0.150 µmol) and the reaction monitored by UV-Vis and Fluorescence.

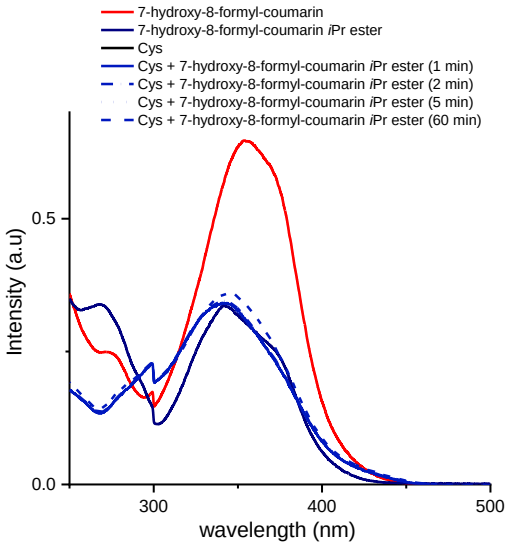


Figure S 28 – UV spectra (250-500 nm) of reactions at 1 and 60 min (blue), from cysteine (black) and 7-hydroxy-8-formyl-coumarin *i*Pr ester **14** (red).

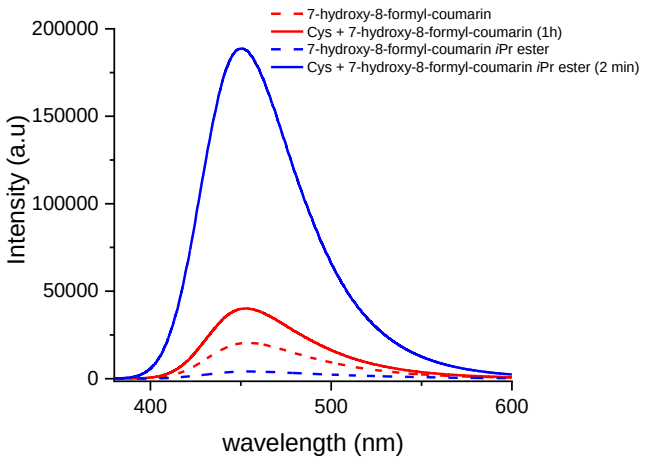
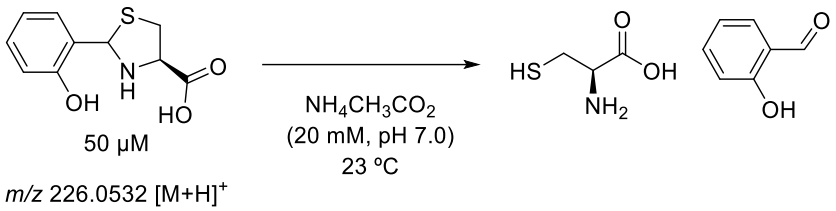


Figure S 29 – Emission spectra (370-600 nm) λ_{exc} 373 nm, Exc bandwidth 3.0 nm, Em bandwidth 5.0 nm with low sensitivity.

7. Phenolic thiazolidine stability



Independent stock solutions of (4*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid **7** in 1:1 ACN:Ammonium acetate 20mM, pH7.0) were prepared for each experiment.

Stability assays were performed by adding (4*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid **7** stock solution (5 µL, 0.050 µmol) in $NH_4CH_3CO_2$ 20mM, pH 4.5, 7.0 or 9.0 (995 µL) pre-equilibrated at room temperature (23 °C).

The reversibility of phenolic thiazolidine to form free cysteine and salicylaldehyde was monitored by LC-HRMS. The EIC (Extracted Ion Chromatogram) of base peak at *m/z* 226.0532, within a δ range of 6 ppm was used to determine the disappearance of the starting thiazolidine. Calibration curves were also performed to convert the peaks' intensity into concentration in molar (M) in order to determine the k_{obs} and $t_{1/2}$ for each tested pH.

LC-MS were acquired for 10 min in positive mode and the HPLC runs were carried out with a gradient of A (Milli Q water) and B (acetonitrile). The mobile phase was t = 0 min, 5% B; t = 4 min, 95% B; t = 6 min, 95% B; t = 7 min, 5% B; t = 10 min, stop, at a flow rate of 0.2 mL/min.

Table S 1 – Base peak at m/z 226.0532 areas vs concentrations of (4*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid **7** in $NH_4CH_3CO_2$ 20 mM, pH 7.0.

[**7**] (M)	Peak Area
5.00E-05	25430612419
4.00E-05	15098850263
3.00E-05	13733279913
2.00E-05	7825450779
1.00E-05	4720104458

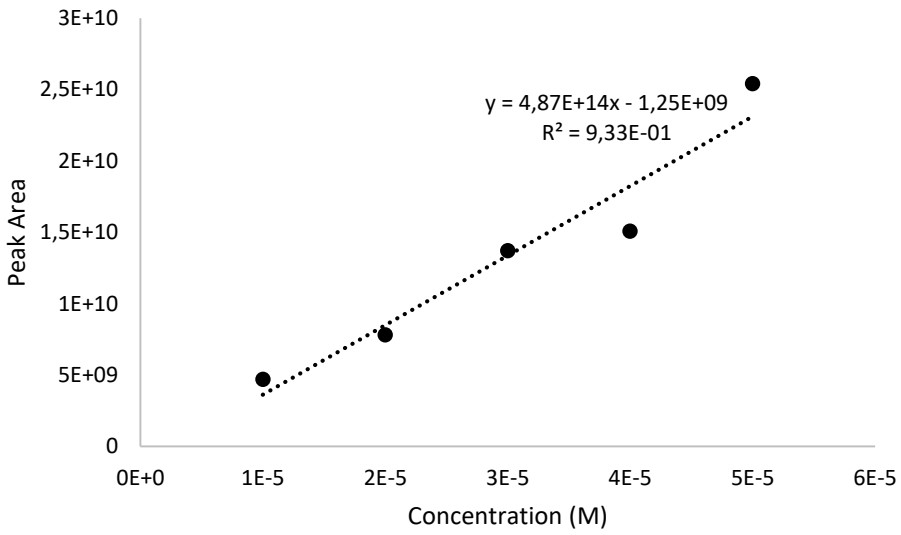


Figure S 30 – Calibration curve of (4R)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid **7** (10 µM to 50 µM) in $NH_4CH_3CO_2$ 20 mM, pH 7.0.

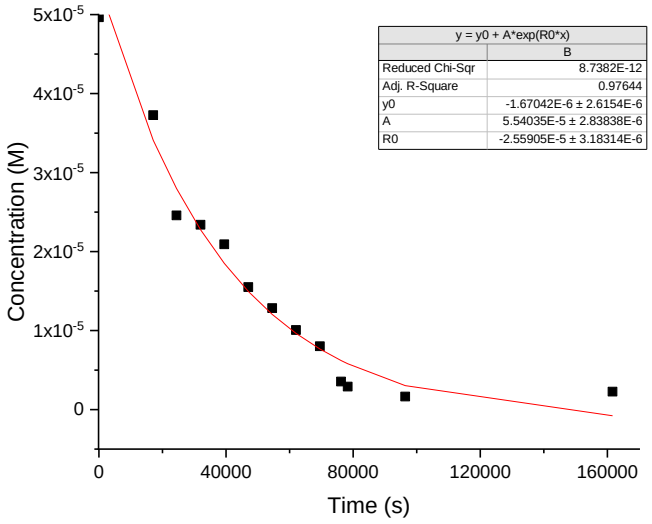


Figure S 31 – Plot of Phenolic thiazolidine **7** (M) *vs* time (s) was used to determine the first-order constant (k_{obs}) and $t_{1/2}$ at pH 7.0.

reaction	k_{obs}	$t_{1/2}$ (s)
Triplicates	2.56E-05	2.71E+04
	2.19E-05	3.17E+04
	2.26E-05	3.07E+04
Average ± Std Dev	2.34E-05 ± 1.60E-06	2.98E+04 ± 1.98E+03

Table S 2 – Base peak at m/z 226.0532 areas *vs* concentrations of (4*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid **7** in $NH_4CH_3CO_2$ 20 mM, pH 4.5.

[**7**] (M)	Peak Area
5.00E-05	21767237517
4.00E-05	18356211449
3.00E-05	13058818200
1.00E-05	5503454838

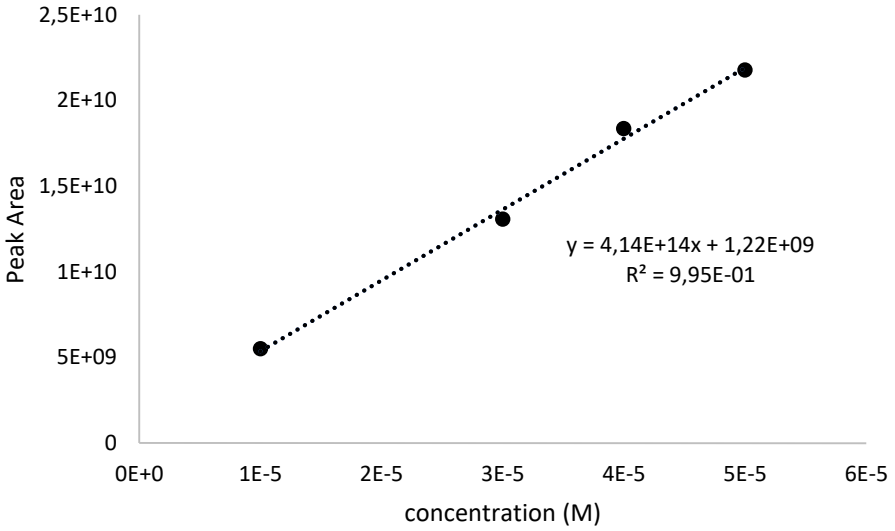


Figure S 32 – Calibration curve of (4*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid **7** (10 µM to 50 µM) in $NH_4CH_3CO_2$ 20 mM, pH 4.5.

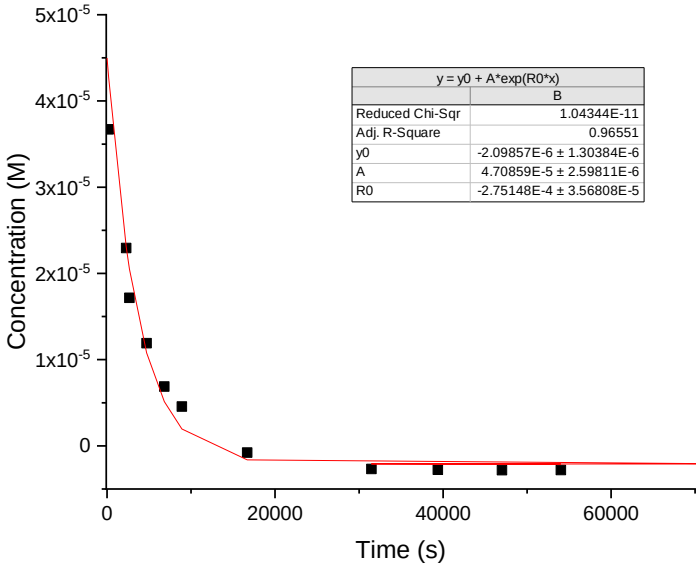


Figure S 33 – Plot of Phenolic thiazolidine **7** (M) *vs* time (s) was used to determine the first-order constant (k_{obs}) and $t_{1/2}$ at pH 4.5.

Table S 3 – k_{obs} and $t_{1/2}$ obtained from the plots Phenolic thiazolidine (M) vs time (s) at pH 4.5.

reaction	k_{obs}	$t_{1/2}$ (s)
Triplicates	2.68E-04	2.58E+03
	2.77E-04	2.51E+03
	2.75E-04	2.52E+03
Average ± Std Dev	2.73E-04 ± 3.53E-06	2.54E+03 ± 3.77E+01

Table S 4 – Base peak at m/z 226.0532 areas vs concentrations of (4*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylic acid **7** in $NH_4CH_3CO_2$ 20 mM, pH 9.0.

[**7**] (M)	Peak Area
5.00E-05	23211215598
4.00E-05	16453064928
2.00E-05	7898663557
1.00E-05	2881701760

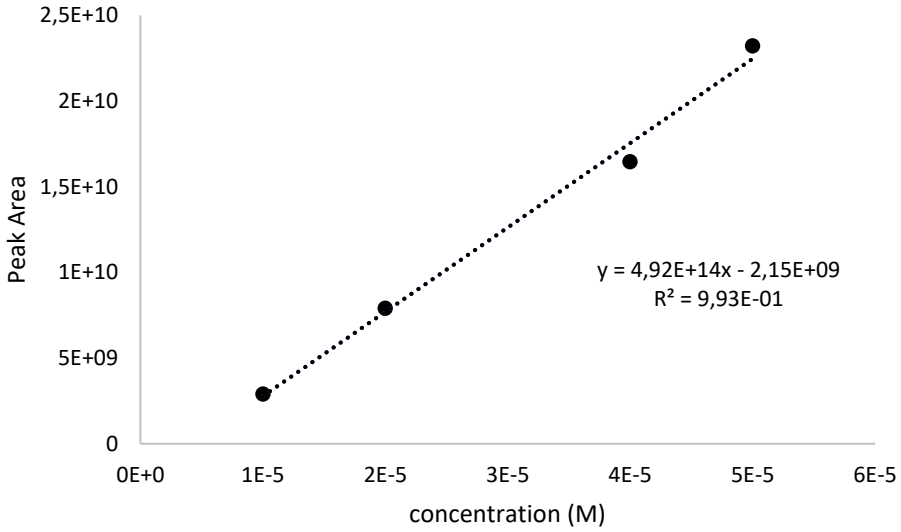


Figure S 34 – Calibration curve of (4*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carboxylicacid **7** (10 µM to 50 µM) in $NH_4CH_3CO_2$ 20 mM, pH 9.0.

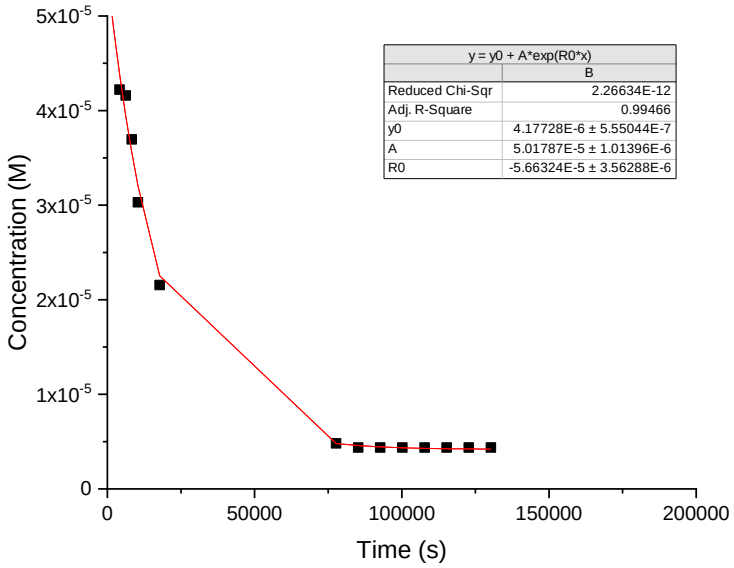


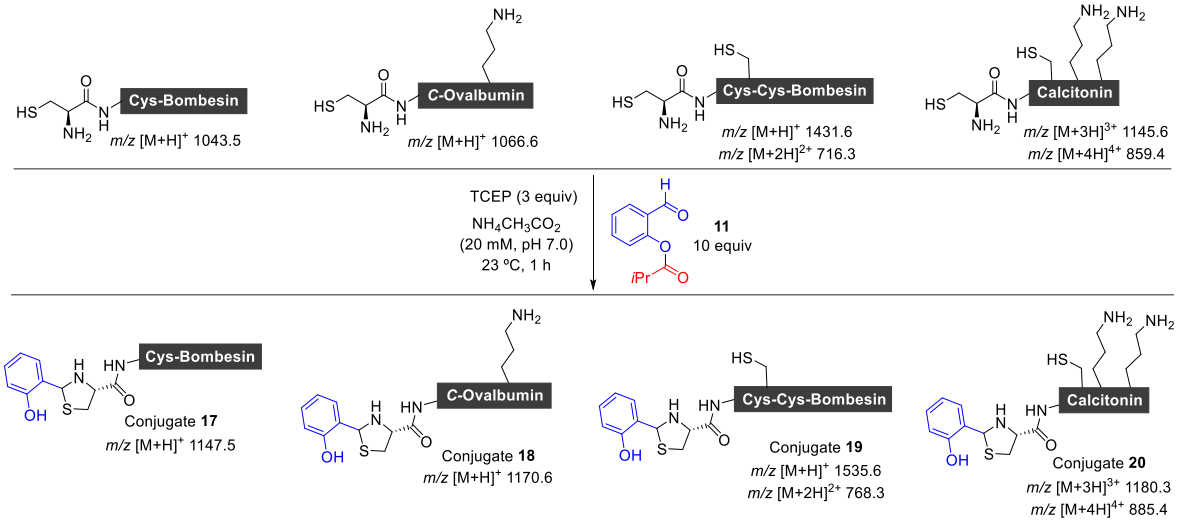
Figure S 35 – Plot of Phenolic thiazolidine **7** (M) *vs* time (s) was used to determine the first-order constant (k_{obs}) and $t_{1/2}$ at pH 9.0.

