Electronic Supplementary Material (ESI) for ChemComm.

Supplemental Information

Rapid, green disulphide bond formation in water using the corrin dicyanocobinamide

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Detailed experimental, materials, and methods

Materials and instrumentation

Dimethylformamide (DMF), dichloromethane (DCM), diethyl ether, trifluoroacetic acid (TFA), triisopropyl silane (TIPS), *N,N*'-diisopropylcarbodiimide (DIC), piperidine, NaCl, α-cyano-4-hydroxy-cinnamic acid (CHCA), and HPLC grade acetonitrile (MeCN) were purchased from Sigma Aldrich (Milwaukee, WI, USA). Fmoc-protected amino acids, Oxyma Pure, and ProTide rink-amide resin were purchased from CEM (Matthews, NC, USA). Dithiothreitol (DTT) was purchased from MP Biomedicals, LLC (Solon, OH, USA).

Deionized water was prepared in-house and filtered through a Nalgene Rapid-Flow 0.2 µm PES vacuum filter prior to use. 50 mM HEPES stock solution was prepared by dissolving 11.915 HEPES free acid and 6.83 g NaCl in a total volume of 1 L of deionized water. The solution was diluted to 20 mM and the pH was adjusted to 7 with KOH.

Peptides were synthesized in-house on a microwave assisted CEM Liberty Blue peptide synthesizer and cleaved from the support resin using a CEM Razor. Reverse-phase high performance liquid chromatography (RP-HPLC) was performed on an Agilent 1100 Series instrument with an Agilent ZORBAX 300SB-C8 (5 µm, 9.4 x 250 mm), Agilent Eclipse XDB-C18 column (3.5 µm, 4.6 x 100 mm), or Agilent Eclipse XDB-C18 column (5 µm, 4.6 x 150 mm). MALDI-TOF MS was conducted on a Bruker microflex with an MSP 96 polished steel BC target and CHCA matrix. High resolution mass spectrometry (HRMS) was performed on a Thermo Orbitrap Fusion Lumos at the SUNY Upstate Proteomics & Mass Spectrometry Core Facility.

For spin filtration studies, Amicon Ultra – 15 centrifugal filters (Ultracel – 3K) were used (Merck Millipore Ltd., Cork, Ireland).

HPLC Methods

All HPLC analyses were done in reverse phase with a gradient system of 0.1% TFA in water (solvent A) and 0.1% TFA in MeCN (solvent B). The diode array detector was set to 280 nm and 360 nm to detect the peptides and Cbi, respectively. In the case a peptide did not have an aromatic residue to detect at 280 nm, 220 nm was used to detect peptide bonds.

Method A was used for peptide purification and developed on an Agilent ZORBAX 300SB-C8 column (5 µm, 9.4 x 250 mm) using the following gradient: 10%-75% ACN over 15 min, 95% ACN for 5 min, 10% ACN for 5 min. Methods B-D were used for analytical studies. Method B was developed on an Eclipse Plus C18 column (3.5 µm, 4.6 x 100 mm) using the following gradient: 1-70% ACN for 15 min, 95% ACN for 5 min, 1% ACN for 5 min. Method C was developed on an Agilent Eclipse XDB-C18 column (5 µm, 4.6 x 150 mm) using the following gradient: 18% ACN for 5 min, 18-60% ACN for 5 min, 95% ACN for 2.5 min, 18% ACN for 2.5 min. Method D was developed on an Agilent Eclipse XDB-C18 column (5 µm, 4.6 x 150 mm) using the following gradient: 20% ACN for 5 min, 20-75% ACN for 5 min, 95% ACN for 2.5 min, 20% ACN for 2.5 min.

Peptide synthesis

Peptides were synthesized following solid-phase chemistry at a 0.025 mmol scale on rink amide resin on an automatic peptide synthesizer. Fmoc-protected amino acids were prepared at 0.2 M in DMF.

The activator and activator base used for coupling were Oxyma Pure (0.25 M) and DIC (0.125 M), respectively. The deprotection reagent used between couplings was 20% piperidine in DMF.

After synthesis, the resin-bound peptides were transferred to the cleavage instrument with DCM, the DCM was removed under vacuum filtration, and the deprotection/cleavage solution was added (95% TFA, 2.5% TIPS, and 2.5% H_2O) and left to incubate for 40-45 min at 40°C. The peptides were precipitated with cold (-20°C) diethyl ether and centrifuged for 10 min at 4,000 rpm to obtain a crude pellet.

Purification was achieved by RP-HPLC using method A. The peptide was then flash frozen with liquid nitrogen and lyophilized to a dry powder. Purity traces were obtained using methods B-D and >90% purity was ensured before further experimentation.

Cbi synthesis

Cbi was prepared in-house as described by *Gryko et al*.,[1] however, it is also commercially available from Sigma-Aldrich. Cyanocobalamin (100 mg, 0.074 mmol), NaCN (13 mg, 0.25 mmol), and EtOH (6.6 mL) were added to a microwave reaction vessel with a magnetic stir bar and sealed with a cap. The vessel was heated to 120 °C for 10 min using 300 W. The mixture was transferred to a flask using EtOH and reduced to minimal volume by rotary evaporation. The concentrated mixture was then loaded onto a normal phase flash chromatography cartridge and eluted using an isocratic method of MeOH in EtOAc (2:1 v:v) at a flow rate of 5 mL/min. The major violet fraction was isolated, dried by rotary evaporation, dissolved in iPrOH, and vacuum filtered through a celite plug to remove any excess cyanide and silica. The filtrate was then dried, redissolved in water, and lyophilized. ^{1}H NMR (400 MHz, D_2O) δ 5.891 (s, 1H), 3.907 (m, *J*=5.95 Hz, 1H), 3.839 (d, *J*=8.23, 1H), 3.748 (d, *J*=10.44, 1H), 3.654 (q, *J*=7.11, 2H), 3.572 (q, *J*=7.08 Hz, 1H), 3.404 (dd, *J*=7.00,4.78, 1H), 3.312-3.151 (3H, overlapped), 2.914-2.857 (m, 1H), 2.748-2.712 (m, 4H), 2.600-2.380 (6H, overlapped), 2.316-2.231 (14H, overlapped), 2.148-2.069 (m, 4H), 2.030-1.943 (m, 1H), 1.915 (s, 3H), 1.891-1.744 (m, 3H), 1.683 (s, 3H), 1.532 (s, 3H), 1.489 (s, 3H), 1.430 (s, 3H), 1.312 (s, 3H), 1.184 (t, *J*=7.12 Hz, 7H), 1.151 (d, *J*=6.36 Hz, 3H); UV−vis (H_2O) λ 276, 312, 367, 503, 539, 580 nm; MS (MALDI-TOF) calculated for $C_{48}H_{73}CoN_{11}O_8$ ($[M-2CN+H]^+$) 991.11, found 990.72; HPLC t_R values on method B: 1.839 and 2.105 min; method C: 1.989 and 2.837 ; and method D: 1.852 and 2.282.

Peptide Oxidation Reactions

DMSO reactions. 10% DMSO was prepared in 20 mM HEPES solution. A peptide sample (0.0019 mmol) was dissolved in the 10% DMSO in a 20 mL clear glass scintillation vial with a stir bar. The solution was stirred at room temperature and aliquots were taken periodically for HPLC analysis to observe a retention time shift.

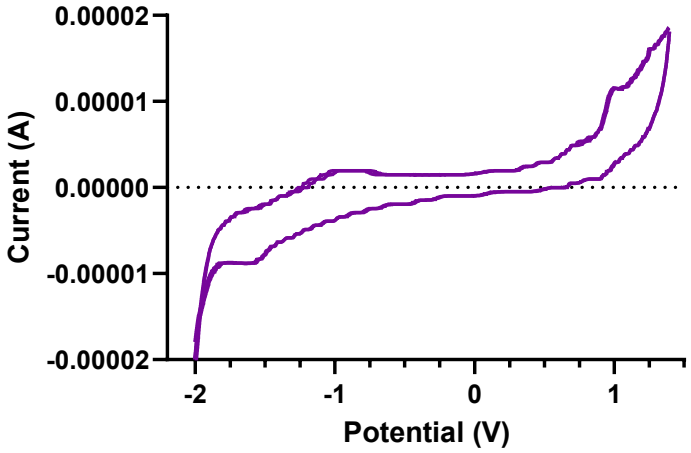
Cbi reactions. Cbi (2 mg, 0.0019 mmol) and peptide (0.0019 mmol) were combined in 2 mL of 20 mM HEPES solution in a 20 mL clear glass scintillation vial with a stir bar. The solution was allowed to stir at room temperature open to air and aliquots were taken periodically for HPLC analysis to observe a retention time shift. In the case no retention time shift was observed, the solution was allowed to stir for 1 hour after which the peptide was purified via HPLC and immediately lyophilized and subsequently submitted for HRMS.

Air restricted reaction. 20 mM HEPES was degassed through three rounds of freeze-pump-thaw. Cbi (1 mg, 0.95 µmol) and peptide (0.95 µmol) were combined in 1 mL of 20 mM HEPES solution in a 2

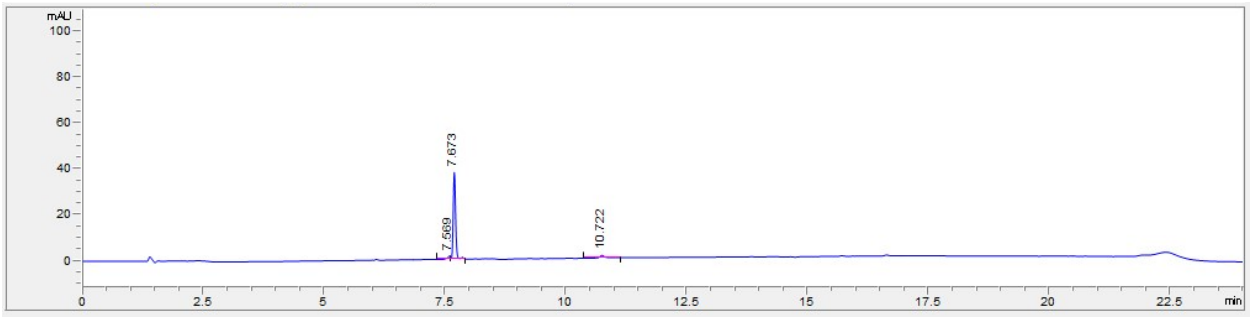
mL autosampler vial and the headspace was purged with argon. The sample was submitted for periodic HPLC analysis to observe a retention time shift.