Table S 5 – k_{obs} and $t_{1/2}$ obtained from the plots Phenolic thiazolidine (M) *vs* time (s) at pH 9.0.

reaction	k_{obs}	$t_{1/2}$ (s)
Triplicates	5.13E-05	1.35E+04
	5.66E-05	1.22E+04
	6.87E-05	1.01E+04
Average ± Std Dev	5.88E-05 ± 7.27E-06	1.20E+04 ± 6.41E+02

8. Peptide scope

8.1 General procedure for the ESI-MS and LC-HRMS assays with peptides



To a solution of the peptide (5.00 nmol) in ammonium acetate solution 20 mM, pH 7.0 (500 µL) was added a tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (1.0 mg/mL in water, 3.5 mM) (4.29 µL, 15.0 nmol) and the solution mixed for 1-2h at 23 °C. Then, the 2-formylphenyl isobutyrate **11** (10.0 mM in ACN) (5 µL, 50.0 nmol) was added. After 1h the reaction was monitored in Positive Mode of ESI^+-MS and LC-HRMS using HPLC method A and B.

8.2 Reaction between Laminin and 2-formylphenyl isobutyrate 11

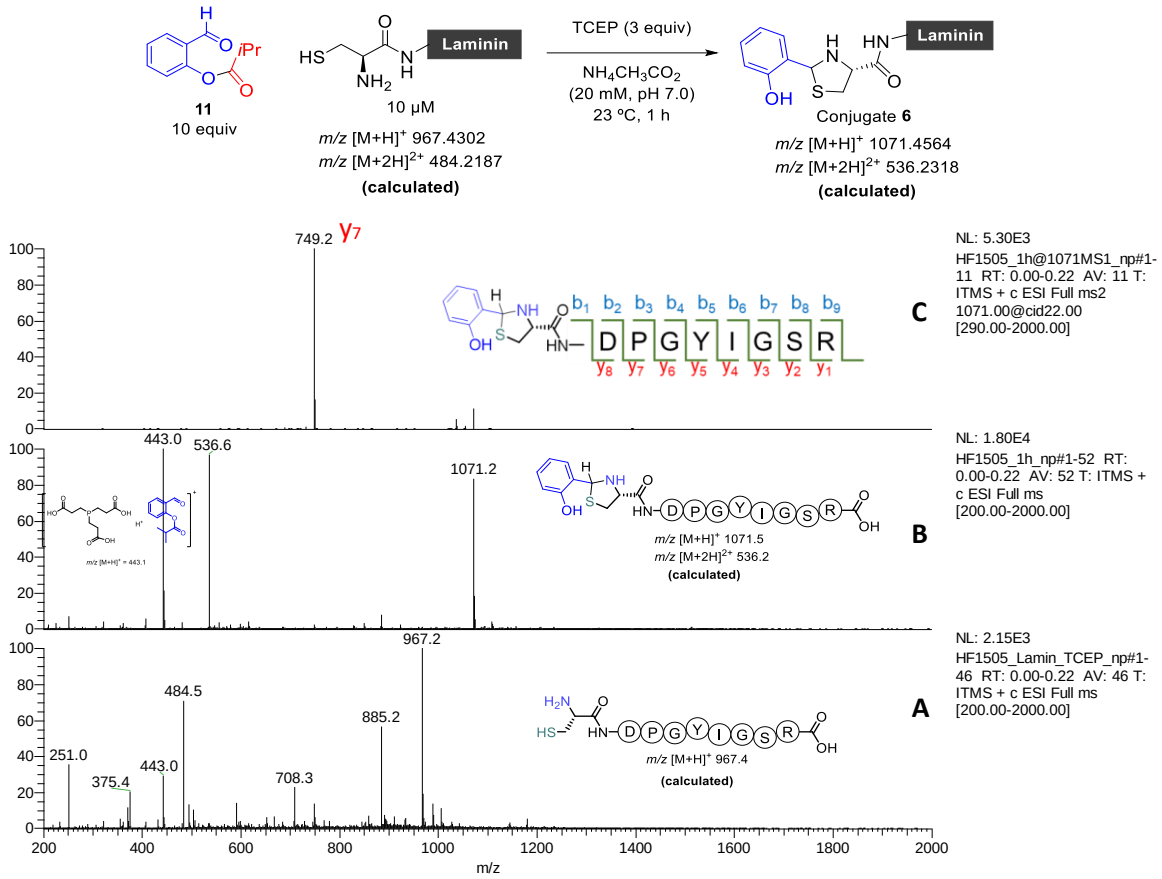


Figure S 36 – (**A**) ESI$^+$-MS spectrum of Laminin; (**B**) ESI$^+$-MS spectrum of the reaction of Laminin peptide with 2-formylphenyl isobutyrate **11**; (**C**) MS fragmentation spectrum of *m/z* [M+H]$^+$ 1071.4. MS fragmentation of the peaks correspondent to the expected product *m/z* resulted in the same profile to afford a daughter peak of *m/z* 749.4. This fragment confirms that the Laminin modification occurred in the *N*-terminus of the peptide.

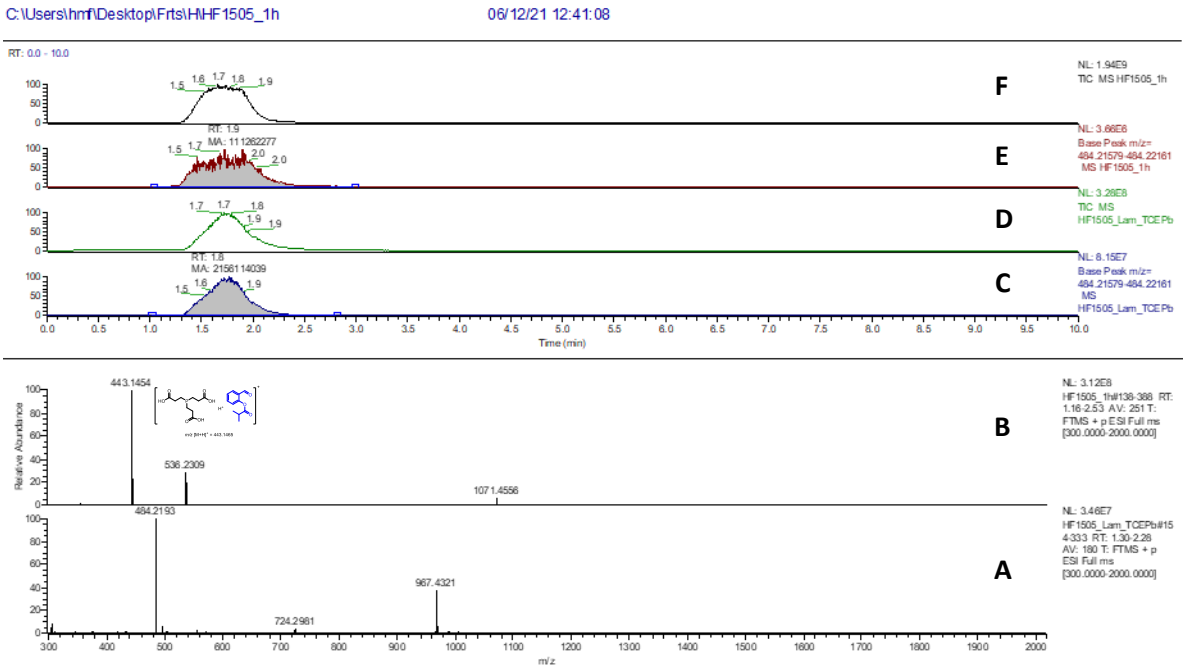


Figure S 37 – LC-HRMS analysis to Laminin peptide reaction with 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, using HPLC method B: (**A**) HRMS ESI$^+$-MS spectrum of the peak at 1.8 min from the solution of Laminin peptide; (**B**) HRMS ESI$^+$-MS spectrum of the peak at 1.8 min from the reaction solution between Laminin peptide and 2-formylphenyl isobutyrate **11**; (**C**) EIC chromatogram of Laminin peptide in the solution of reaction with 2-formylphenyl isobutyrate (base peak *m/z* 484.2187, within δ 6 ppm range); (**D**) Total ion current (TIC) chromatogram of the reaction after 60 min addition of 2-formylphenyl isobutyrate; (**E**) EIC chromatogram of Laminin peptide in the solution before addition of 2-formylphenyl isobutyrate (base peak *m/z* 484.2187, within δ 6 ppm range); (**F**) Total ion current (TIC) chromatogram of the solution of peptide before addition of 2-formylphenyl isobutyrate. Comparing the EICs before and after the addition of 2-formylphenyl isobutyrate we observed a 95% conversion.

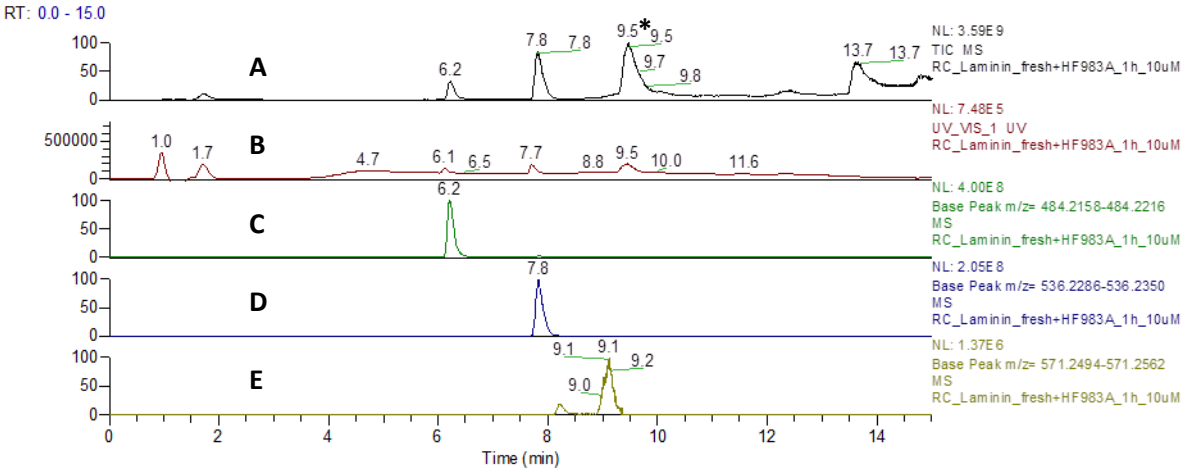


Figure S 38 – HPLC analysis to reaction between Laminin peptide and 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, using HPLC method A. (**A**) Total ion current (TIC) chromatogram. **RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Laminin peptide (base peak *m/z* 484.2187 $[M+2H]^{2+}$, within δ 6 ppm range); (**D**) EIC chromatogram of modified Laminin peptide (base peak *m/z* 536.2318 $[M+2H]^{2+}$, within δ 6 ppm range); (**E**) EIC chromatogram of modified Laminin peptide with *N*-acetylation (base peak *m/z* 571.2528 $[M+2H]^{2+}$, within δ 6 ppm range).

8.3 Reaction between *C*-Ovalbumin and 2-formylphenyl isobutyrate 11

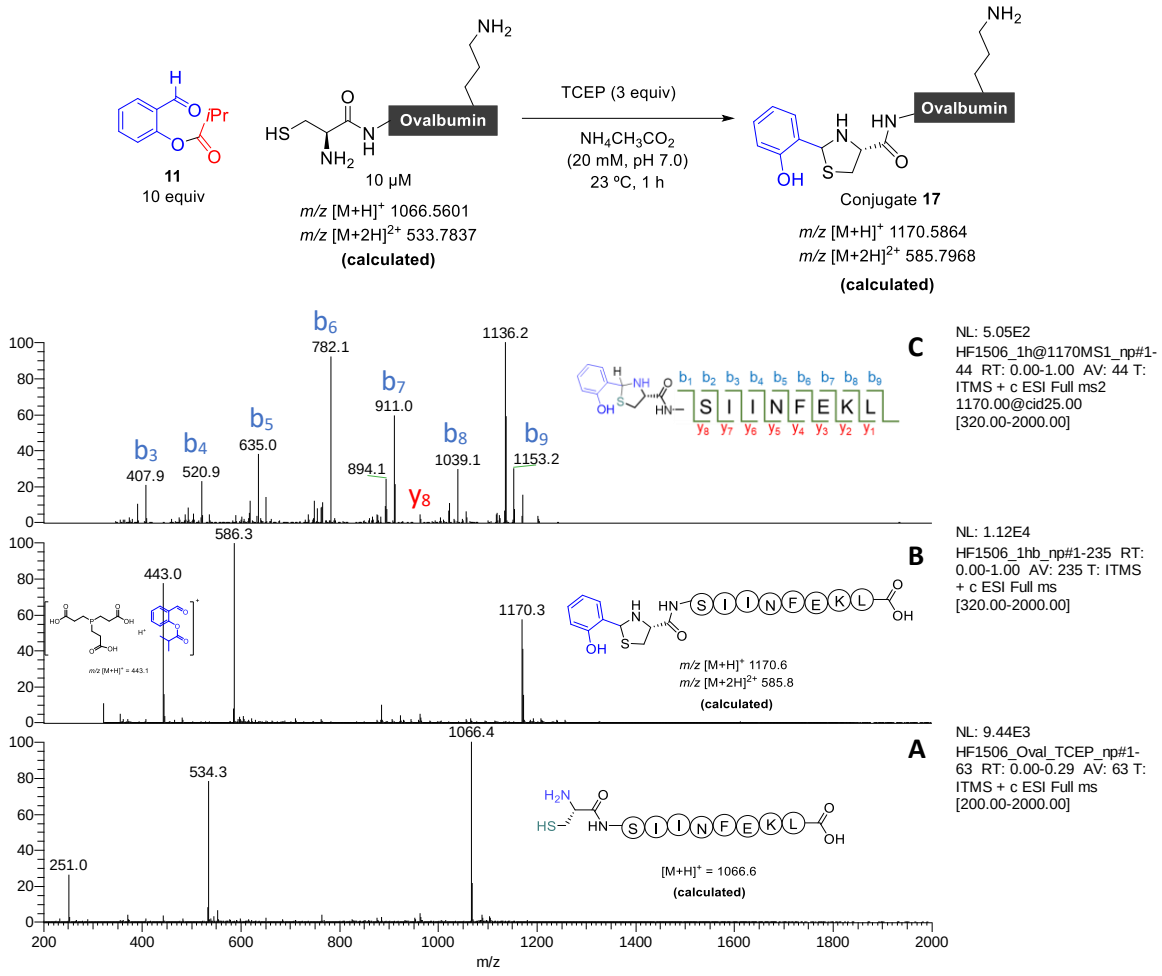


Figure S 39 – (**A**) ESI⁺-MS spectrum of *C*-Ovalbumin; (**B**) ESI⁺-MS spectrum of the reaction of C-Ovalbumin peptide with 2-formylphenyl isobutyrate **11**; (**C**) MS fragmentation spectrum of conjugate **17** *m/z* [M+H]⁺ 1071.4. MS fragmentation peaks y_8 and $b_{4\text{-}9}$ confirm that the *C*-Ovalbumin modification occurred in the *N*-terminus of the peptide.