Table S1. Peptide MW and amount (mg) used per reaction (equivalent to 0.0019 mmol).

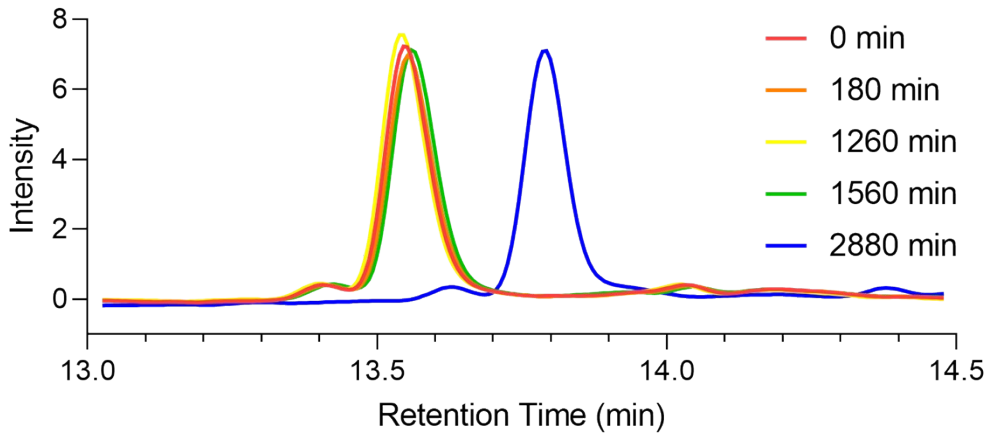
Peptide	Reduced MW (g/mol)	Amt. used per reaction (mg)
OT	1050	2.0
AVP	1086	2.1
SST	1639	3.1
ET-1	2495	4.8
***Tr*CART-1**	2076	4.0
***Tr*CART-2**	2780	5.3
***Tr*CART-3**	4234	8.1



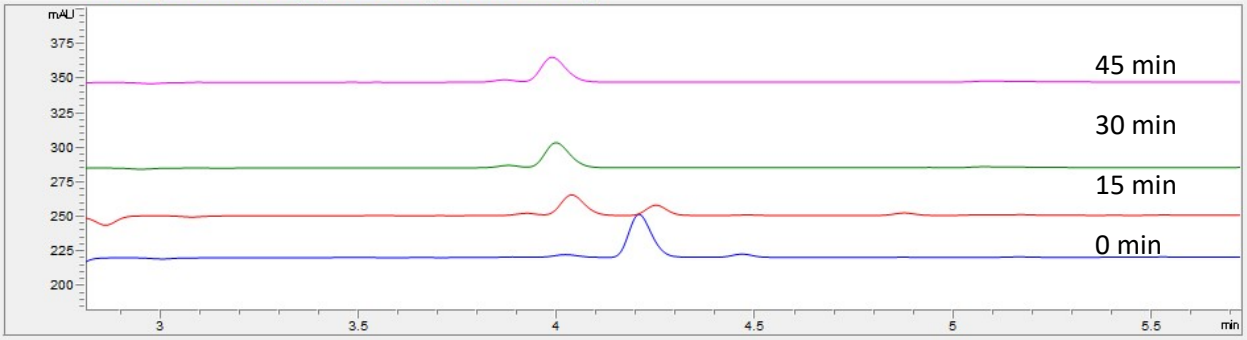
S1. Cyclic Voltammogram of dicyanocobinamide (Cbi)**.** Measurements were carried out in DMF at a scan rate of 100 mV s^{-1} with Fc/Fc^{+} employed as an internal standard and are reported vs. Fc/Fc^{+}.



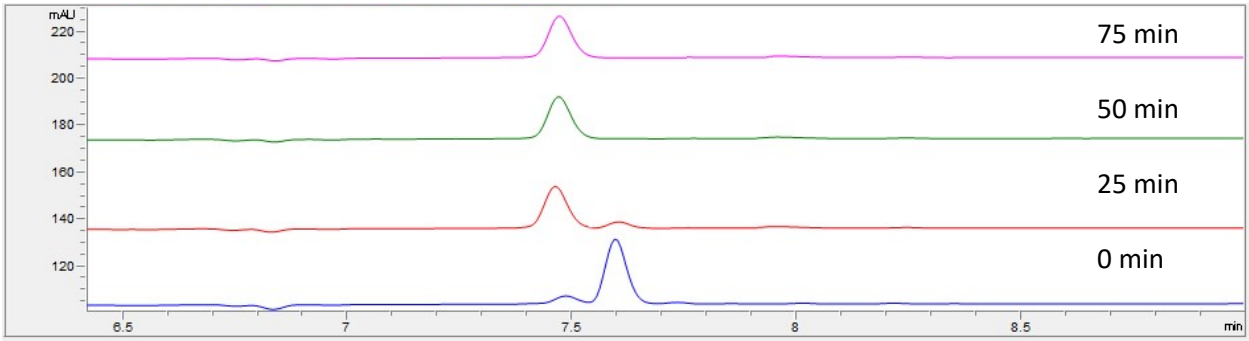
S2. HPLC purity trace (280 nm) of reduced **OT** showing 90% purity at an elution time of 7.673 min on method B.



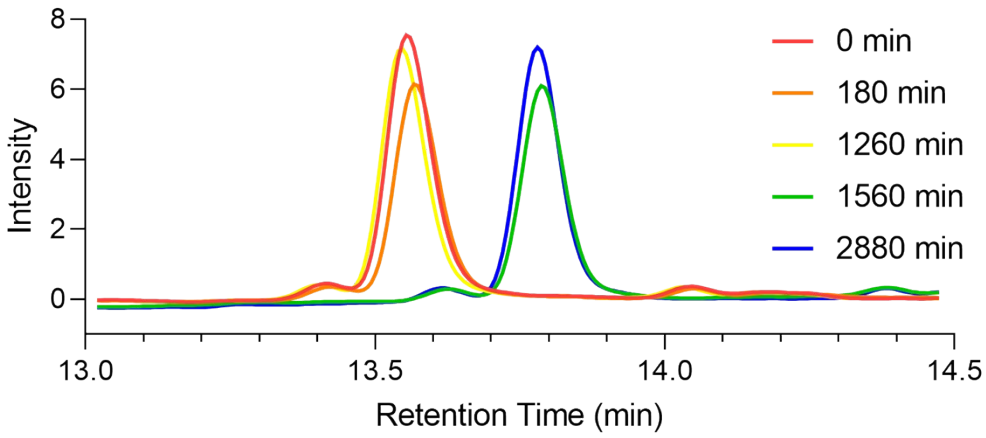
S3. HPLC traces (280 nm) of **OT** reacted with 10% DMSO showing a retention time shift at 2880 min. HPLC method B was used with an Agilent ZORBAX 300SB-C8 (5 µm, 9.4 x 250 mm) column at 2 mL/min flow rate.



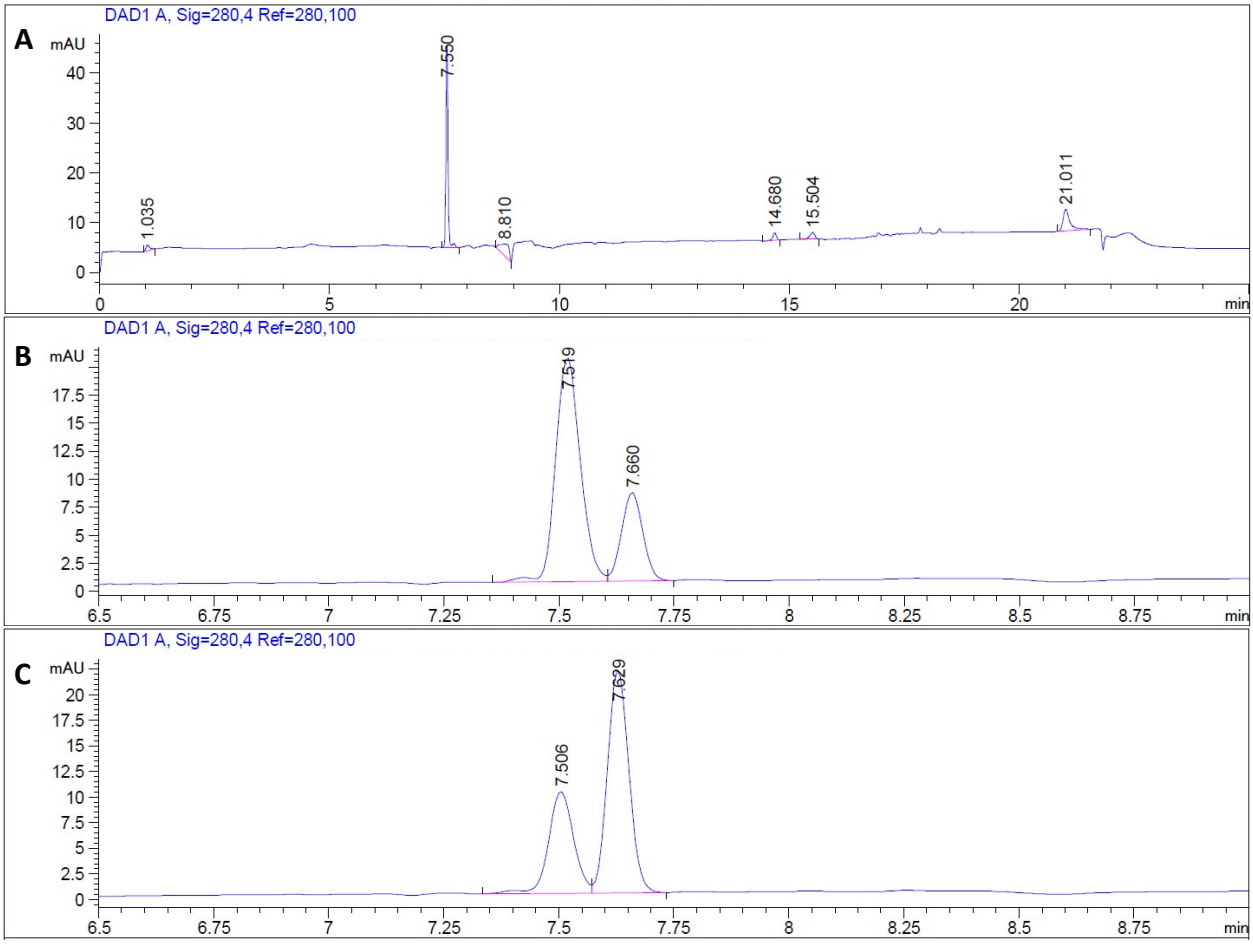
S4. HPLC traces (280 nm) of **OT** reacted with 1 molar equivalent of Cbi on method C showing a complete retention time shift at 30 min.