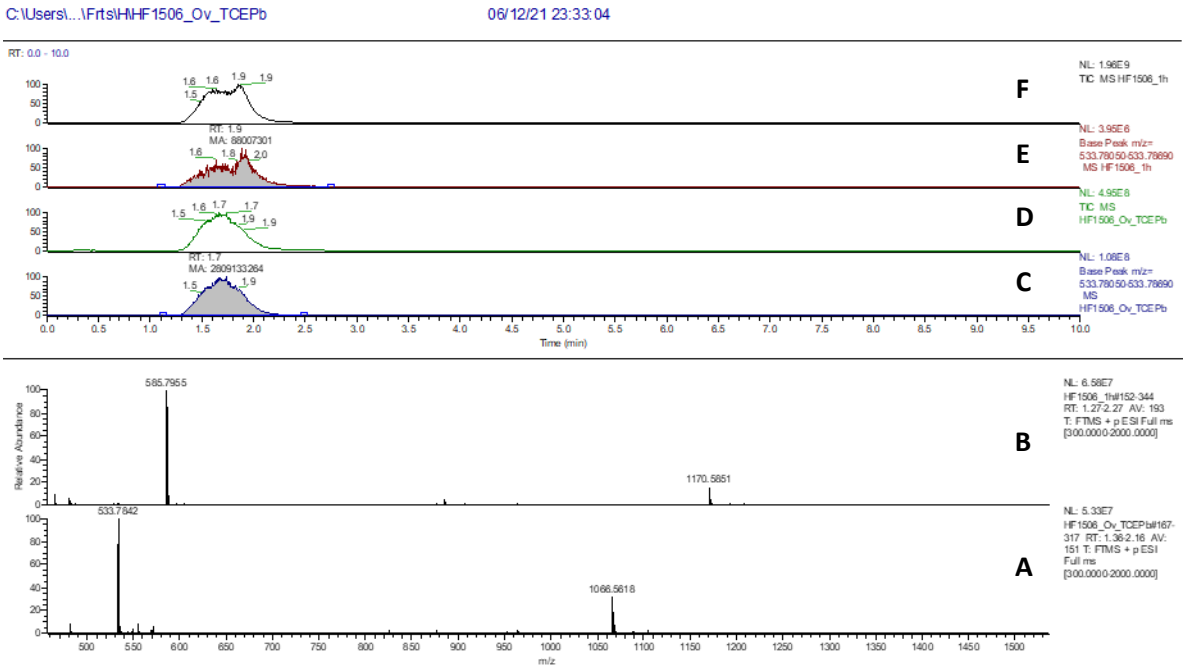


Figure S 40 – LC-HRMS analysis to *C*-Ovalbumin peptide reaction with 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, using HPLC method B: (**A**) HRMS ESI^{+}-MS spectrum of the peak at 1.8 min from the solution of *C*-Ovalbumin peptide; (**B**) HRMS ESI^{+}-MS spectrum of the peak at 1.8 min from the reaction solution between *C*-Ovalbumin peptide and 2-formylphenyl isobutyrate **11**; (**C**) EIC chromatogram of *C*-Ovalbumin peptide in the solution of reaction with 2-formylphenyl isobutyrate (base peak *m/z* 533.7837, within δ 6 ppm range); (**D**) Total ion current (TIC) chromatogram of the reaction after 60 min addition of 2-formylphenyl isobutyrate; (**E**) EIC chromatogram of *C*-Ovalbumin peptide in the solution before addition of 2-formylphenyl isobutyrate (base peak *m/z* 533.7837, within δ 6 ppm range); (**F**) Total ion current (TIC) chromatogram of the solution of peptide before addition of 2-formylphenyl isobutyrate. Comparing the EICs before and after the addition of 2-formylphenyl isobutyrate we observed a 97% conversion.

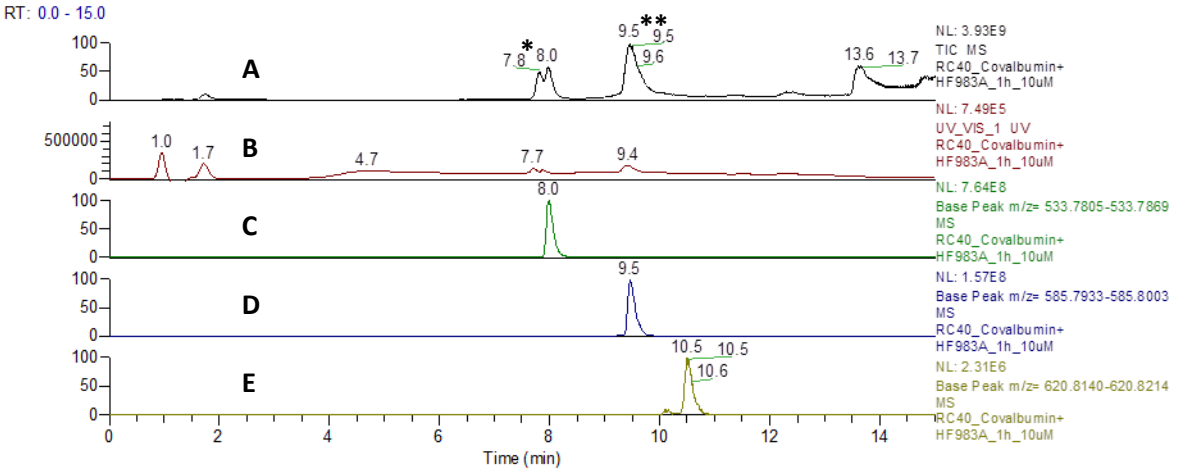
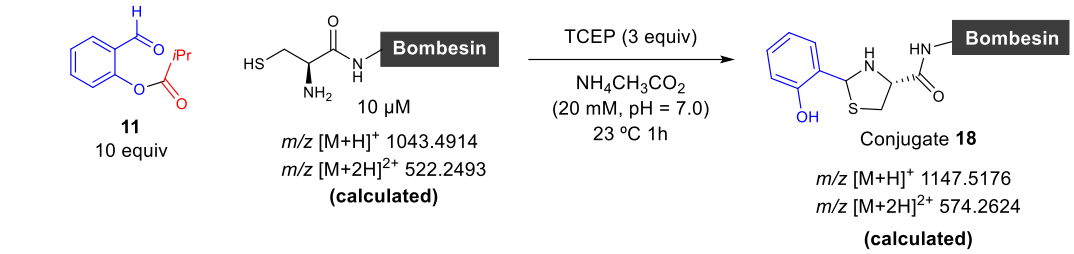


Figure S 41 – HPLC analysis to reaction between *C*-Ovalbumin peptide and 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, suing HPLC method A. (**A**) Total ion current (TIC) chromatogram. ***RT 7.8 min *C*-Ovalbumin with TCEP adduct; ****RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of *C*-Ovalbumin peptide (base peak *m/z* 533.7837 $[M+2H]^{2+}$, within δ 6 ppm range); (**D**) EIC chromatogram of modified *C*-Ovalbumin peptide (base peak *m/z* 585.7968 $[M+2H]^{2+}$, within δ 6 ppm range); (**E**) EIC chromatogram of modified *C*-Ovalbumin peptide with *N*-acetylation (base peak *m/z* 620.8177 $[M+2H]^{2+}$, within δ 6 ppm range).

8.4 Reaction between Bombesin and 2-formylphenyl isobutyrate 11



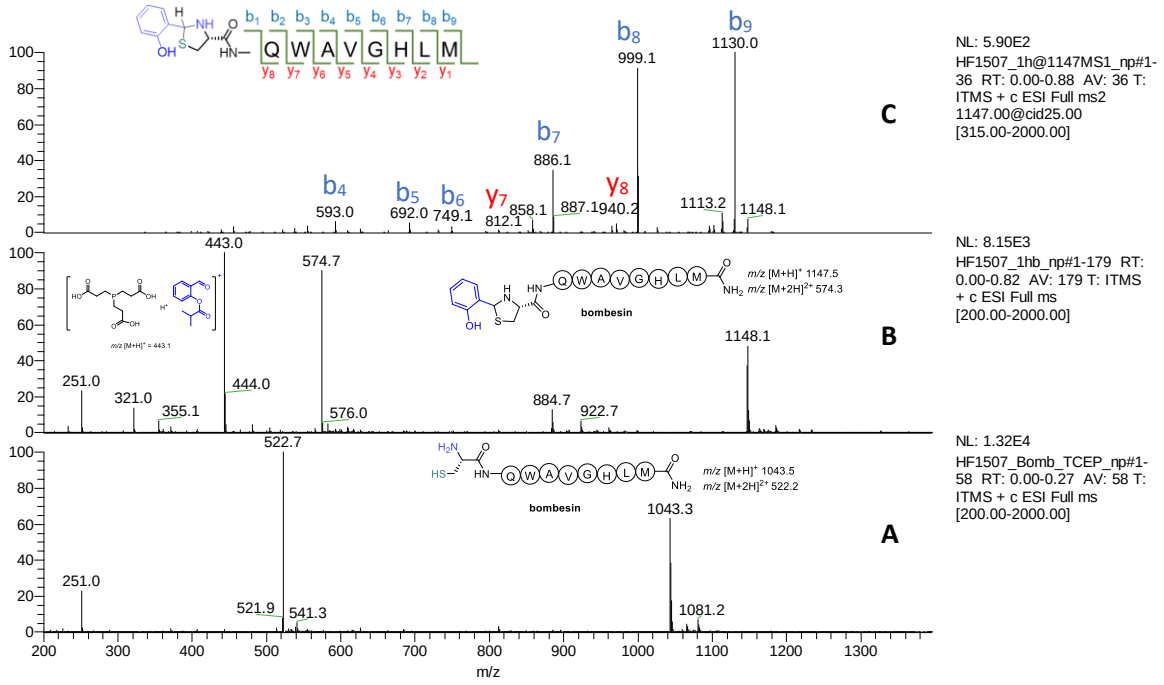


Figure S 42 – (**A**) ESI+-MS spectrum of Cys-Bombesin; (**B**) ESI+-MS spectrum of the reaction of Cys-Bombesin peptide with 2-formylphenyl isobutyrate **11**; (**C**) MS fragmentation spectrum of conjugate **18** *m/z* $[M+H]^+$ 1147. MS fragmentation peaks $y_{7,8}$ and $b_{4\text{-}9}$ confirm that the Cys-Bombesin modification occurred in the *N*-terminus of the peptide.

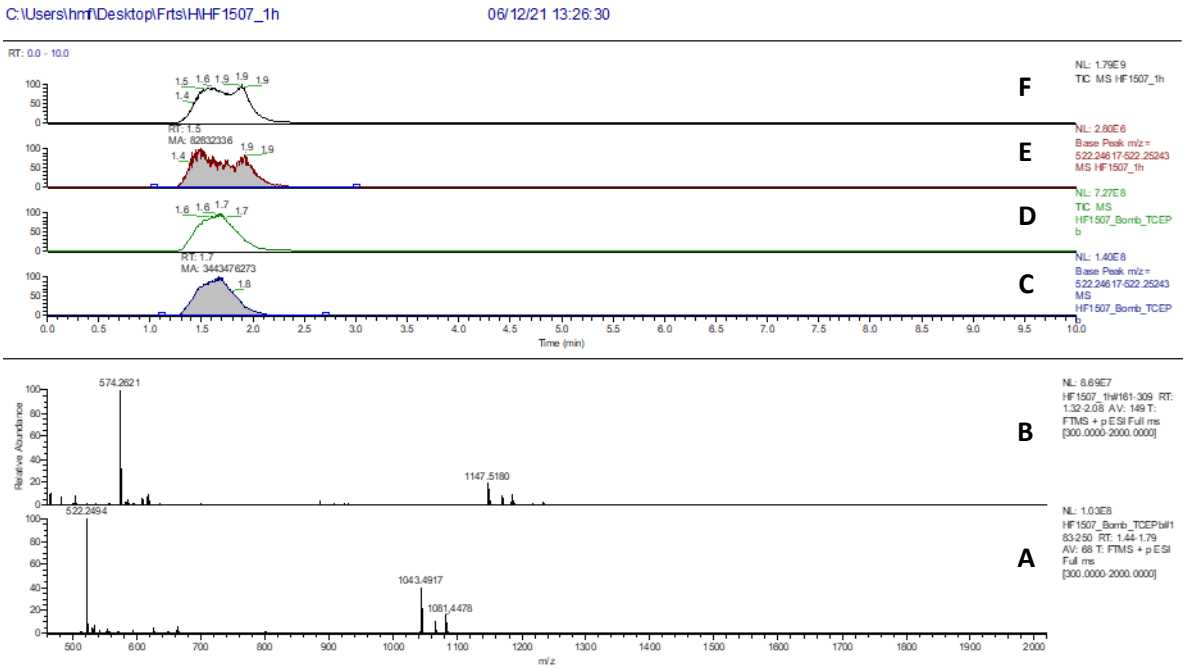


Figure S 43 – LC-HRMS analysis to Cys-Bombesin peptide reaction with 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, using HPLC method B: (**A**) HRMS ESI$^+$-MS spectrum of the peak at 1.8 min from the solution of Cys-Bombesin peptide; (**B**) HRMS ESI$^+$-MS spectrum of the peak at 1.8 min from the reaction solution between Cys-Bombesin peptide and 2-formylphenyl isobutyrate **11**; (**C**) EIC chromatogram of Cys-Bombesin peptide in the solution of reaction with 2-formylphenyl isobutyrate (base peak *m/z* 522.2493, within δ 6 ppm range); (**D**) Total ion current (TIC) chromatogram of the reaction after 60 min addition of 2-formylphenyl isobutyrate; (**E**) EIC chromatogram of Cys-Bombesin peptide in the solution before addition of 2-formylphenyl isobutyrate (base peak *m/z* 522.2493, within δ 6 ppm range); (**F**) Total ion current (TIC) chromatogram of the solution of peptide before addition of 2-formylphenyl isobutyrate. Comparing the EICs before and after the addition of 2-formylphenyl isobutyrate we observed a 98% conversion.

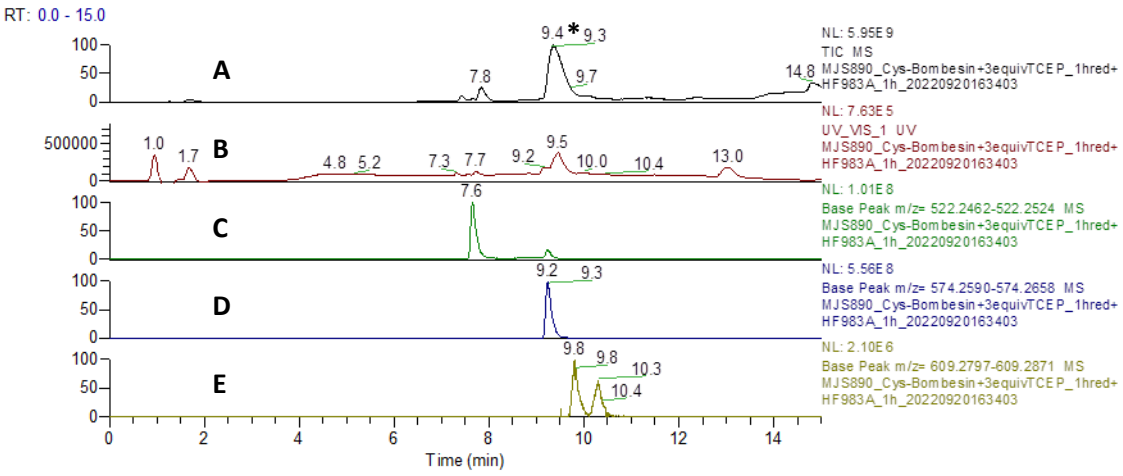


Figure S 44 – HPLC analysis to reaction between Cys-Bombesin peptide and 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, using HPLC method A. (**A**) Total ion current (TIC) chromatogram. *****RT 9.4 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Cys-Bombesin peptide (base peak *m/z* 522.2493 $[M+2H]^{2+}$, within δ 6 ppm range); (**D**) EIC chromatogram of modified Cys-Bombesin peptide (base peak *m/z* 574.2624 $[M+2H]^{2+}$, within δ 6 ppm range); (**E**) EIC chromatogram of modified Cys-Bombesin peptide with *N*-acetylation (base peak *m/z* 609.2834 $[M+2H]^{2+}$, within δ 6 ppm range).

8.5 Reaction between Cys-Cys-Bombesin and 2-formylphenyl isobutyrate 11

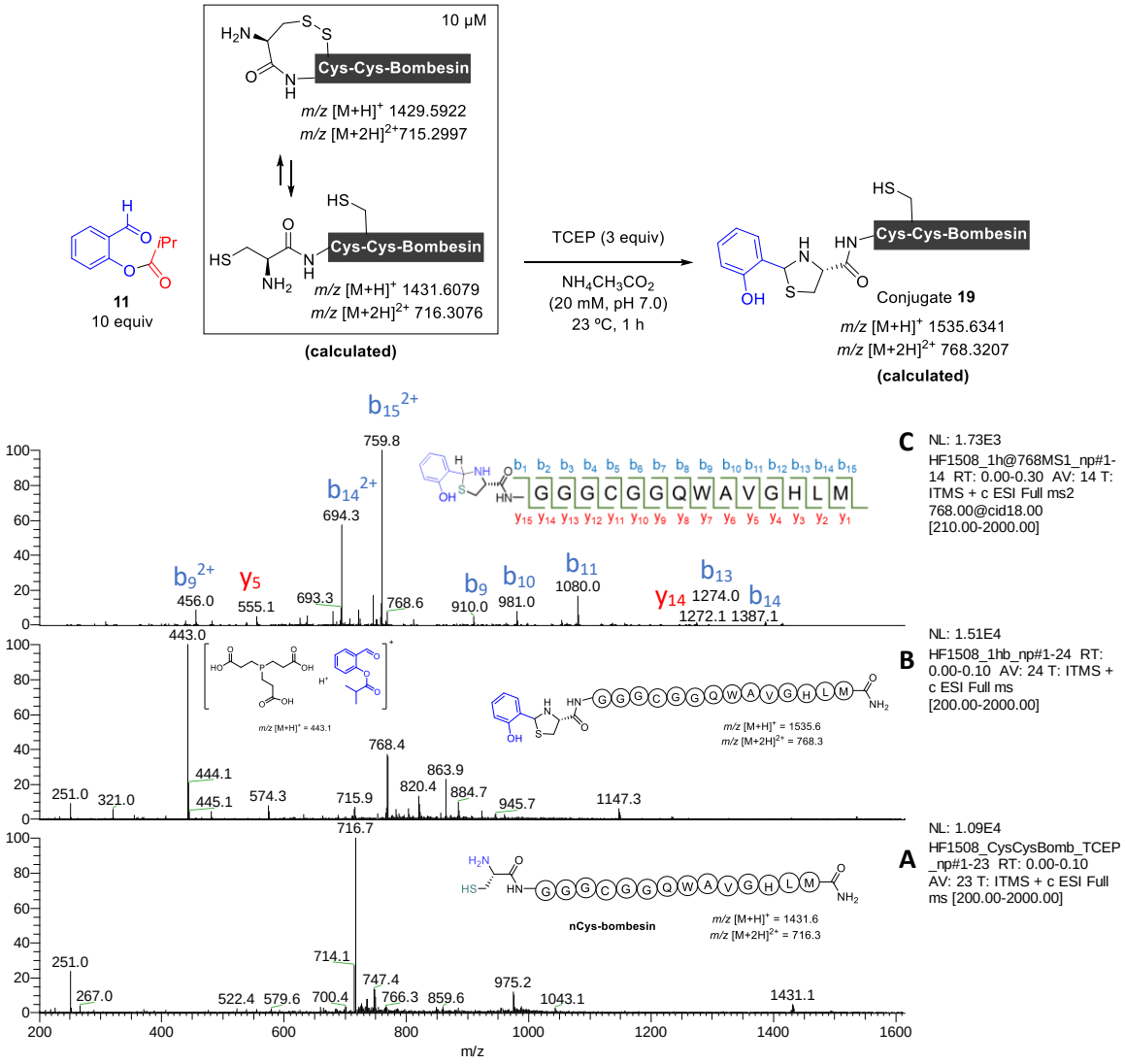


Figure S 45 – (**A**) ESI⁺-MS spectrum of Cys-Cys-Bombesin; (**B**) ESI⁺-MS spectrum of the reaction of Cys-Cys-Bombesin peptide with 2-formylphenyl isobutyrate **11**; (**C**) MS fragmentation spectrum of conjugate **19** *m/z* [M+2H]²⁺ 768.4. MS fragmentation peaks $y_{14,5}$ and $b_{9\text{-}15}$ confirm that the Cys-Cys-Bombesin modification occurred in the *N*-terminus of the peptide.

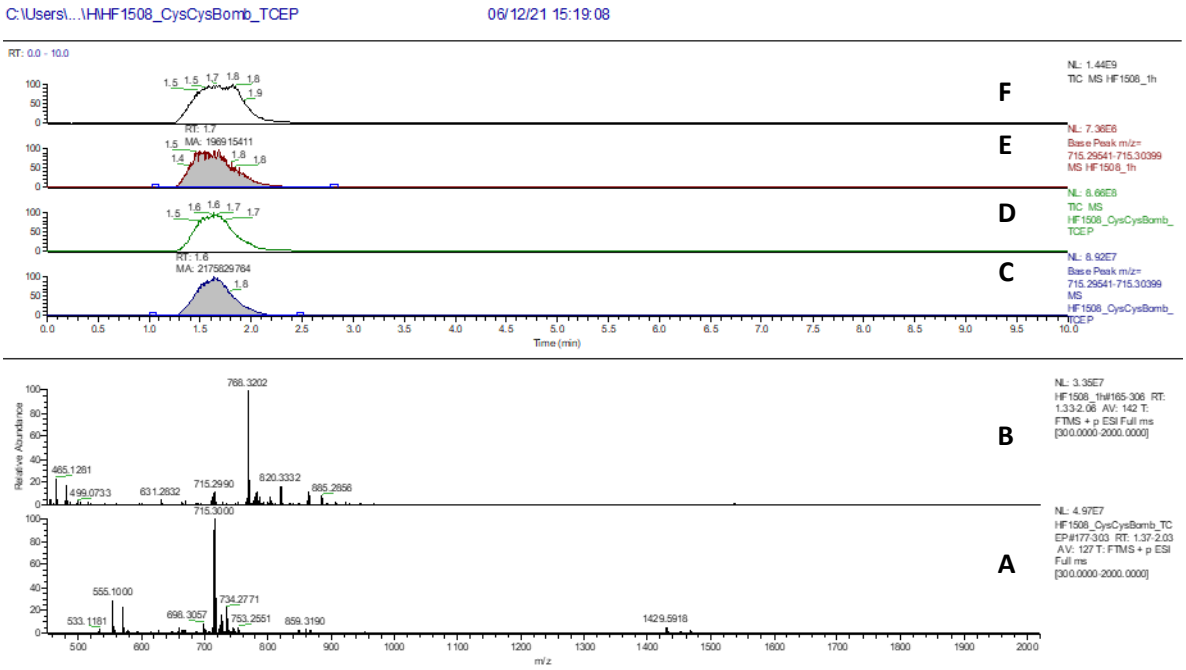


Figure S 46 – LC-HRMS analysis to Laminin peptide reaction with 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, using HPLC method B: (**A**) HRMS ESI^{+}-MS spectrum of the peak at 1.8 min from the solution of Cys-Cys-Bombesin peptide; (**B**) HRMS ESI^{+}-MS spectrum of the peak at 1.8 min from the reaction solution between Cys-Cys-Bombesin peptide and 2-formylphenyl isobutyrate **11**; (**C**) EIC chromatogram of Cys-Cys-Bombesin peptide in the solution of reaction with 2-formylphenyl isobutyrate (base peak *m/z* 715.2997, within δ 6 ppm range); (**D**) Total ion current (TIC) chromatogram of the reaction after 60 min addition of 2-formylphenyl isobutyrate; (**E**) EIC chromatogram of Cys-Cys-Bombesin peptide in the solution before addition of 2-formylphenyl isobutyrate (base peak *m/z* 715.2997, within δ 6 ppm range); (**F**) Total ion current (TIC) chromatogram of the solution of peptide before addition of 2-formylphenyl isobutyrate. Comparing the EICs before and after the addition of 2-formylphenyl isobutyrate we observed 91% conversion.

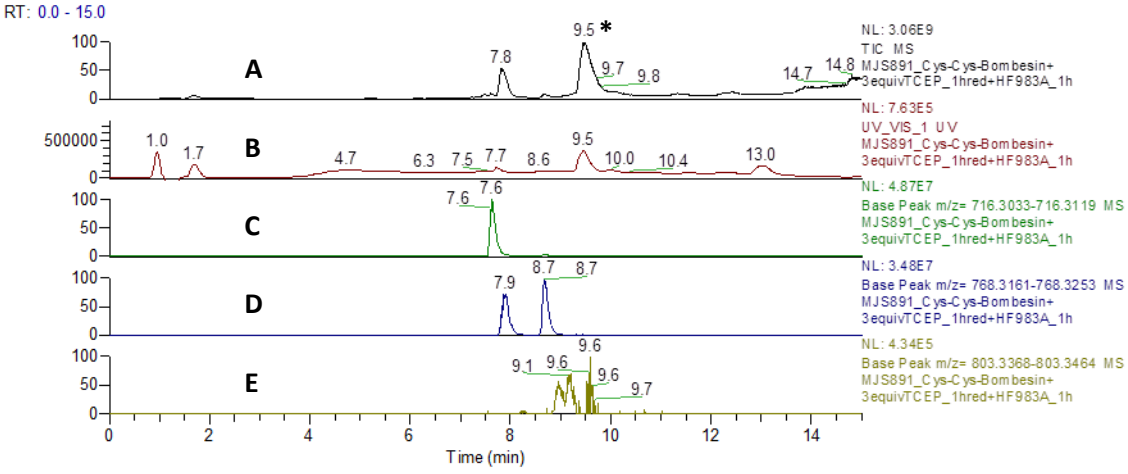


Figure S 47 – HPLC analysis to reaction between Cys-Cys-Bombesin peptide and 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, using HPLC method A. (**A**) Total ion current (TIC) chromatogram. *****RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Cys-Cys-Bombesin peptide (base peak *m/z* 716.3076 $[M+2H]^{2+}$, within δ 6 ppm range); (**D**) EIC chromatogram of modified Cys-Bombesin peptide (base peak *m/z* 768.3207 $[M+2H]^{2+}$, within δ 6 ppm range); (**E**) EIC chromatogram of modified Cys-Cys-Bombesin peptide with *N*-acetylation (base peak *m/z* 803.3416 $[M+2H]^{2+}$, within δ 6 ppm range).

8.6 Reaction between Calcitonin and 2-formylphenyl isobutyrate 11

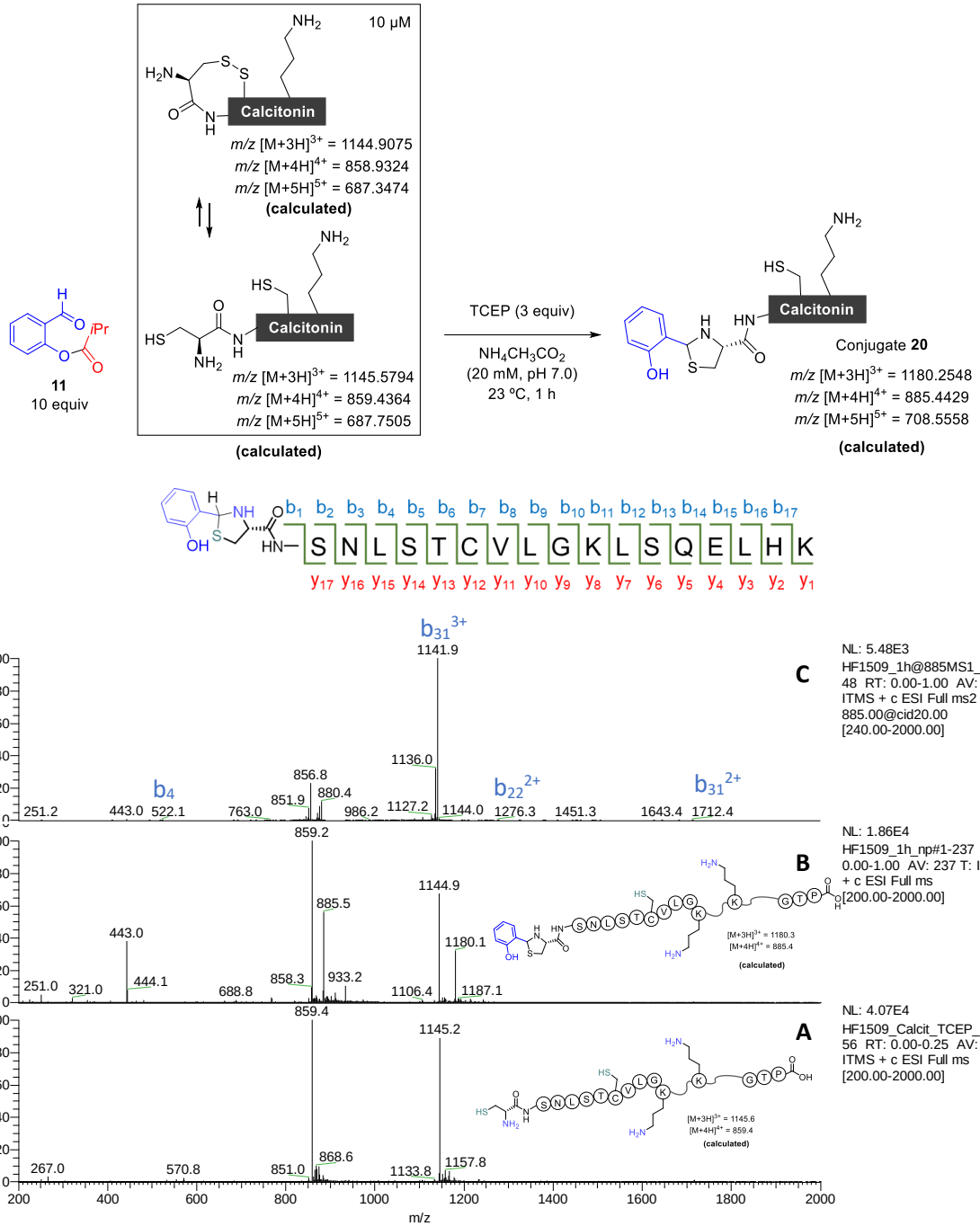


Figure S 48– (**A**) ESI⁺-MS spectrum of Calcitonin Salmon; (**B**) ESI⁺-MS spectrum of the reaction of Calcitonin peptide with 2-formylphenyl isobutyrate **12**; (**C**) MS fragmentation spectrum of conjugate **20** *m/z* [M+4H]$^{4+}$ 885.5. MS fragmentation peaks $b_{4,22,31,32}$ indicate that the Calcitonin modification occurred in the *N*-terminus of the peptide.

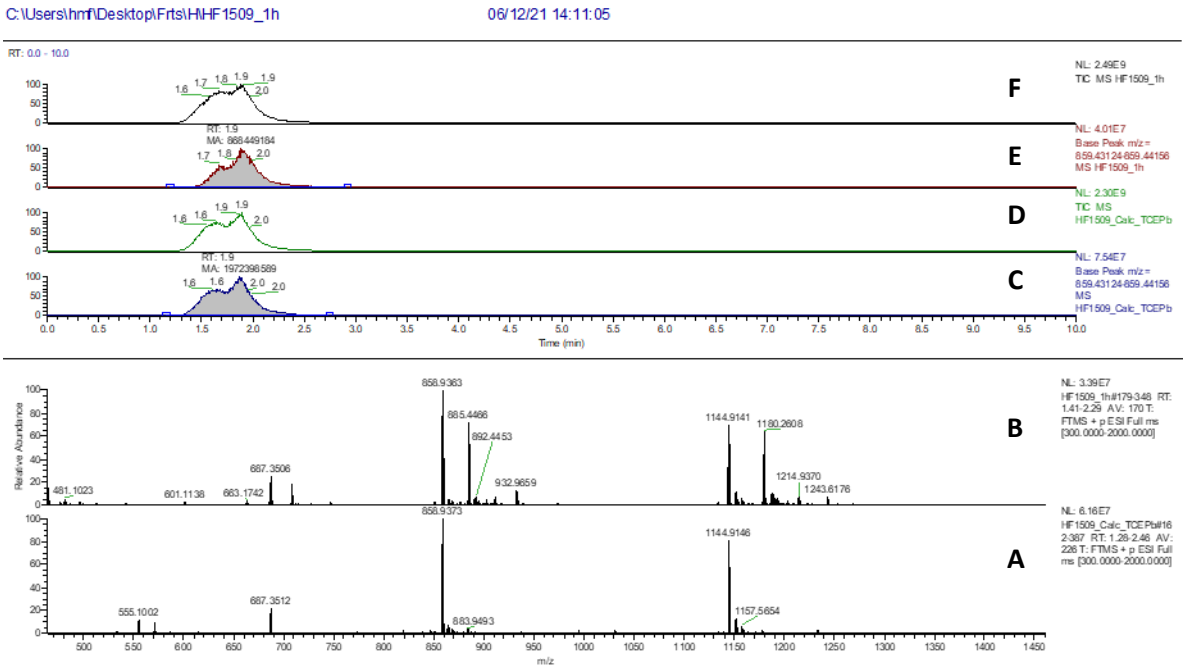


Figure S 49 – LC-HRMS analysis to Laminin peptide reaction with 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, using HPLC method B: (**A**) HRMS ESI^{+}-MS spectrum of the peak at 1.8 min from the solution of Calcitonin peptide; (**B**) HRMS ESI^{+}-MS spectrum of the peak at 1.8 min from the reaction solution between Calcitonin peptide and 2-formylphenyl isobutyrate **11**; (**C**) EIC chromatogram of Calcitonin peptide in the solution of reaction with 2-formylphenyl isobutyrate (base peak *m/z* 859.4364, within δ 6 ppm range); (**D**) Total ion current (TIC) chromatogram of the reaction after 60 min addition of 2-formylphenyl isobutyrate; (**E**) EIC chromatogram of Calcitonin peptide in the solution before addition of 2-formylphenyl isobutyrate (base peak *m/z* 859.4364, within δ 6 ppm range); (**F**) Total ion current (TIC) chromatogram of the solution of peptide before addition of 2-formylphenyl isobutyrate. Comparing the EICs before and after the addition of 2-formylphenyl isobutyrate we observed a 56% conversion.