S5. HPLC traces (280 nm) of **OT** reacted with 0.5 molar equivalences of Cbi on method B showing a complete retention time shift at 50 min.

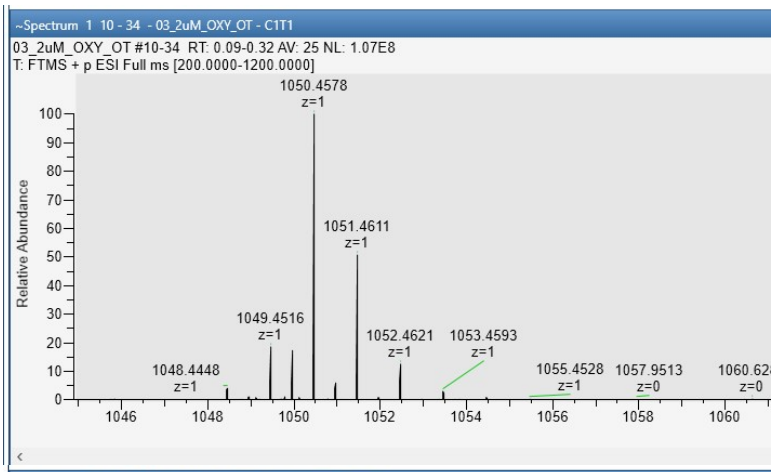


S6. HPLC traces (280 nm) of **OT** reacted with 0.1 molar equivalences of Cbi showing a retention time shift at 1560 min. HPLC method B was used with an Agilent ZORBAX 300SB-C8 (5 µm, 9.4 x 250 mm) column at 2 mL/min flow rate.

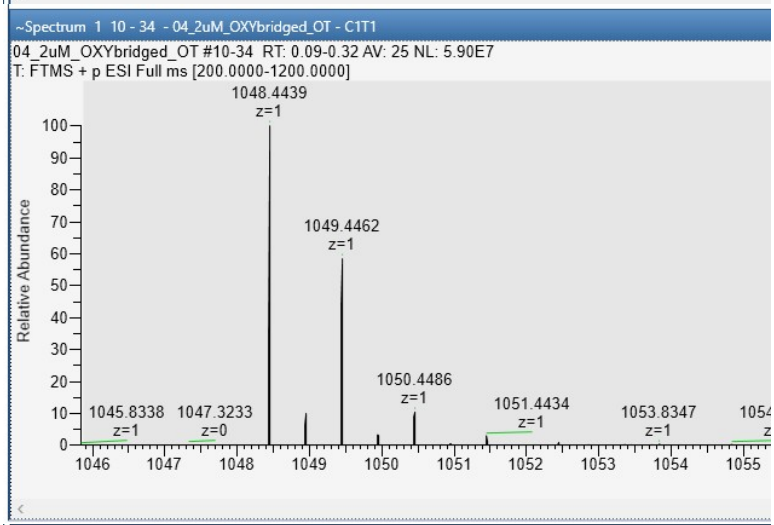


S7. HPLC traces (280 nm) of **OT** on method B**:** (A) a full range trace of oxidized OT with a retention time of 7.550 min, (B) a zoomed in trace of 1 mM OT treated with 10 mM of DTT upon initial mixing, and (C) a zoomed in trace of 1 mM OT treated with 10 mM of DTT after 25 min of mixing. The oxidized OT peak at 7.506 min shrinks and the reduced OT peak at 7.629 min grows in as the DTT reduces the OT.