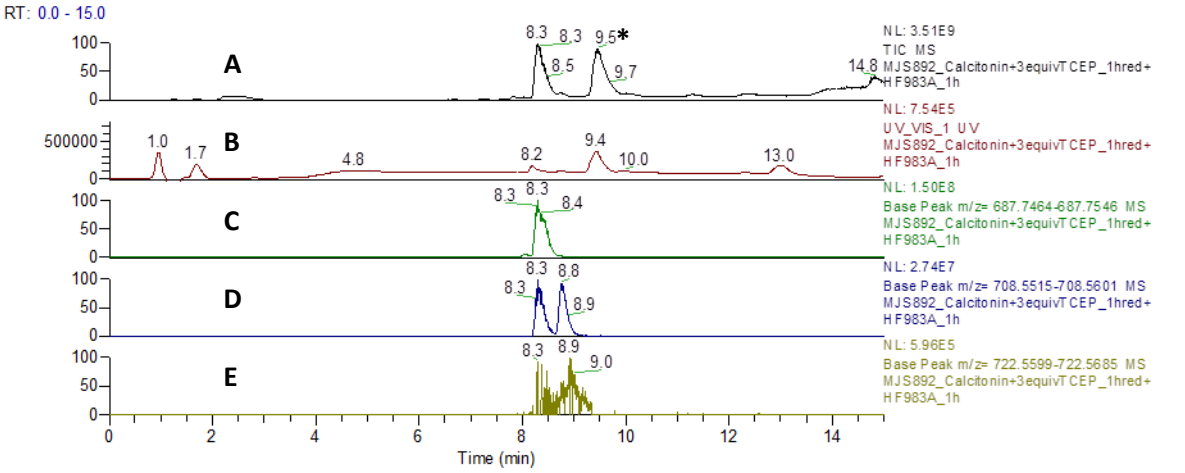
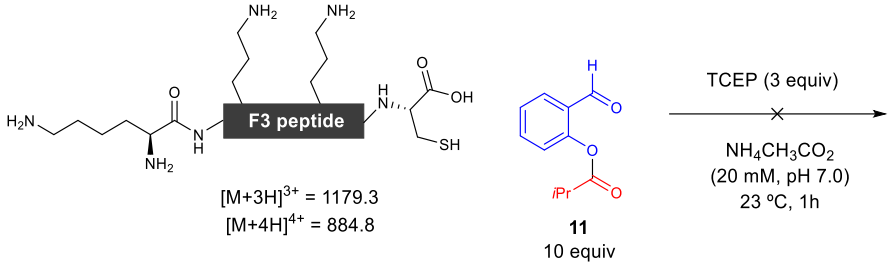


Figure S 50 – HPLC analysis to reaction between Calcitonin peptide and 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0, using HPLC method A. (**A**) Total ion current (TIC) chromatogram. *****RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of Calcitonin peptide (base peak *m/z* 687.7505 $[M+5H]^{5+}$, within δ 6 ppm range); (**D**) EIC chromatogram of modified Calcitonin peptide (base peak *m/z* 708.5558 $[M+5H]^{5+}$, within δ 6 ppm range); (**E**) EIC chromatogram of modified Calcitonin peptide with *N*-acetylation (base peak *m/z* 722.5642 $[M+5H]^{5+}$, within δ 6 ppm range).

8.7 Reaction between F3 peptide and 2-formylphenyl isobutyrate 11



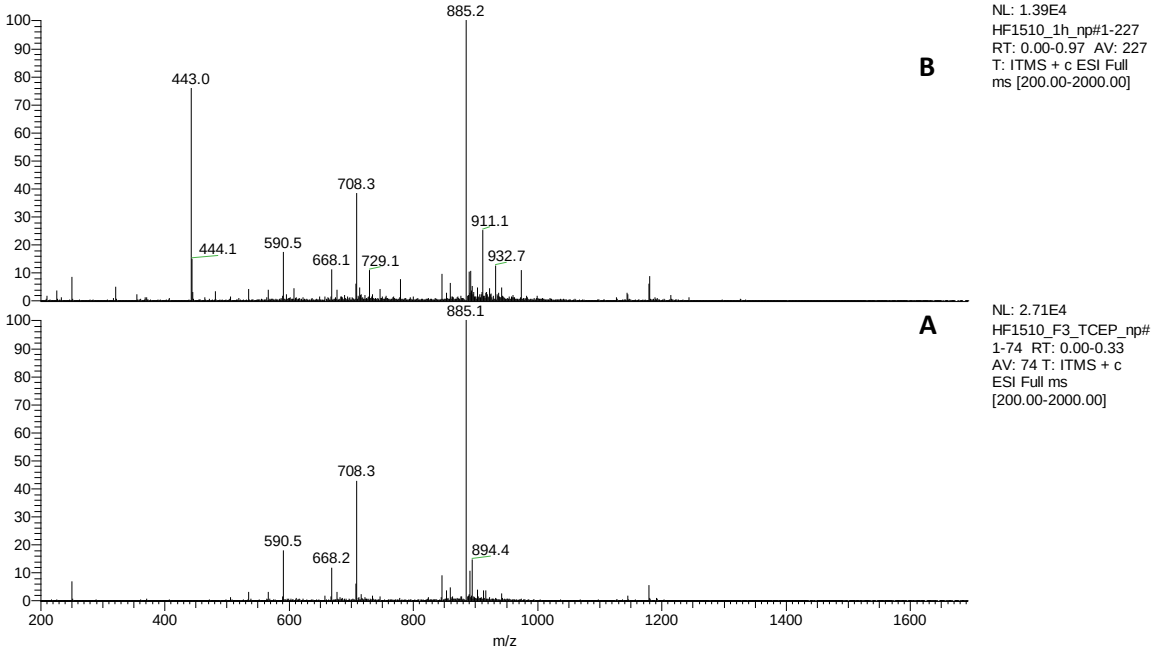


Figure S 51 – (**A**) – ESI^{+}-MS spectrum of F3 peptide; (**B**) - ESI^{+}-MS spectrum of the reaction of F3 peptide with 2-formylphenyl isobutyrate **11**. No conjugate was detected.

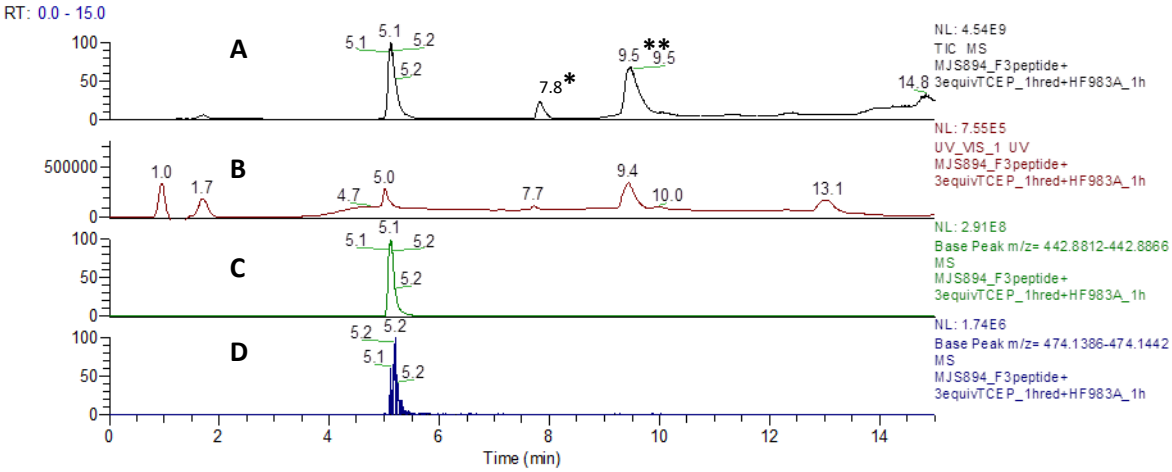
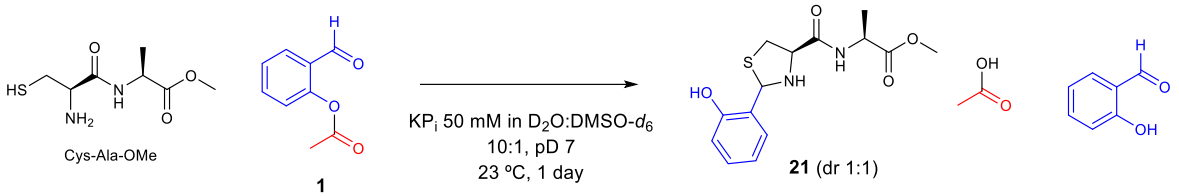


Figure S 52 – HPLC analysis to reaction between F3 peptide and 2-formylphenyl isobutyrate **11** after 60 min at 10 µM in Ammonium acetate 20 mM, pH 7.0. (**A**) Total ion current (TIC) chromatogram. *RT 7.8 min product of TCEP reaction with 2-formylphenyl isobutyrate **11**;**RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm; (**C**) EIC chromatogram of F3 peptide (base peak *m/z* 687.7505 [M+5H]$^{5+}$, within δ 6 ppm range); (**D**) EIC chromatogram of modified F3 peptide (base peak *m/z* 708.5558 [M+5H]$^{5+}$, within δ 6 ppm range); (**E**) EIC chromatogram of modified F3 peptide with *N*-acetylation (base peak *m/z* 722.5642 [M+5H]$^{5+}$, within δ 6 ppm range).

8.8 Reaction between Cys-Ala peptide and *O*-Salicylaldehyde methylester 1



To a solution of methyl *L*-cysteinyl-*L*-alaninate (2.03 mg, 9.84 µmol) in KPi 50 mM in D_2O, pD 7 (500 µL)was added 2-formylphenyl acetate **1** (2 mg, 0.012 mmol)in DMSO-d_6 (50.0 µl). The reaction was monitored by ^{1}H-NMR and ESI$^+$-HRMS. In addition to the diastereoisomers mixture of the phenolic thiazolidine **21**, also salicylaldehyde and acetic acid were formed upon degradation of the 2-formylphenyl acetate added in excess, and the peptide partially dimerized through disulfide bond. Methyl ((4*R*)-2-(2-hydroxyphenyl)thiazolidine-4-carbonyl)-*L*-alaninate **21**, **^{1}H NMR** (400 MHz, D_2O) δ 7.47 (d, *J* = 7.7 Hz, 1H), 7.38 – 7.27 (m, 1H), 7.06 – 6.93 (m, 2H), 5.90 (s, 0.5H), 5.79 (s, 0.5H), 4.50 (m, 1.5H), 4.10 (t, *J* = 7.9 Hz, 0.5H), 3.79 (s, 3H), 3.52 (dd, *J* = 10.6, 7.1 Hz, 0.5H), 3.45 (dd, *J* = 11.1, 7.1 Hz, 0.5H), 3.29 – 3.09 (m, 1H), 1.46 (d, *J* = 7.5 Hz, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 180.3, 180.0, 176.4, 176.3, 164.6, 164.3, 155.7, 155.2, 131.9, 131.5, 129.7, 128.7, 122.2, 122.1, 117.7, 117.5, 68.6, 67.2, 66.2, 54.54, 54.52, 50.4, 50.3, 30.7, 17.4, 17.3. **HRMS** Calculated for $C_{14}H_{19}N_2O_4S$ $[M+H]^+$: 311.1060, found 311.1055.

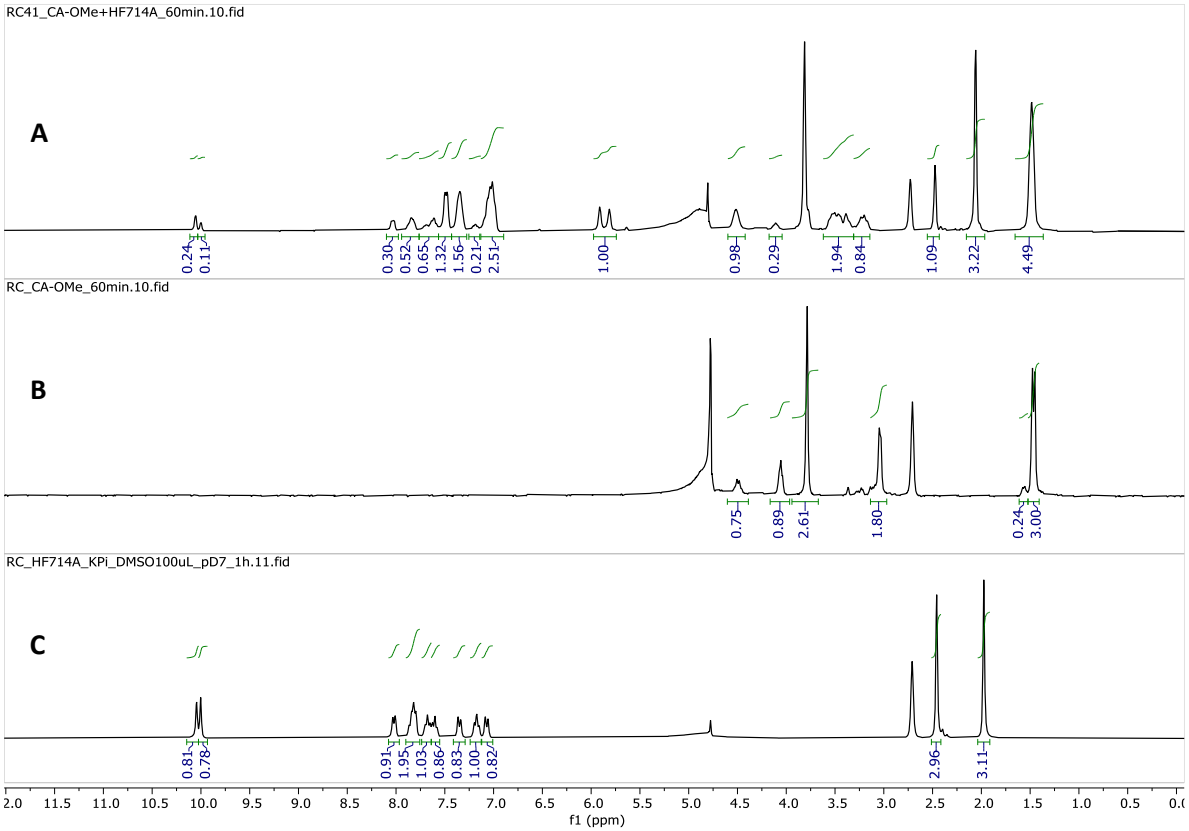


Figure S 53 – ^{1}H NMR (300 MHz) spectra in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1 of: **A**) Cys-Ala peptide reaction with 1.2 equiv of 2-formylphenyl acetate **1**, after 60 min; **B**) Cys-Ala peptide, after 60 min; **C**) 2-formylphenyl acetate **1**, 60 min.

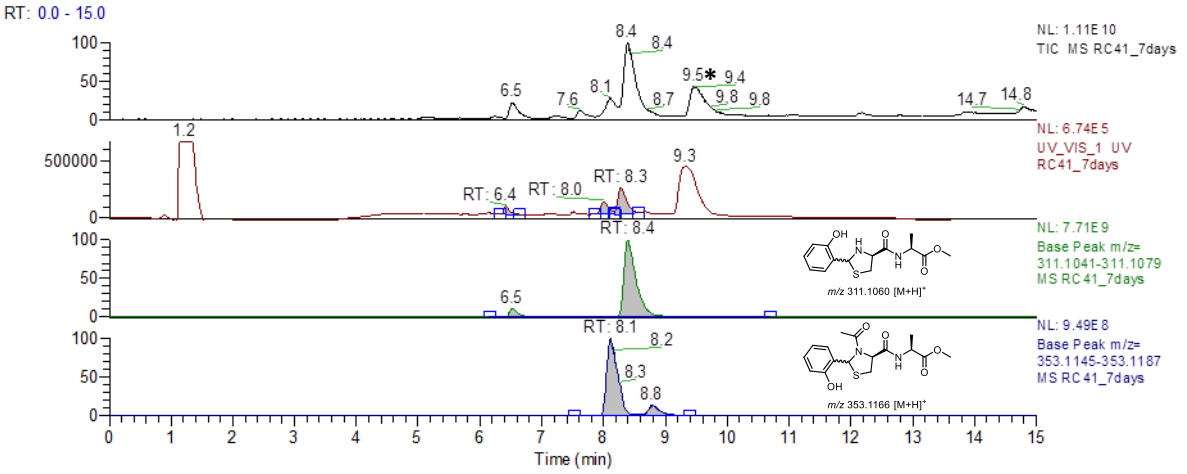


Figure S 54. HPLC-MS analysis to reaction between Cys-Ala-OMe peptide and 2-formylphenyl acetate **1** after 7 days at 20 mM in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1. (**A**) Total ion current (TIC) chromatogram; ***RT 9.5 min phtalate contamination; (**B**) UV chromatogram, detection at 210 nm. According to AUC of peaks of obtained products, phenolic thiazolidine **21** is the major product, over *N*-acetylated phenolic thiazolidine (3:1); (**C**) EIC chromatogram of phenolic thiazolidine **21** (base peak *m/z* 311.1060 $[M+H]^+$, within δ 6 ppm range); (**D**) EIC chromatogram of *N*-acetylated phenolic thiazolidine (base peak *m/z* 353.1166 $[M+H]^+$, within δ 6 ppm range).

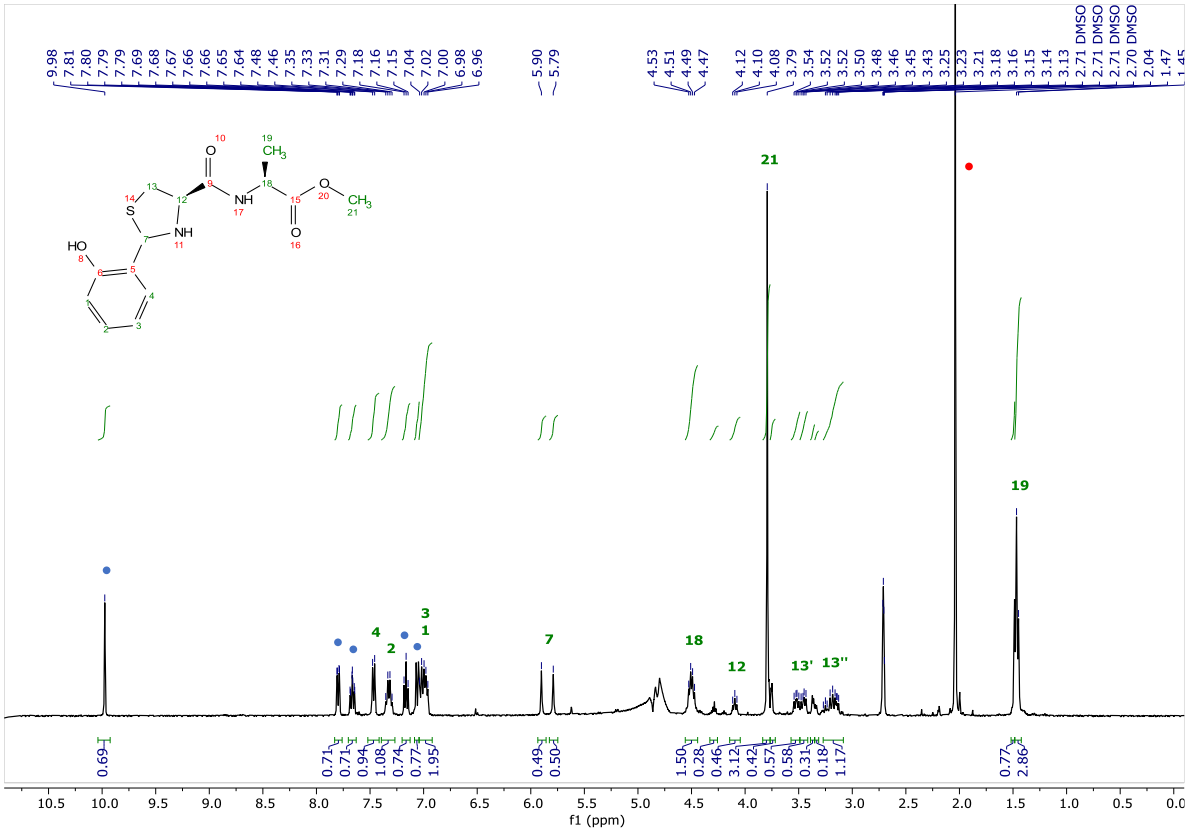


Figure S 55 – ^1H NMR (400 MHz) spectrum of Cys-Ala-OMe peptide reaction with 1.2 equiv of 2-formylphenyl acetate in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1, after 8 h. • Salicylaldehyde and • acetic acid peaks.

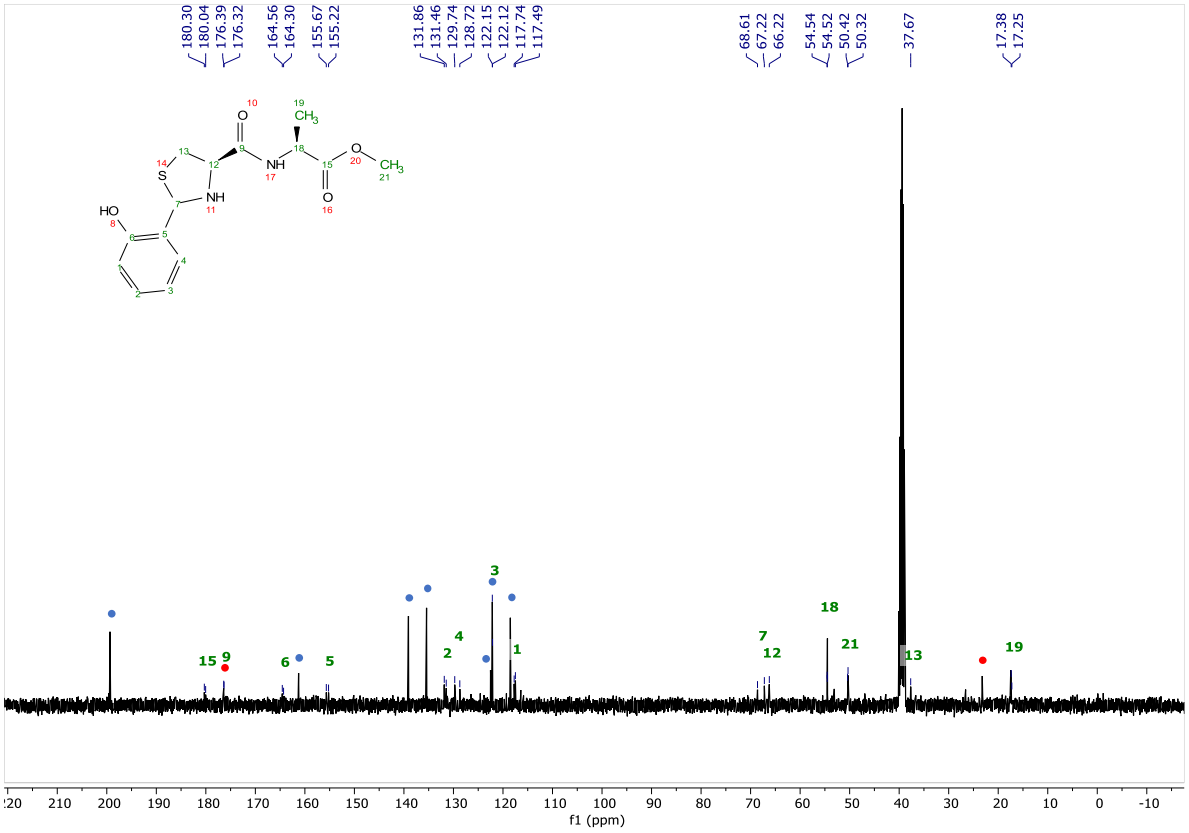


Figure S 56 ^{13}C-NMR (101 MHz) spectrum of Cys-Ala peptide reaction with 1.2 equiv of 2-formylphenyl acetate in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1, after 8 h. • Salicylaldehyde and • acetic acid peaks.

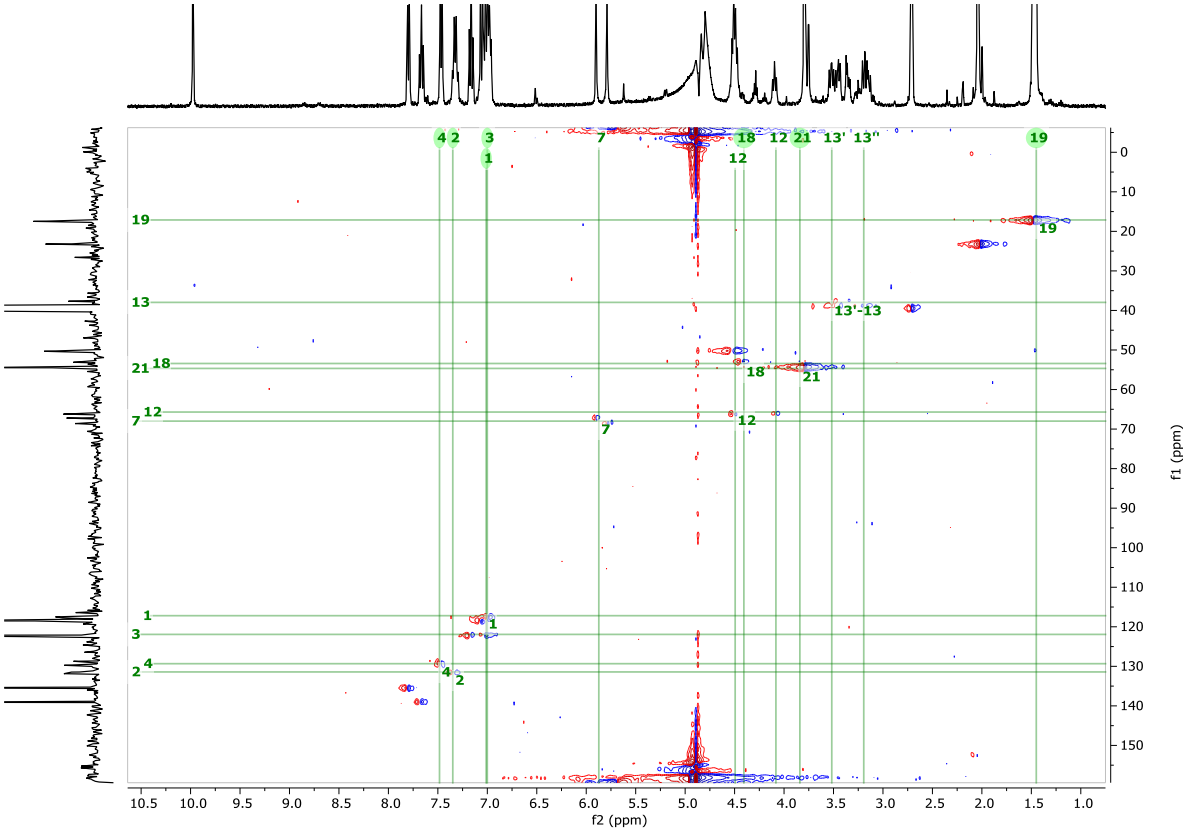


Figure S 57 – HSQC spectrum of Cys-Ala peptide reaction with 1.2 equiv of 2 formylphenyl acetate **11** in KP_i 50 mM (pD 7) in D_2O:DMSO-d_6 10:1, after 3 days.

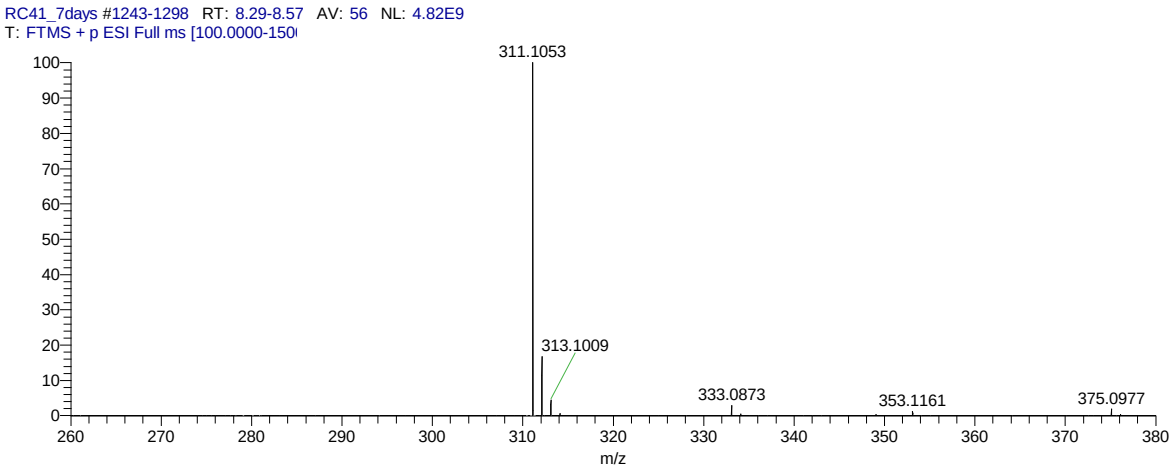


Figure S 58 – ESI^+-HRMS of phenolic thiazolidine **21** after 7 days.