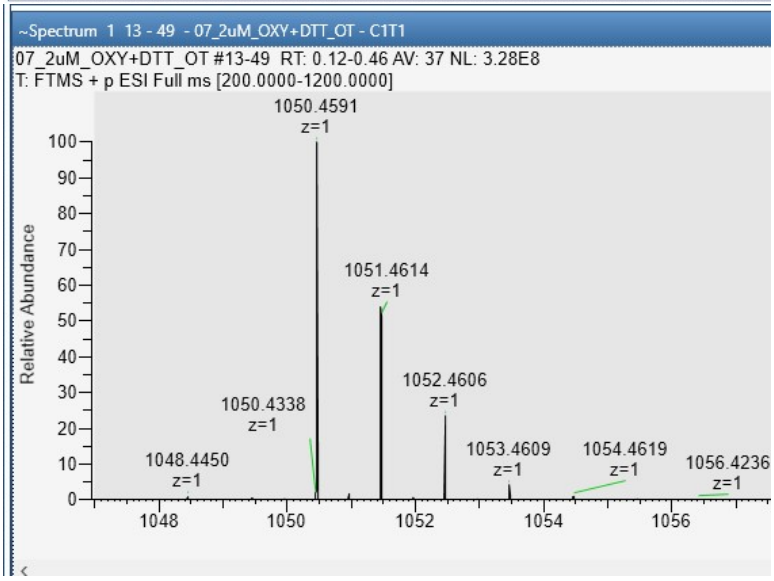
A



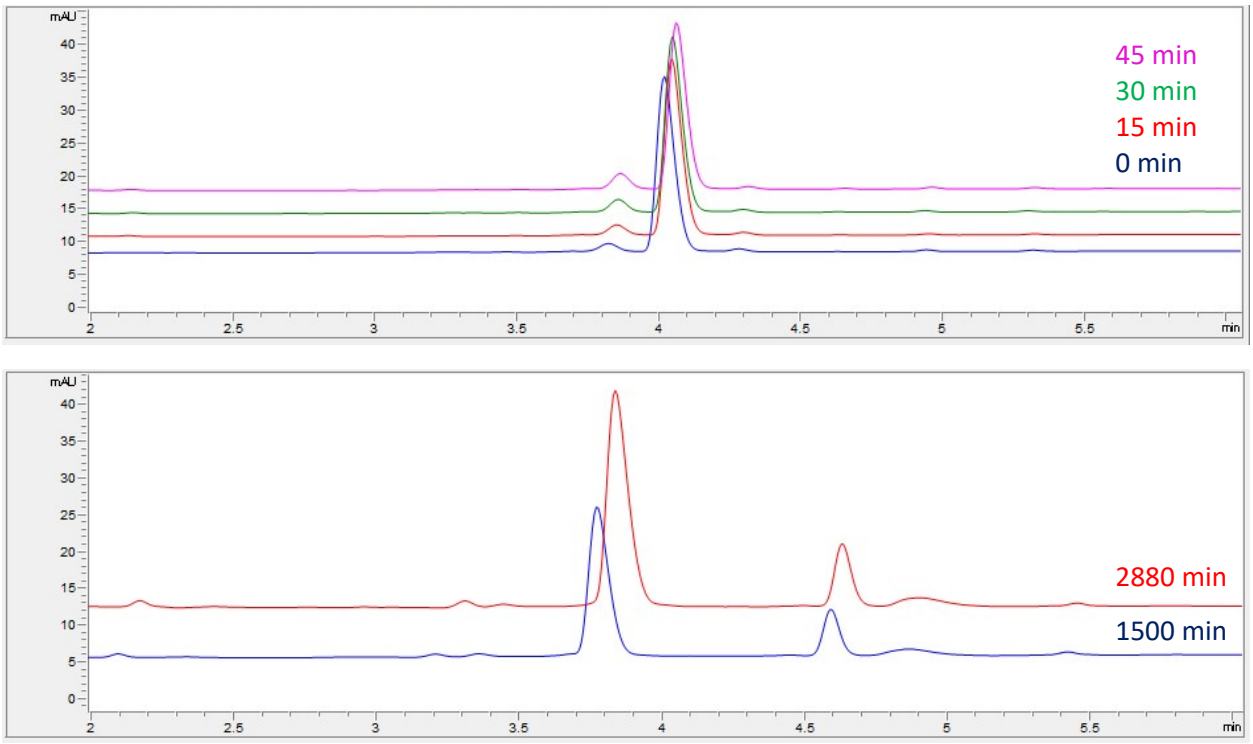
B



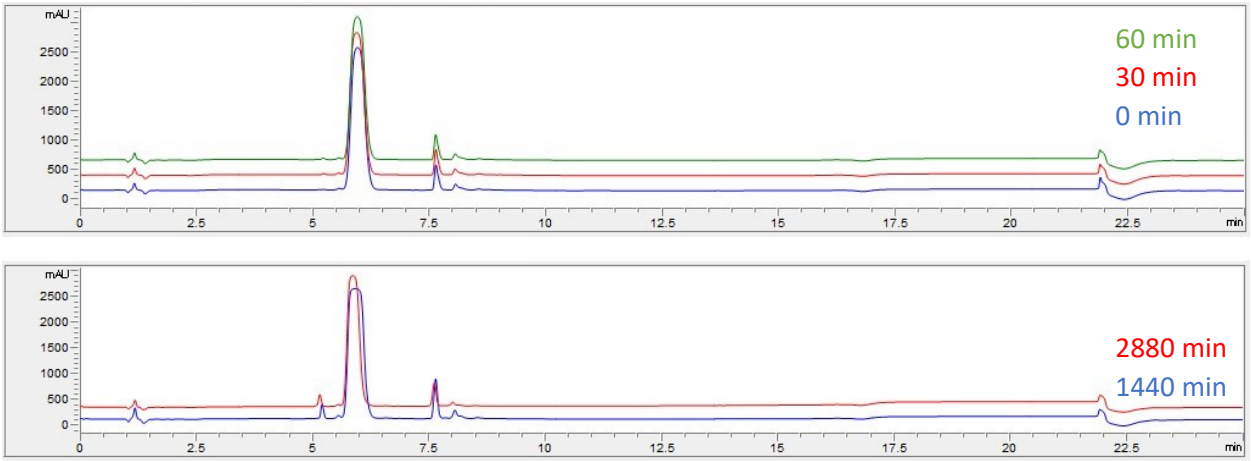
C



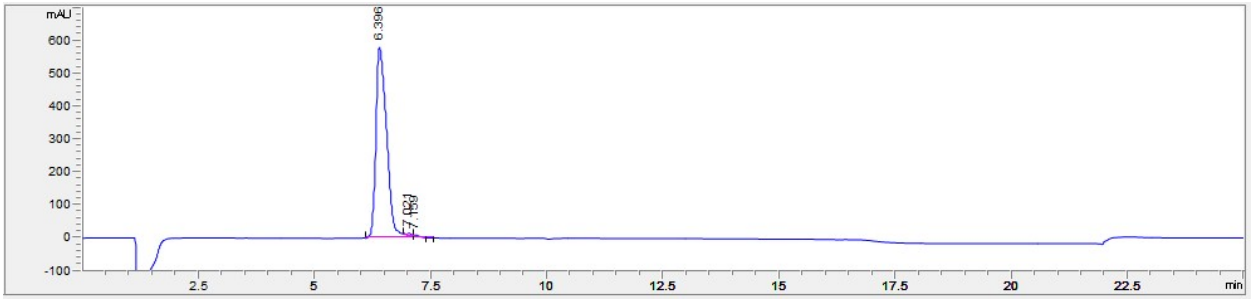
S8. HRMS of (A) reduced **OT** with the expected mass of 1050 m/z, (B) oxidized **OT** obtained from reaction with Cbi for 1 hour with the expected mass of 1048 m/z, and (C) oxidized **OT** reacted with DTT with the expected mass of 1050 m/z.



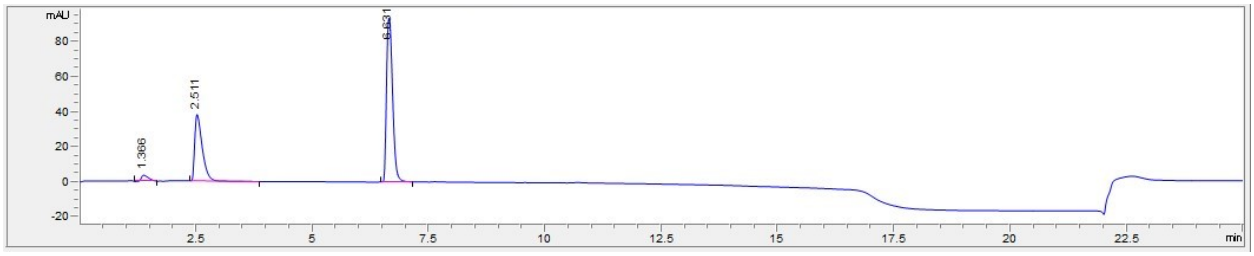
S9. HPLC traces (280 nm) of **OT** reacted with 1 molar equivalent of $CoCl_2$ on method C showing a retention time shift at 1500 min but incomplete conversion out to 2880 min. A side product was observed at a retention time of ~4.6 min.



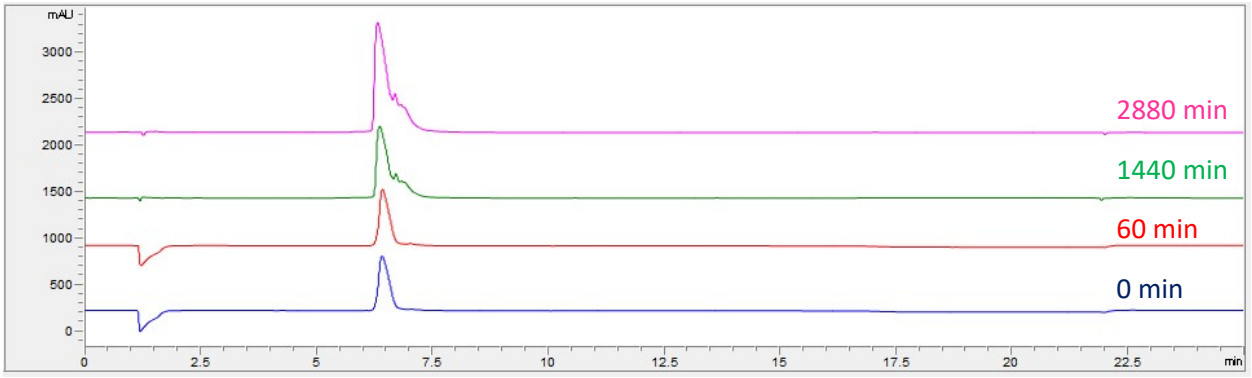
S10. HPLC traces (280 nm) of OT reacted with B_{12} via method B over 2880 min. The large band at 6 min is B_{12} while **OT** was observed at ~7.63 min consistent with its reduced state. Side-products were detected at ~5.2 and ~8.0 min but their identities could not be confirmed.



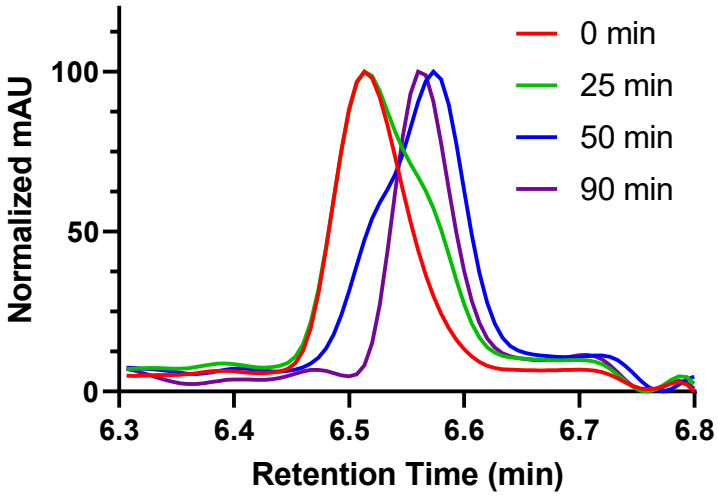
S11. HPLC purity trace (280 nm) of reduced **AVP** showing 99% purity at an elution time of 6.396 min on method B.



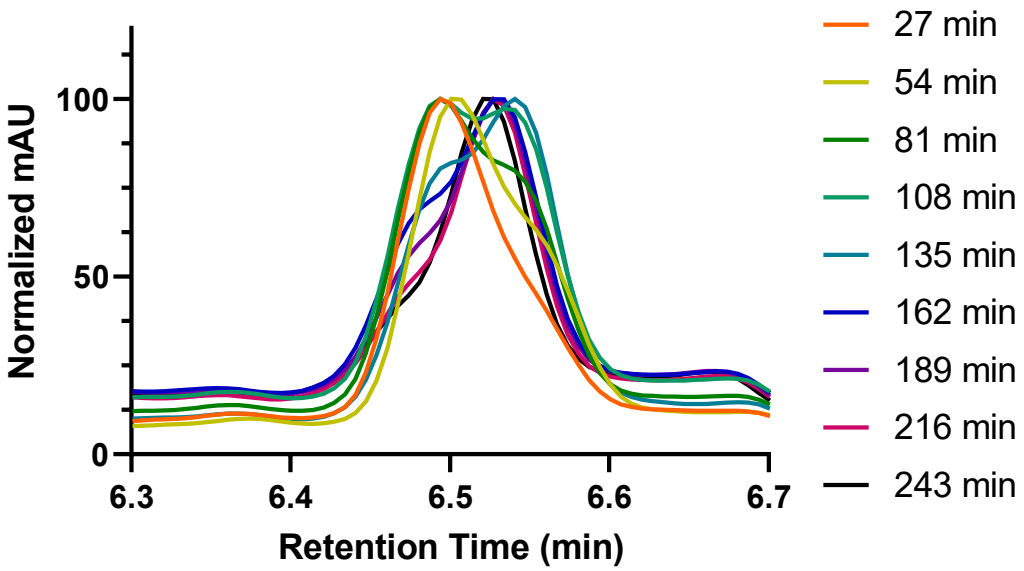
S12. HPLC trace of AVP commercial standard showing the oxidized peptide retention time at 6.631 min on method B.



S13. HPLC traces (280 nm) of **AVP** in 10% DMSO over the course of 48 hours on method B showing little conversion of reduced **AVP** (6.41 min) to oxidized **AVP** (6.67 min).

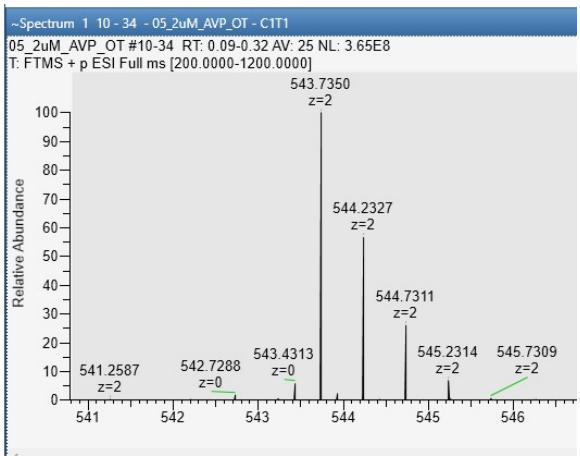


S14. HPLC traces (280 nm) of **AVP** upon reaction with Cbi showing an elution time shift from 6.494 min to 6.527 min in 90 min on method B.