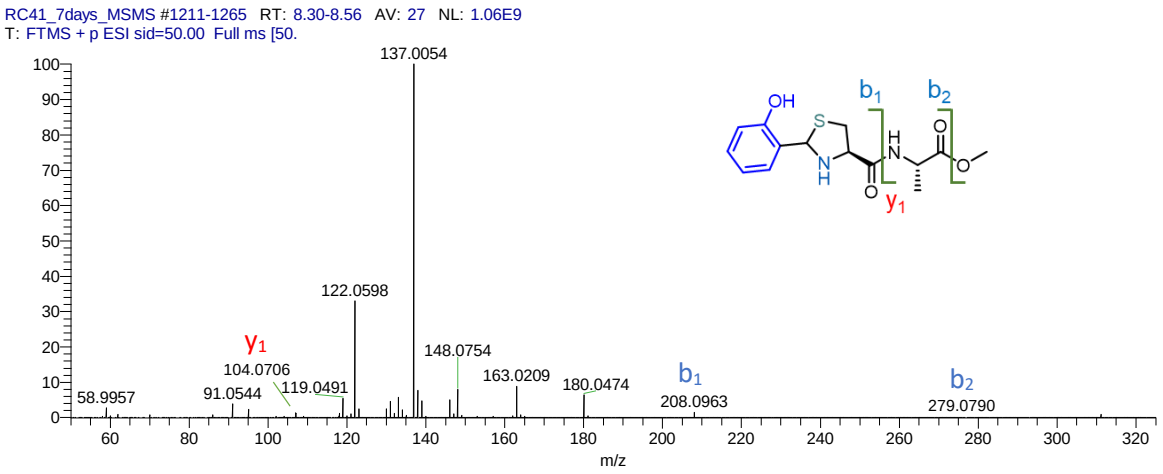
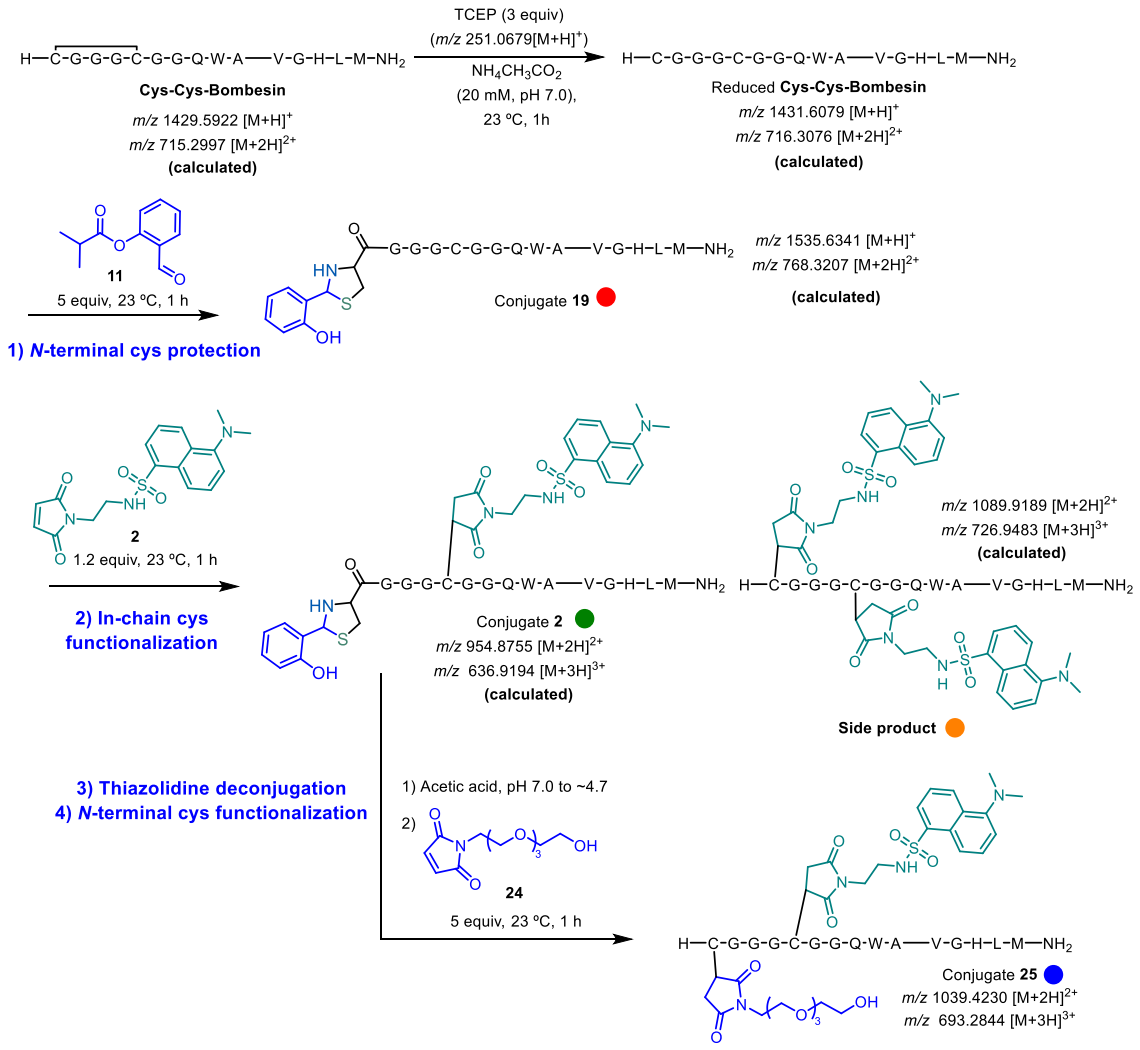


Figure S 59 – ESI$^+$-HRMS/MS spectrum of phenolic thiazolidine **21** after 7 days. MS fragmentation peaks y_1 and $b_{1\text{-}2}$ confirm that the Cys-Ala-OMe modification occurred in the *N*-terminus of the peptide.

9. Orthogonal dual modification of Cys-Cys-bombesin peptide



1) *N*-terminal cys protection

To a solution of *N*-Cys-Cys-Bombesin (5 mg, 3.50 µmol) in ammonium acetate 20 mM pH 7.0 (14 mL) was added Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (0.1 M in MQ water) (0.105 mL, 10.49 µmol) and the solution mixed for 60 min at 23 °C. Then, 2-formylphenyl isobutyrate **11** (0.1 M in ACN) (0.175 mL, 0.017 mmol) and allowed to react for 1 h for complete *N*-terminal cys protection. The reaction was monitored by ESI$^+$-MS after 1 h to afford the phenolic thiazolidine of *N*-Cys-Cys-Bombesin *m/z* 768.4 $[M+2H]^{2+}$.

2) In-chain Cys functionalization

Dansyl-Maleimide **22** (10 mM in ACN) (0.420 mL, 4.20 µmol) was added and allow to react for 1h, to afford conjugate **23**, as confirmed by ESI$^+$-MS *m/z* 955.5 $[M+2H]^{2+}$ and a side product with the installation of two dansyl-maleimides *m/z* 1090.0 $[M+2H]^{2+}$.

3) Deprotection and 4) *N*-terminal Cys functionalization

The pH was adjusted to 4.7 with 350 µL of pure acetic acid and PEG-Maleimide **24** (0.175 mL, 0.017 mmol) was added immediately after for thiazolidine deprotection and installation of the second maleimide unit. The final construct **25** structure was validated by ESI$^+$-MS and MS/MS analysis to the reaction mixture after 2 h.

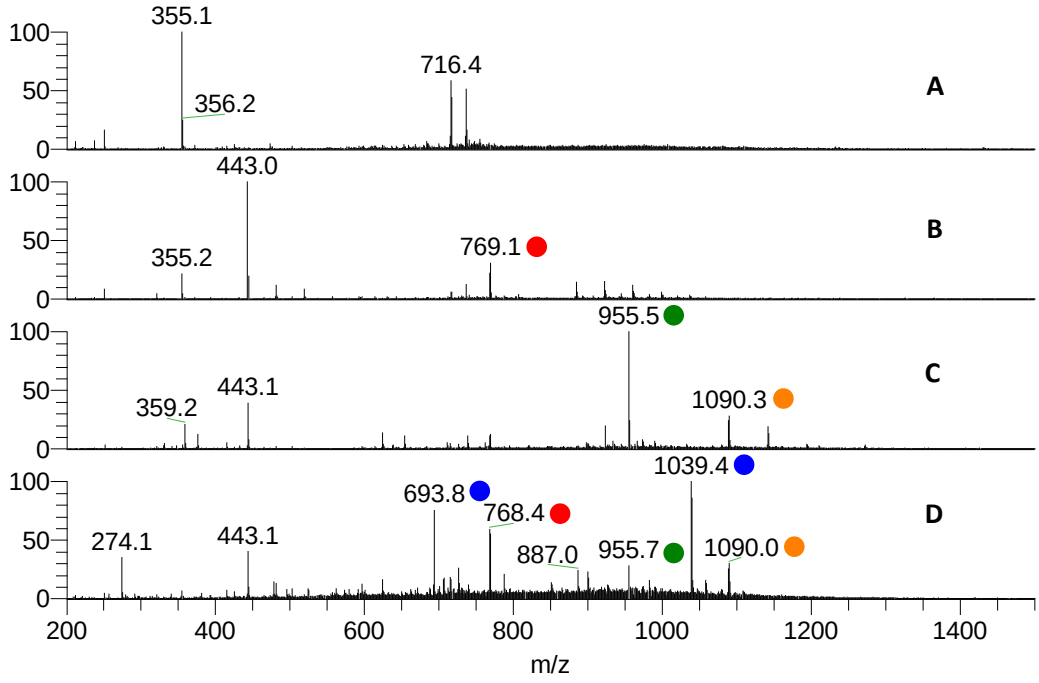
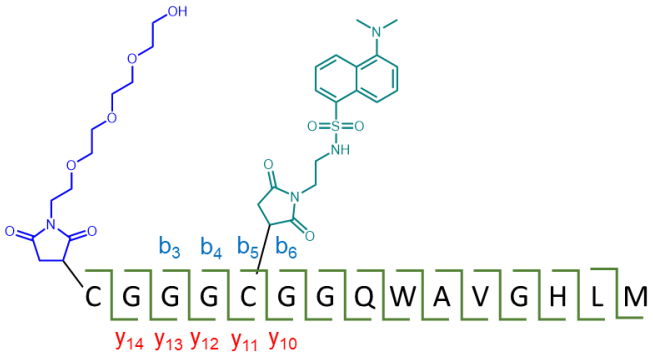


Figure S 60 – ESI$^+$-MS analysis to the orthogonal dual modification of Cys-Cys-bombesin, using 2-formylphenyl isobutyrate **11** as *N*-terminal Cys protecting reagent. (**A**) Cys-Cys-bombesin (0.25 mM) in ammonium acetate reduction with TCEP (3 equiv) for 1 h at 23°C, in ammonium acetate 20 mM, pH7.0; (**B**) *N*-terminal Cys protection with 5 equiv of 2-formylphenyl isobutyrate **11**; (**C**) In-chain Cys functionalization with Dansyl-maleimide **22** (1.2 equiv) for 1 h; (**D**) acidification until pH ~4.7 with acetic acid for thiazolidine deconjugation followed by *N*-terminal Cys functionalization with PEG_4-maleimide **23** (5 equiv). *peak *m/z* 443.0 is a TCEP adduct with 2 formylphenyl isobutyrate **11**.



Fragment ion	Fragment	Calculated	Found
b_3^+	C-PEGMaleimide-G	491.1806	491.1809
b_4^+	C-PEGMaleimide-GG	548.2021	548.2015
b_5^+	C-PEGMaleimide-GGC-DansylMaleimideG	1024.3209	1024.3203
b_6^+	C-PEGMaleimide-GGC-DansylMaleimide-	1081.3424	1081.3409
y_{10}^{2+}	GG-Bombesin	1054.5251	1054.5245
y_{11}^{2+}	C-DansylMaleimide-GG-Bombesin	765.8256	765.8236
y_{12}^{2+}	GC-DansylMaleimide-GG-Bombesin	794.3363	794.3359
y_{13}^{2+}	GGC-DansylMaleimide-GG-Bombesin	822.8471	822.8465
y_{14}^{2+}	GGGC-DansylMaleimide-GG-Bombesin	851.3578	851.3562

Atomic coordinates of the optimized species

CH_3COOH

H	1.000665	1.659271	1.684799
O	4.001004	1.710590	1.389791
C	2.831849	1.964426	1.168675
O	1.895818	1.389077	1.946100
C	2.340576	2.883125	0.093407
H	1.817956	3.726096	0.554958
H	1.629471	2.353228	-0.546211
H	3.181662	3.243287	-0.496020

A

C	-3.648157	-1.737486	-0.576276
C	-3.873232	-2.864092	0.216638
H	0.697134	-1.385992	-0.295443
C	-3.230092	-3.004002	1.445991
C	-2.361409	-2.009080	1.884037
O	-3.080709	3.058984	0.265994
C	-2.130877	-0.864505	1.111672
H	-3.498180	2.777120	-0.558236
C	-2.785224	-0.754511	-0.123550
O	-2.602318	0.377093	-0.914203
C	-1.216808	0.156547	1.662658
H	-0.712206	-0.138442	2.596906
O	-1.028645	1.268714	1.188646
C	-1.487076	0.413391	-1.689872
O	-0.751021	-0.543859	-1.798009
C	-1.295074	1.747405	-2.327476
S	2.930892	2.768516	0.720158
C	2.377943	1.085746	1.157558
C	1.673868	0.379810	0.004323
C	2.547111	0.343418	-1.240665
O	3.717164	-0.270806	-1.032380
O	2.220113	0.788136	-2.325320
N	1.252522	-0.946999	0.436408
H	-4.130791	-1.614965	-1.540373
H	-4.551290	-3.635306	-0.134547
H	-3.405253	-3.882570	2.057497
H	-1.851685	-2.104017	2.839482
H	-2.427122	2.362863	0.450349
H	-0.617943	1.653203	-3.175363
H	-0.847875	2.411365	-1.577982
H	-2.252920	2.171722	-2.633229
H	1.710261	3.244072	0.414908
H	1.700227	1.123536	2.012914
H	3.277997	0.538645	1.452247
H	0.788097	0.954339	-0.273949
H	4.222602	-0.300374	-1.863793
H	2.067775	-1.531617	0.611183

TS_{AB}

C	-3.618826	-1.766193	-0.552434
C	-3.677723	-2.889786	0.270526
H	0.413564	-1.107669	-0.401712
C	-2.824413	-2.996058	1.368080
C	-1.921665	-1.971789	1.639648
O	-3.416374	2.673361	0.585952
C	-1.846506	-0.833635	0.831439
H	-3.858904	2.299723	-0.187236
C	-2.706888	-0.758303	-0.269051
O	-2.741209	0.372105	-1.086691
C	-0.864404	0.238577	1.219794
H	-0.462991	0.079974	2.232118
O	-0.936490	1.410863	0.765705
C	-1.648651	0.659167	-1.833987
O	-0.734981	-0.127115	-1.973176
C	-1.737577	2.019405	-2.438566
S	3.509102	2.396163	0.848985
C	2.330569	1.065728	1.251041
C	1.649204	0.494454	0.011263
C	2.660082	0.022709	-1.023540

O	3.510489	-0.880315	-0.530443
O	2.684787	0.405178	-2.175886
N	0.752563	-0.588404	0.408451
H	-4.271565	-1.658849	-1.412886
H	-4.390285	-3.678401	0.051303
H	-2.864815	-3.869003	2.011026
H	-1.257606	-2.041406	2.498428
H	-2.561086	2.199524	0.614400
H	-0.988447	2.123640	-3.221956
H	-1.548413	2.752223	-1.647373
H	-2.740486	2.195392	-2.832561
H	2.593850	3.272199	0.397530
H	1.572391	1.436550	1.943679
H	2.911126	0.288530	1.755698
H	1.038698	1.265761	-0.470687
H	4.126396	-1.165037	-1.229092
H	1.191784	-1.231259	1.068613

B

C	-3.680314	-1.747128	-0.434172
C	-3.655398	-2.849187	0.416067
H	0.316465	-0.587718	-0.831189
C	-2.667431	-2.945619	1.395064
C	-1.717288	-1.934708	1.516331
O	-3.286135	2.460068	0.835054
C	-1.717571	-0.817884	0.673139
H	-3.811662	2.085422	0.116733
C	-2.716759	-0.753860	-0.302576
O	-2.872045	0.353008	-1.140238
C	-0.641909	0.249120	0.887436
H	-0.311885	0.164365	1.938162
O	-0.878027	1.478354	0.464784
C	-1.894444	0.670579	-2.011763
O	-0.947557	-0.061768	-2.229539
C	-2.149026	1.987739	-2.661640
S	3.751479	2.024717	1.377356
C	2.221674	1.036876	1.418072
C	1.753949	0.632929	0.020929
C	2.881731	0.011252	-0.790696
O	3.361529	-1.088128	-0.212375
O	3.291034	0.465015	-1.837042
N	0.636569	-0.342814	0.116543
H	-4.440169	-1.639469	-1.201520
H	-4.407690	-3.624517	0.313668
H	-2.641489	-3.797867	2.066193
H	-0.952353	-1.999172	2.287881
H	-2.379465	2.058986	0.703187
H	-1.434513	2.145181	-3.467577
H	-2.035685	2.768218	-1.902411
H	-3.173241	2.026526	-3.038818
H	3.222664	3.098940	0.764981
H	1.435047	1.600249	1.920949
H	2.453188	0.147392	2.010738
H	1.382383	1.495648	-0.533454
H	4.080725	-1.463594	-0.752614
H	0.947915	-1.199143	0.590753

TS_{BC}

C	-3.783668	-1.747523	-0.444691
C	-3.773315	-2.829752	0.430667
H	0.392032	-0.875862	-0.808901
C	-2.750543	-2.964623	1.371798
C	-1.745820	-2.002864	1.436911
O	-3.272110	2.269071	1.090940
C	-1.738120	-0.909920	0.566154
H	-3.939301	1.824695	0.551912
C	-2.765519	-0.800941	-0.375297
O	-2.857235	0.289323	-1.218340
C	-0.673527	0.167836	0.683223
H	-0.428707	0.316805	1.744813
O	-0.945420	1.311497	0.033540
C	-1.654762	0.786258	-1.727655
O	-0.823212	-0.012890	-2.179556
C	-1.839377	2.176245	-2.253046
S	3.539917	2.338740	0.968814
C	2.100596	1.239226	1.176372
C	1.723147	0.549220	-0.131989
C	2.919468	-0.145633	-0.768959
O	3.461831	-1.049631	0.043430
O	3.324432	0.100350	-1.883703
N	0.650789	-0.460363	0.096960
H	-4.568382	-1.622684	-1.184067
H	-4.566315	-3.569042	0.376066
H	-2.741961	-3.807733	2.054565
H	-0.954603	-2.085181	2.179891
H	-2.415855	1.960655	0.712854
H	-0.859499	2.630645	-2.404725
H	-2.437950	2.781417	-1.571921
H	-2.350727	2.105060	-3.218582
H	2.922015	3.237380	0.182675
H	1.254370	1.817542	1.548913
H	2.384378	0.499850	1.930519
H	1.331710	1.264451	-0.856104
H	4.224766	-1.470832	-0.392760
H	0.980772	-1.202786	0.725677