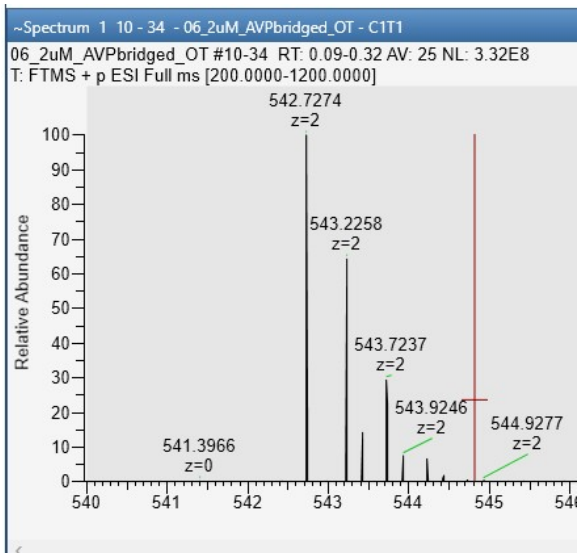


S15. HPLC traces (280 nm) of AVP upon reaction with Cbi in degassed H_2O on method B. These experiments were performed in a 2 mL auto sampling vial with a septum cap at room temperature. The reaction progressed to completion after 4 hours as opposed to 90 minutes when the reaction is open to air.

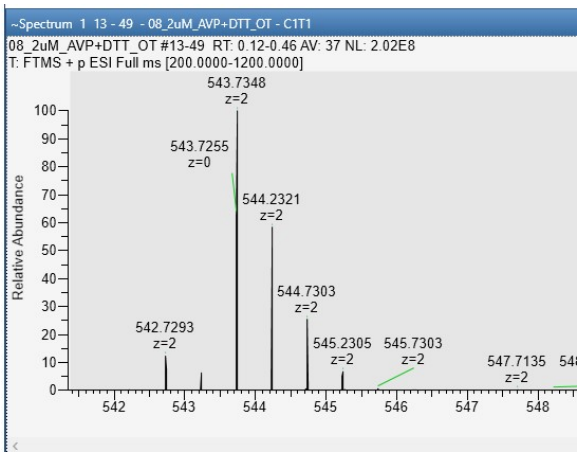
A



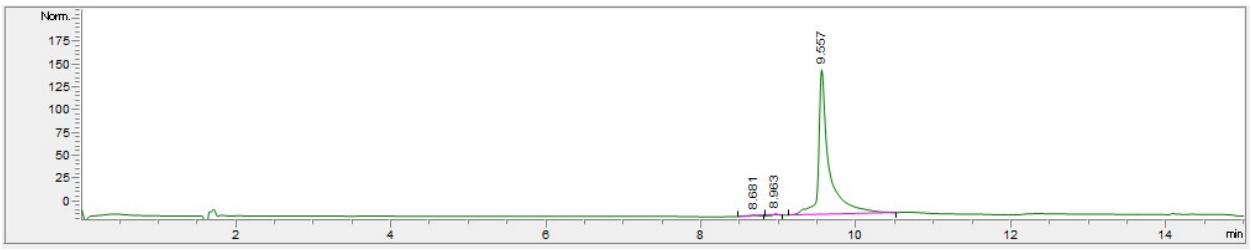
B



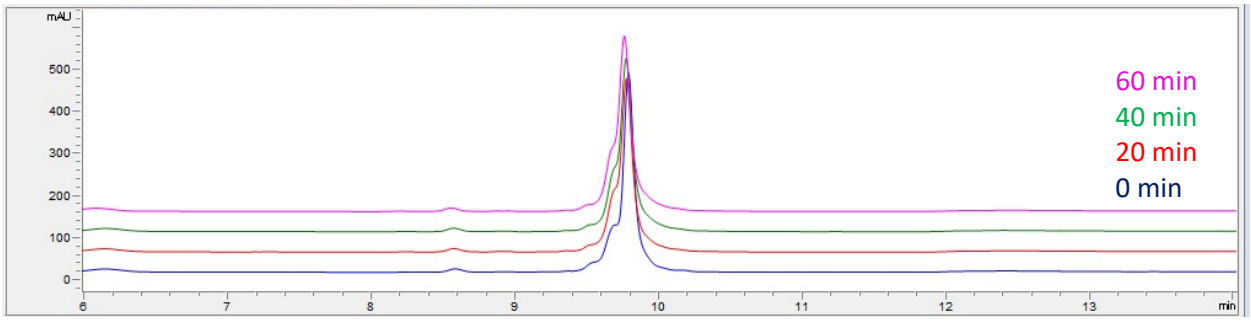
C



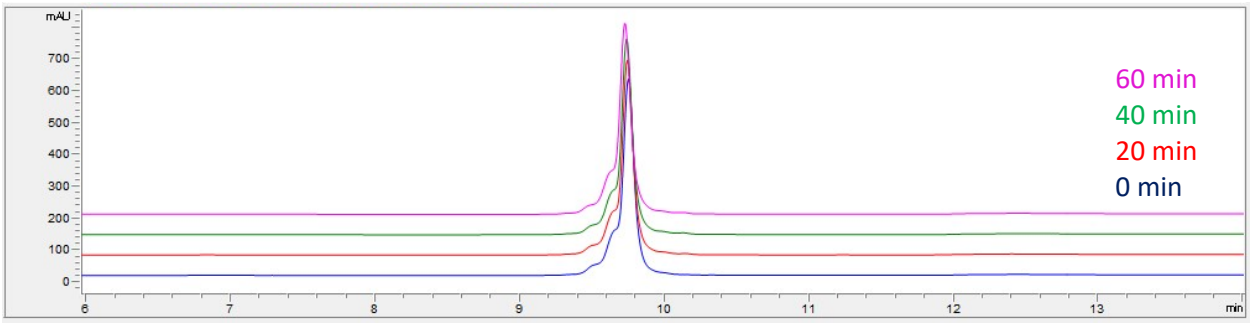
S16. HRMS of (A) reduced AVP with the expected mass of 543 m/z (z=2, 1086 parent mass), (B) oxidized AVP obtained from reaction with Cbi for 90 min with the expected mass of 542 m/z (z=2, 1084 parent mass), and (C) **oxidized AVP reacted with DTT with the expected mass of 543 m/z (z=2, 1086 parent mass).**



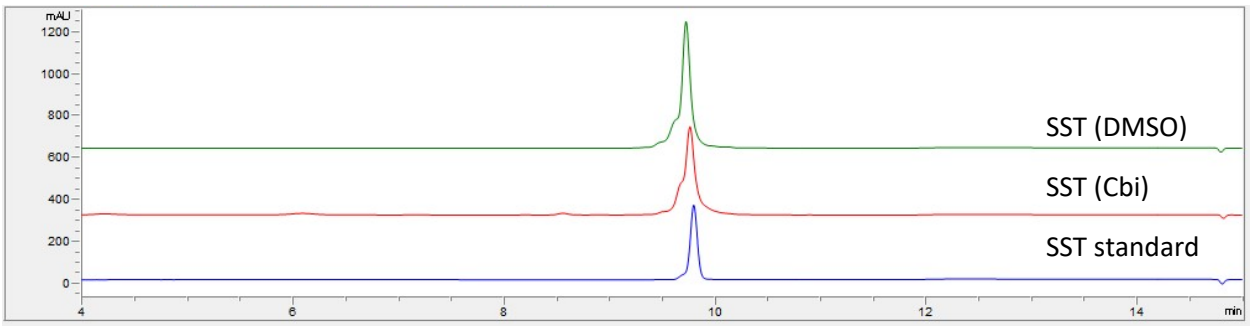
S17. HPLC purity trace (280 nm) of SST showing an elution time of 9.557 min and purity of 98.9% on method C.



S18. HPLC traces (280 nm) of **SST** oxidation with Cbi for 1 hour showing no shift in elution time over the course of oxidation on method C.

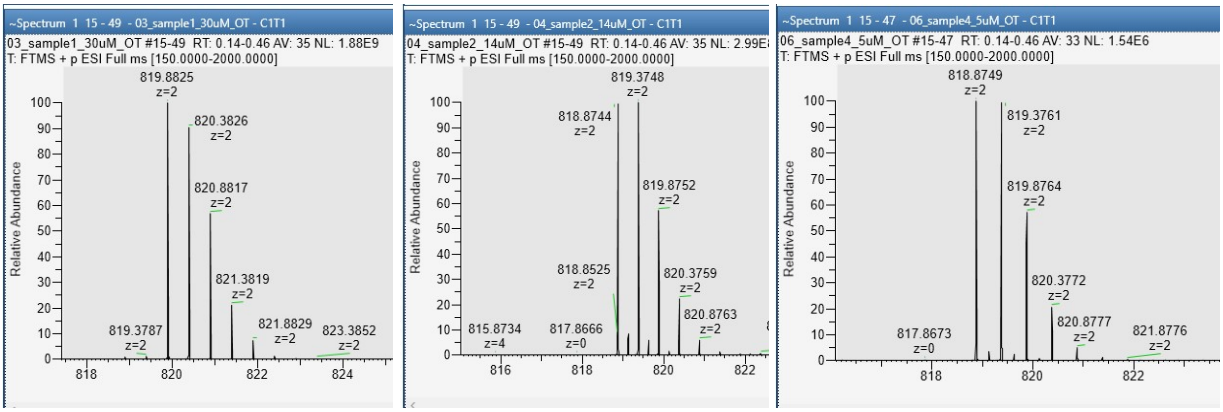


S19. HPLC traces (280 nm) of SST oxidation with DMSO for 1 hour showing no shift in elution time over the course of oxidation on method C.

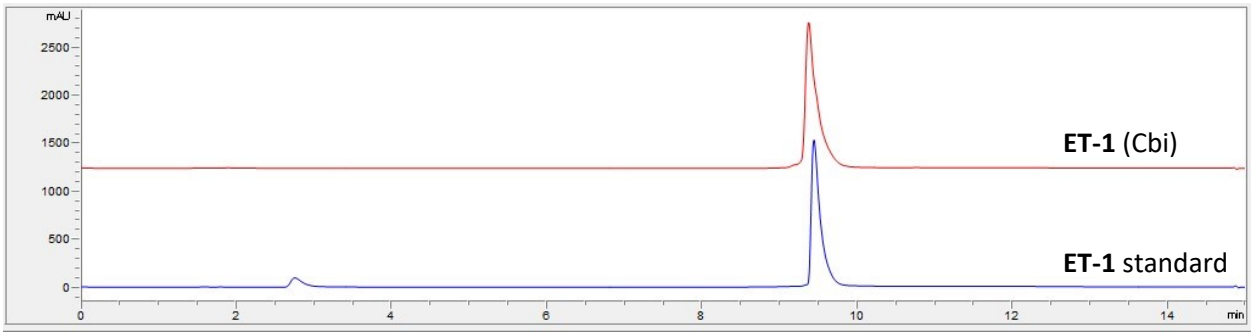


S20. HPLC trace (280 nm) of SST reacted with Cbi or DMSO for 1 hour and a commercial SST standard all showing the same retention time shift of ~9.5 min on method C.

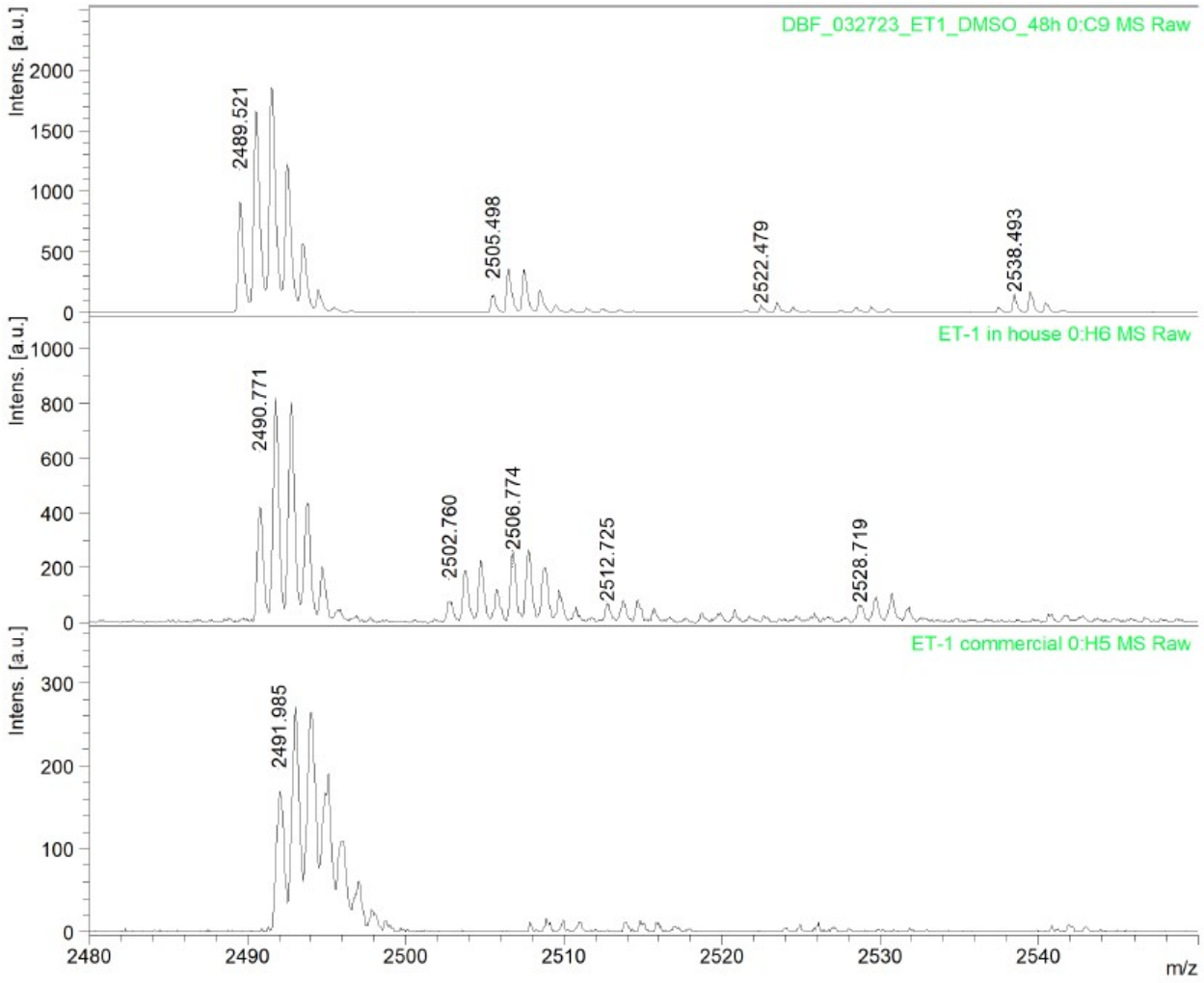
A B C



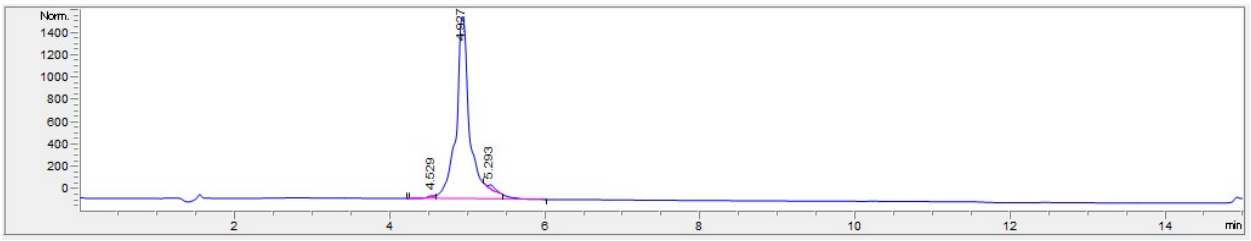
S21. HRMS of (A) SST reduced with the expected mass of 819.8825 m/z (z=2, 1637.764 monoisotopic mass), (B) oxidized SST obtained from reaction with Cbi with 1 hour with the expected mass of 818.844 m/z (z=2, 1635.749 monoisotopic mass), and (C) SST oxidized with DMSO for 48 hours with the expected mass of 818.875 m/z (z=2, 1635.749 monoisotopic mass).



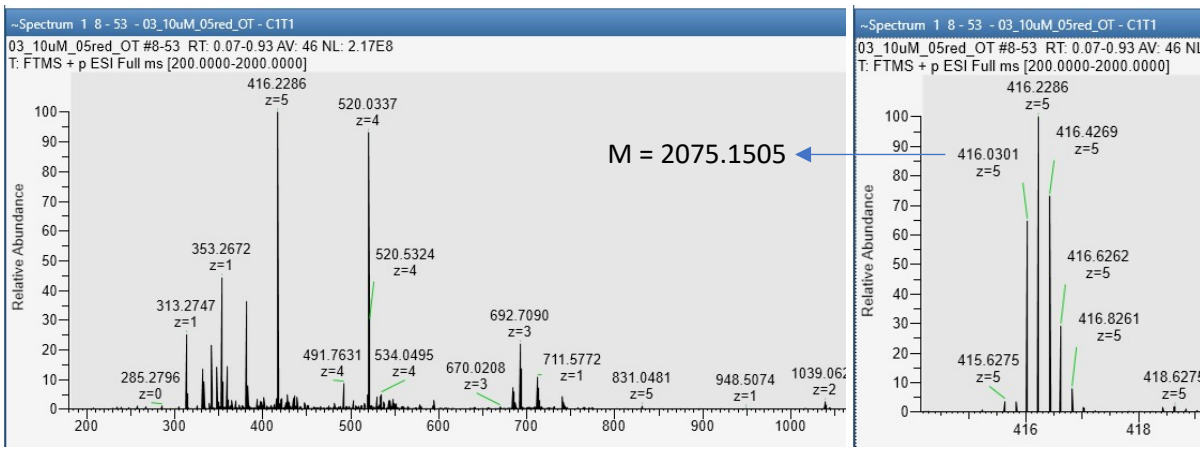
S22. HPLC purity trace of oxidized ET-1 obtained from reaction with Cbi for 1 hour and commercial standard on method D with elution times of 9.377 and 9.446 minutes, respectively.



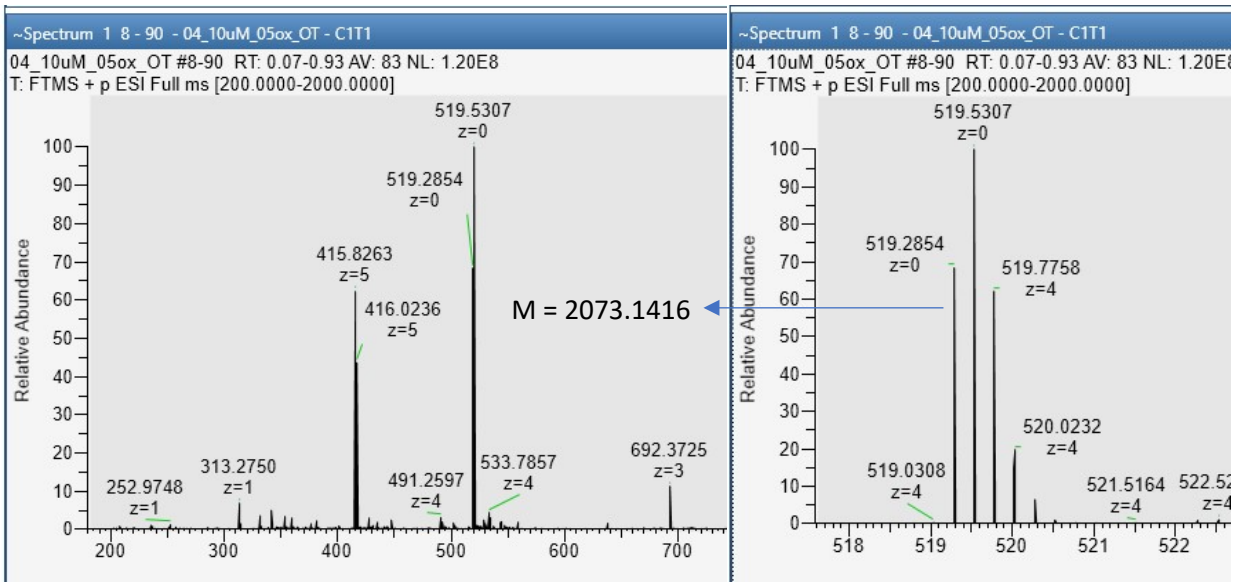
S23. MALDI-ToF MS of (top) **ET-1** oxidized with 10% DMSO for 48 hours, (middle) **ET-1** oxidized with Cbi after 1 hour and (bottom) commercial **ET-1.**