C

C	-3.705410	-1.806565	-0.227981
C	-3.664056	-2.681363	0.852484
H	-0.016730	-0.351434	-1.753032
C	-2.632798	-2.601213	1.793421
C	-1.645284	-1.632911	1.647720
O	-3.546868	2.249794	0.714494
C	-1.674108	-0.746949	0.568885
H	-4.068412	1.601422	0.222227
C	-2.703154	-0.846449	-0.365530
O	-2.791746	0.024321	-1.426074
C	-0.631803	0.324912	0.407265
H	-0.530187	0.906556	1.331664
O	-1.054914	1.291267	-0.559180
C	-1.613373	0.760306	-1.742120
O	-0.703772	-0.069973	-2.401759
C	-2.033479	1.901318	-2.628497
S	3.849677	1.509366	1.880311
C	2.377129	0.467920	1.604890
C	1.788370	0.632318	0.204948
C	2.811939	0.350788	-0.882191
O	3.417397	-0.826866	-0.716455
O	3.036421	1.089793	-1.819909
N	0.641214	-0.257200	-0.022078
H	-4.497777	-1.855036	-0.968289
H	-4.441588	-3.430922	0.961261
H	-2.605865	-3.283704	2.636110
H	-0.844017	-1.547114	2.378622
H	-2.653115	2.151672	0.349688
H	-1.154931	2.498484	-2.878112
H	-2.771032	2.520007	-2.113536
H	-2.469143	1.496004	-3.544052
H	3.210628	2.691029	1.845051
H	1.614147	0.693254	2.353341
H	2.708371	-0.563781	1.751559
H	1.455216	1.662186	0.048331
H	4.038742	-0.983602	-1.449915
H	0.791105	-1.153127	0.441836

C'

C	-3.527245	-1.997868	-0.180119
C	-3.607395	-2.765805	0.977499
H	-2.356494	-1.677779	-4.042141
C	-2.700496	-2.566821	2.020826
C	-1.714647	-1.592483	1.900248
O	-3.165694	-1.459988	-3.541386

C	-1.616635	-0.813997	0.745136
H	-2.903111	-0.968759	-2.655737
C	-2.528171	-1.034246	-0.283533
O	-2.499247	-0.256546	-1.439427
C	-0.557363	0.244817	0.603523
H	-0.611720	0.945076	1.449221
O	-0.840583	1.079910	-0.519186
C	-1.260572	0.459071	-1.698754
O	-0.372545	-0.480777	-2.173538
C	-1.585363	1.541413	-2.698322
S	3.715475	2.227991	1.752832
C	2.618436	0.767255	1.688991
C	1.840521	0.643604	0.386030
C	2.706367	0.277825	-0.834288
O	2.086175	0.220887	-1.950480
O	3.925973	0.049978	-0.692329
N	0.763033	-0.355578	0.477963
H	-4.230875	-2.130811	-0.996754
H	-4.384102	-3.518701	1.064686
H	-2.765983	-3.164025	2.924118
H	-1.009876	-1.420709	2.710699
H	-3.627396	-2.294057	-3.331514
H	-0.660470	2.070952	-2.938352
H	-2.316399	2.240217	-2.288671
H	-1.978784	1.083695	-3.608850
H	2.762486	3.111854	2.089427
H	1.921790	0.825559	2.528905
H	3.259479	-0.105873	1.834764
H	1.385320	1.612224	0.150565
H	0.583914	-0.160297	-2.019021
H	0.914696	-0.960201	1.283966

$TS_{C'D}$

C	-3.554320	-2.017778	-0.183074
C	-3.621794	-2.795978	0.968675
H	-2.244904	-1.599704	-3.939235
C	-2.715413	-2.592758	2.011185
C	-1.740520	-1.607034	1.891647
O	-3.073961	-1.421700	-3.461010
C	-1.652219	-0.820629	0.740923
H	-2.831711	-0.875871	-2.460025
C	-2.567788	-1.038592	-0.287029
O	-2.543357	-0.249581	-1.427989
C	-0.587596	0.236002	0.609167
H	-0.625338	0.911585	1.475649
O	-0.866527	1.098810	-0.485122
C	-1.185339	0.512291	-1.723990
O	-0.338736	-0.355477	-2.202284
C	-1.632224	1.620403	-2.651754
S	3.669420	2.272384	1.620331
C	2.576267	0.806813	1.638906
C	1.813164	0.586070	0.338505
C	2.708802	0.129806	-0.813009
O	2.096752	-0.153844	-1.941640
O	3.926938	0.034585	-0.713943
N	0.725908	-0.388172	0.469926
H	-4.260311	-2.154864	-0.996989
H	-4.389497	-3.558601	1.052471
H	-2.771817	-3.195152	2.911680
H	-1.034312	-1.434051	2.700831
H	-3.496998	-2.280771	-3.289183
H	-0.755531	2.226350	-2.894384
H	-2.396112	2.248483	-2.191114
H	-2.019294	1.179401	-3.573359
H	2.720238	3.165704	1.941571
H	1.861581	0.922119	2.456741
H	3.210696	-0.055073	1.860037
H	1.377580	1.536885	0.008519
H	1.027989	-0.180729	-1.928727
H	0.887283	-0.983325	1.281094

D

C	-3.439487	-2.121970	-0.018076
C	-3.087277	-2.977267	1.019219
H	-5.367050	-0.726050	-3.439903
C	-1.884572	-2.785787	1.694919
C	-1.041800	-1.750923	1.299106
O	-5.432830	-0.670326	-2.476624
C	-1.357006	-0.893343	0.237877
H	-3.872485	-0.471172	-1.744623
C	-2.598400	-1.070926	-0.403394
O	-2.984446	-0.220623	-1.395031
C	-0.388555	0.247157	-0.056375
H	-0.595683	1.060540	0.649342
O	-0.604992	0.915415	-1.319718
C	-0.519032	0.206842	-2.457702
O	-0.250471	-0.979724	-2.489382
C	-0.784277	1.064417	-3.654845
S	2.571964	2.228399	3.003227
C	1.916574	0.734395	2.179912
C	1.890572	0.814529	0.652728
C	3.275919	0.651841	0.031149
O	3.271113	0.297533	-1.257966
O	4.322468	0.846076	0.613173
N	0.995783	-0.147066	0.023778
H	-4.381653	-2.251648	-0.542384
H	-3.757947	-3.783323	1.300346
H	-1.601762	-3.429183	2.521104
H	-0.117007	-1.598894	1.848833
H	-5.852437	-1.498091	-2.204537
H	-0.029907	1.854299	-3.708124
H	-1.762650	1.540209	-3.549561
H	-0.753986	0.458164	-4.558718
H	1.456537	2.964162	2.868439
H	0.900355	0.561765	2.541528
H	2.537136	-0.104687	2.505614
H	1.558922	1.814631	0.339795
H	2.347873	0.079417	-1.506505
H	1.130793	-1.086988	0.383781

E

C	-3.352914	-2.124864	0.469877
C	-3.187605	-3.130862	1.413637
H	2.749600	1.324726	-1.099266
C	-2.034037	-3.167053	2.199302
C	-1.071583	-2.177978	2.036235
O	3.423876	1.366992	-0.385378
C	-1.218663	-1.159278	1.087150
H	-2.875665	1.939904	2.262630
C	-2.373832	-1.141813	0.288793
O	-2.625150	-0.178238	-0.650880
C	-0.085650	-0.152404	1.061384
H	0.185565	0.118458	2.083073
O	-0.555842	1.179063	0.464315
C	-1.547462	1.824313	0.916770
O	-2.117404	1.389819	1.986969
C	-1.980321	3.052461	0.220689
S	0.830504	-2.759745	-3.065241
C	0.407747	-2.193198	-1.379871
C	1.071828	-0.858287	-1.060700
C	0.527389	0.311859	-1.916524
O	-0.681275	0.287773	-2.290947
O	1.329654	1.247810	-2.169732
N	1.076643	-0.538616	0.378007
H	-4.238342	-2.084195	-0.157044
H	-3.957321	-3.887030	1.533206
H	-1.893054	-3.946965	2.939930
H	-0.180622	-2.181785	2.659568
H	4.105278	0.731403	-0.638322
H	-1.743139	3.908097	0.861198
H	-3.063483	3.015379	0.079681
H	-1.462861	3.136747	-0.733136

H	2.142464	-2.943830	-2.836901
H	0.712889	-2.948763	-0.653141
H	-0.680546	-2.127413	-1.376043
H	2.133456	-0.947709	-1.309720
H	-1.829691	-0.023870	-1.248356
H	1.841857	0.103591	0.579288

TS_{EF}

C	-3.342401	-2.147935	0.413641
C	-3.237057	-3.064064	1.451100
H	2.901915	1.220181	-1.256919
C	-2.112290	-3.055943	2.278895
C	-1.120838	-2.108685	2.058805
O	3.399774	1.307156	-0.418346
C	-1.206137	-1.180183	1.011351
H	-2.759672	1.924823	2.536560
C	-2.332949	-1.206847	0.172299
O	-2.543319	-0.350191	-0.864339
C	-0.055904	-0.212813	0.972375
H	0.206891	0.150980	1.963902
O	-0.726085	1.296122	0.391351
C	-1.621823	1.902111	1.013180
O	-2.066370	1.379331	2.123699
C	-2.185481	3.174150	0.495426
S	0.904682	-2.889015	-3.065494
C	0.440521	-2.245908	-1.420040
C	1.135040	-0.916692	-1.150640
C	0.678968	0.214027	-2.102304
O	-0.551129	0.300460	-2.379934
O	1.565984	1.003673	-2.514178
N	1.049904	-0.454703	0.242458
H	-4.205687	-2.144848	-0.244589
H	-4.031563	-3.786419	1.610957
H	-2.015783	-3.766644	3.092492
H	-0.252501	-2.074226	2.712342
H	4.208537	0.794541	-0.543854
H	-2.212884	3.915205	1.297956
H	-3.212753	2.975361	0.172319
H	-1.592039	3.527830	-0.345100
H	2.210686	-3.057391	-2.794511
H	0.698587	-2.975880	-0.650263
H	-0.644369	-2.149085	-1.460173
H	2.206609	-1.057933	-1.313713
H	-1.703214	-0.090514	-1.349602
H	1.844945	0.135468	0.483889

F

C	-3.243418	-1.903493	0.730237
C	-3.284640	-2.819471	1.766721
H	3.180076	1.135149	-1.302686
C	-2.155724	-3.035860	2.566631
C	-1.004272	-2.320652	2.297919
O	3.538917	0.999505	-0.408087
C	-0.915081	-1.429432	1.203555
H	-3.719876	1.637374	1.672567
C	-2.071265	-1.190407	0.425022
O	-2.190652	-0.288891	-0.560002
C	0.390541	-0.773104	1.129666
H	0.802963	-0.480320	2.093819
O	-0.719298	1.724909	1.401624
C	-1.890974	1.964760	1.169252
O	-2.822180	1.389601	1.947754
C	-2.379948	2.882929	0.092030
S	0.510529	-2.386285	-3.469477
C	0.323212	-2.036795	-1.689115
C	1.179144	-0.841033	-1.289372
C	0.797777	0.485741	-1.997380
O	-0.379997	0.906780	-1.809604
O	1.684484	1.057265	-2.662819
N	1.211101	-0.564708	0.156275
H	-4.121859	-1.700924	0.125746
H	-4.207808	-3.353784	1.969704
H	-2.186067	-3.732727	3.396628
H	-0.128065	-2.450672	2.927911
H	4.350500	0.490311	-0.533495
H	-2.881514	3.736960	0.557563
H	-3.107074	2.364179	-0.538256
H	-1.539188	3.228256	-0.507004
H	1.828886	-2.651202	-3.434824
H	0.606545	-2.911981	-1.100920
H	-0.742889	-1.854431	-1.559671
H	2.220715	-1.058005	-1.536624
H	-1.356777	0.161842	-0.943055
H	2.082471	-0.074884	0.403260

G

C	-3.441830	-2.100771	1.903368
C	-3.486596	-3.283416	1.184888
H	4.617587	0.375602	-0.819749
C	-2.326809	-3.811983	0.599101
C	-1.129217	-3.139073	0.736184
O	4.897741	1.001676	-1.514456
C	-1.050746	-1.929334	1.463523
C	-2.231738	-1.416345	2.049198
O	-2.288863	-0.263096	2.759374
C	0.198623	-1.256514	1.737164
H	0.268443	-0.663662	2.648612
S	0.915950	0.555820	-1.607567
C	0.725565	-1.243359	-1.367552
C	1.614110	-1.821129	-0.262538
C	3.099093	-1.510206	-0.558291
O	3.732136	-0.839389	0.301964
O	3.529977	-1.929357	-1.653361
N	1.298170	-1.271618	1.058792
H	-4.332763	-1.677503	2.354898
H	-4.433727	-3.802465	1.075516
H	-2.364409	-4.745329	0.049228
H	-0.231455	-3.575913	0.316414
H	4.572521	0.606832	-2.334353
H	2.176583	0.521086	-2.081511
H	0.956069	-1.767461	-2.295507
H	-0.331986	-1.402047	-1.148055
H	1.512469	-2.908732	-0.248250
H	-1.485516	0.270831	2.688703
H	2.096319	-0.758743	1.449665

TS_{GH}

C	-3.248561	-1.712778	1.452769
C	-3.284389	-3.093998	1.310558
H	3.650495	0.263902	-0.305387
C	-2.100266	-3.818673	1.154178
C	-0.886495	-3.146247	1.116605
O	3.243792	1.152699	-0.259149
C	-0.827354	-1.748530	1.231885
C	-2.027309	-1.039647	1.428058
O	-2.085869	0.318573	1.559082
C	0.473173	-1.040080	1.201163
H	0.626699	-0.260990	1.948649
S	0.236098	0.142160	-0.703690
C	0.845514	-1.371861	-1.485555
C	1.705908	-2.232542	-0.491570
C	3.180327	-2.242537	-0.948298
O	4.003223	-1.545411	-0.291013
O	3.426170	-2.929363	-1.964667
N	1.608405	-1.685660	0.853427
H	-4.156168	-1.135046	1.595066
H	-4.239749	-3.608651	1.337604
H	-2.122548	-4.899570	1.069251
H	0.034903	-3.711305	1.026480
H	3.581343	1.634765	-1.025904
H	1.453128	0.751999	-0.558176
H	1.430072	-1.097297	-2.365510

H	-0.040295	-1.919557	-1.810687
H	1.338988	-3.257254	-0.548795
H	-1.235403	0.712073	1.796885
H	2.458868	-1.171771	1.085643

H

C	-3.339742	-1.890862	1.678342
C	-3.237831	-3.278896	1.705791
H	3.684713	-0.666781	-0.555802
C	-2.007624	-3.890361	1.468539
C	-0.889145	-3.106958	1.188178
O	3.198981	0.852725	-0.004320
C	-0.973471	-1.713344	1.127117
C	-2.215329	-1.117945	1.398456
O	-2.401813	0.246346	1.367150
C	0.216953	-0.843582	0.792792
H	0.359962	-0.082909	1.566151
S	-0.074536	0.080409	-0.832508
C	1.114028	-0.965404	-1.740567
C	1.591285	-2.043007	-0.733086
C	3.023326	-2.465102	-1.027452
O	3.992149	-1.576808	-0.823451
O	3.296464	-3.573056	-1.449881
N	1.460920	-1.559377	0.643170
H	-4.280910	-1.388445	1.879684
H	-4.116677	-3.876765	1.926180
H	-1.914140	-4.970823	1.503915
H	0.069765	-3.585216	1.019291
H	3.892627	1.498713	-0.200656
H	2.386885	1.214484	-0.393740
H	1.937815	-0.344702	-2.100929
H	0.624412	-1.436660	-2.593083
H	0.975437	-2.934349	-0.861210
H	-1.578027	0.726737	1.527270
H	2.213222	-0.907659	0.857061

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