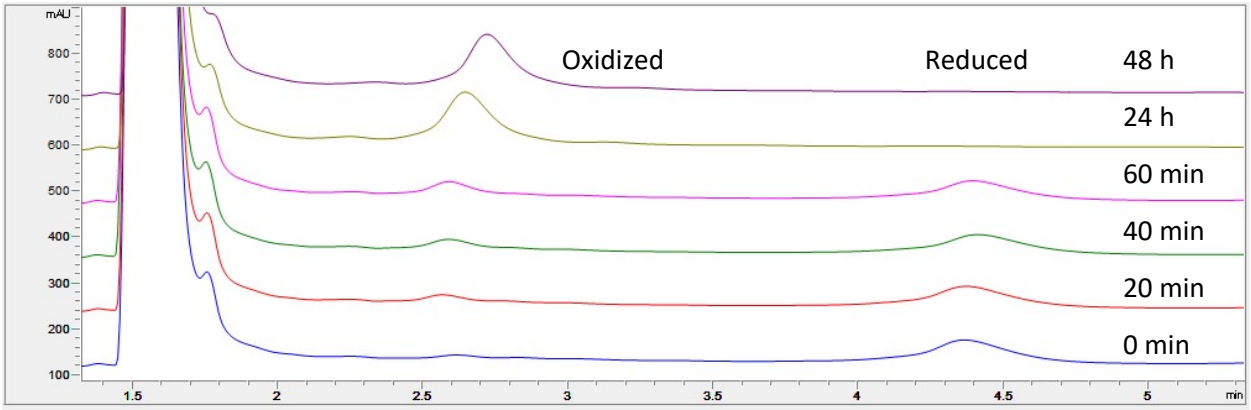
S24. ***Tr*CART-1 HPLC purity trace** on method C with an elution time of 4.927 min with 98% purity on method C.



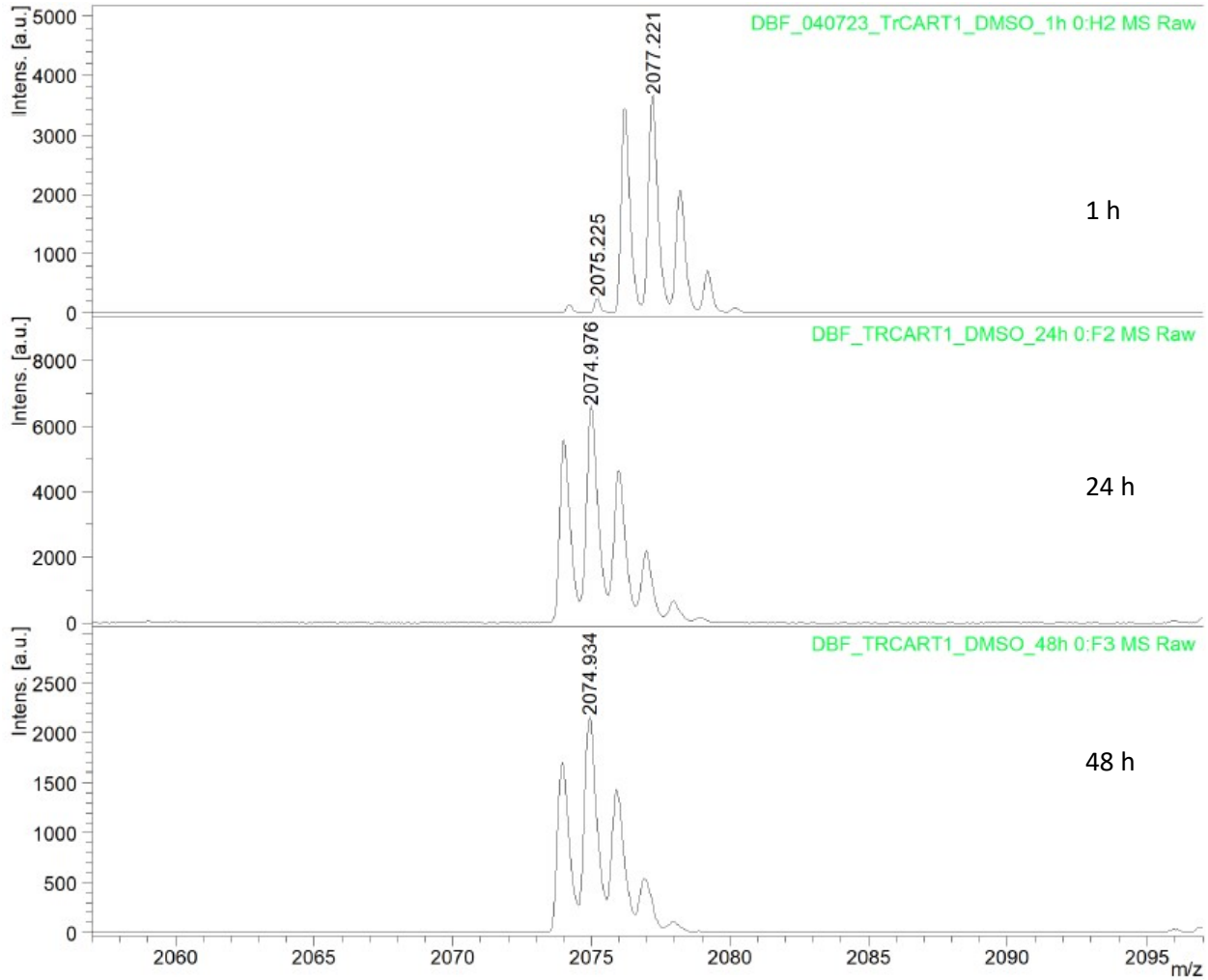
S25. HRMS of reduced *Tr*CART-1 full scan (left) and zoomed (right) with the appropriate mass of 2075.1505 (m/z 416.0301, z=5).



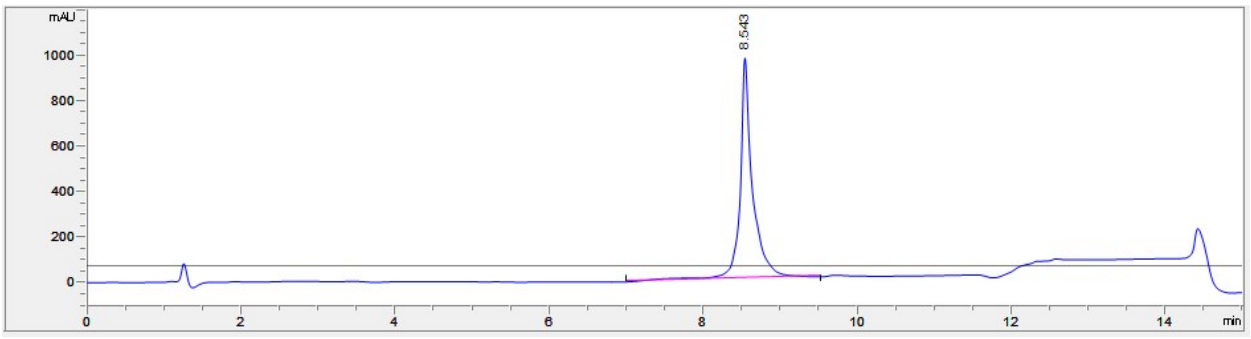
S26. HRMS of oxidized TrCART-1 obtained from reaction with Cbi for 1 hour full scan (left) and zoomed (right) with the appropriate mass of 2073.1416 (m/z 519.2854, z=4).



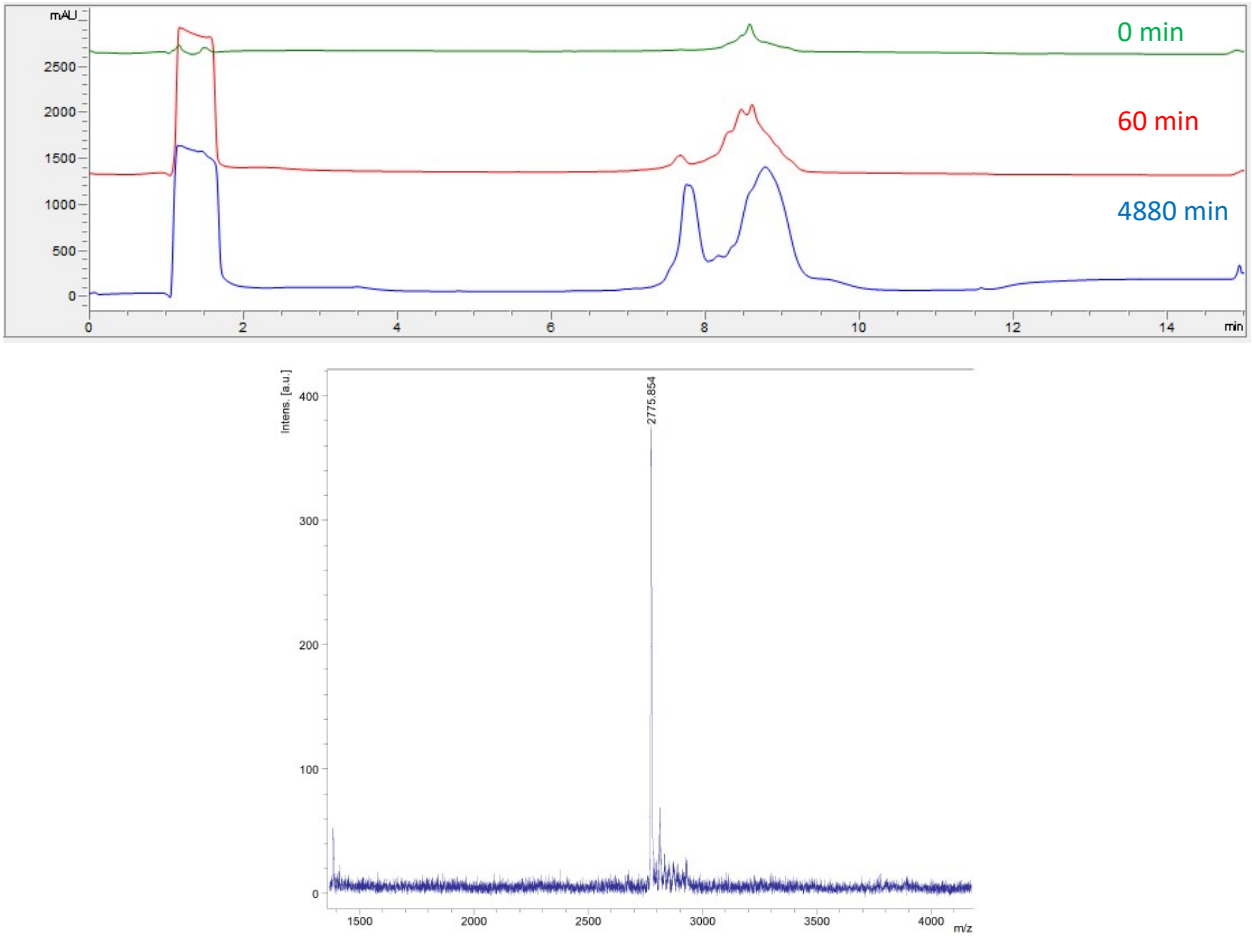
S27. HPLC traces of *Tr*CART-1 in 10% DMSO over 48 hours showing complete oxidation after 24 hours on method C.



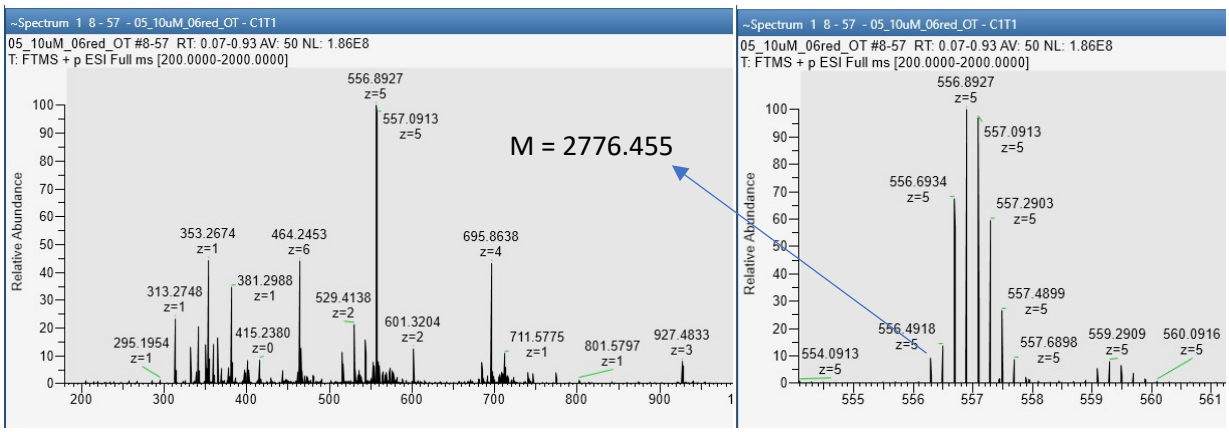
S28. MALDI-ToF MS traces of ***Tr*CART-1** in 10% DMSO over 48 hours showing complete oxidation after 24 hours.



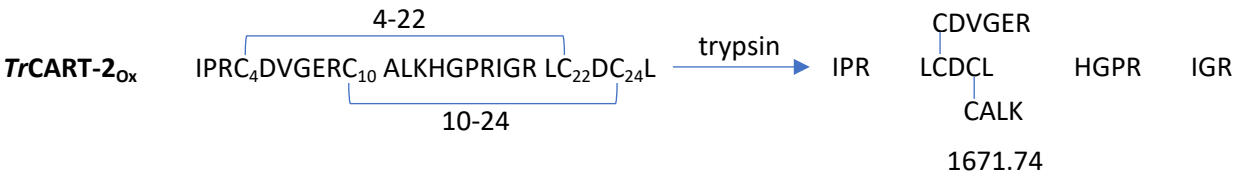
S29. HPLC purity trace of reduced *Tr*CART-2 with an elution time of 8.543 min on method C using an Eclipse Plus C18 column (3.5 µm, 4.6 x 100 mm).



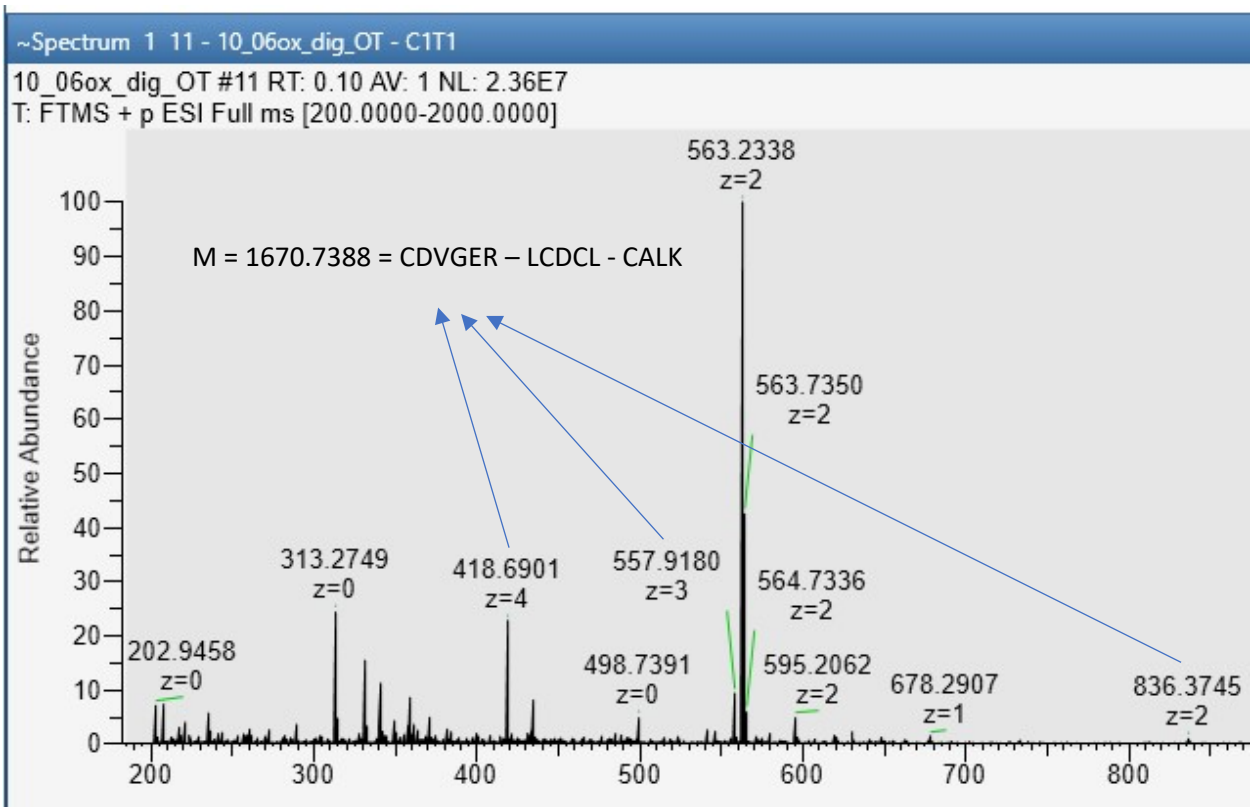
S30. HPLC traces (top) of crude ***Tr*CART-2** in 10% DMSO over 48 hours using method C with an Eclipse Plus C18 column (3.5 µm, 4.6 x 100 mm). The peak at ~7.6 was eluted and the mass, via MALDI-ToF MS (bottom), was found to be that of oxidized ***Tr*CART-2** (2776 m/z).



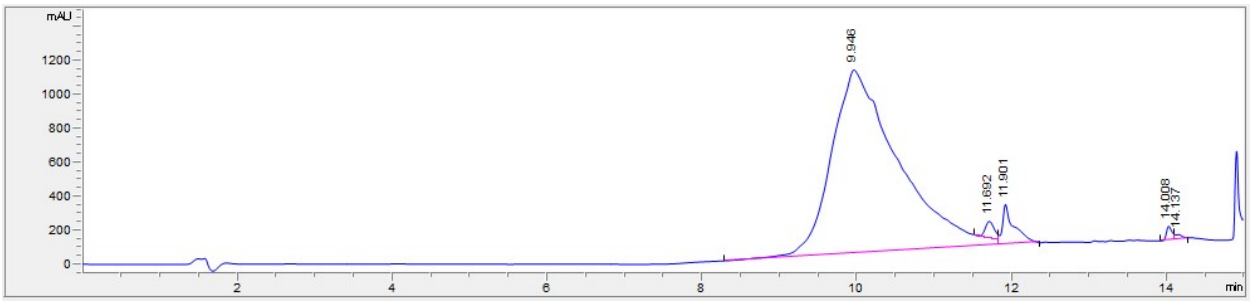
S31. HRMS of reduced *Tr*CART-2 full scan (left) and zoomed (right) with the appropriate mass of 2077.6455 (m/z 556.2910, z=5).



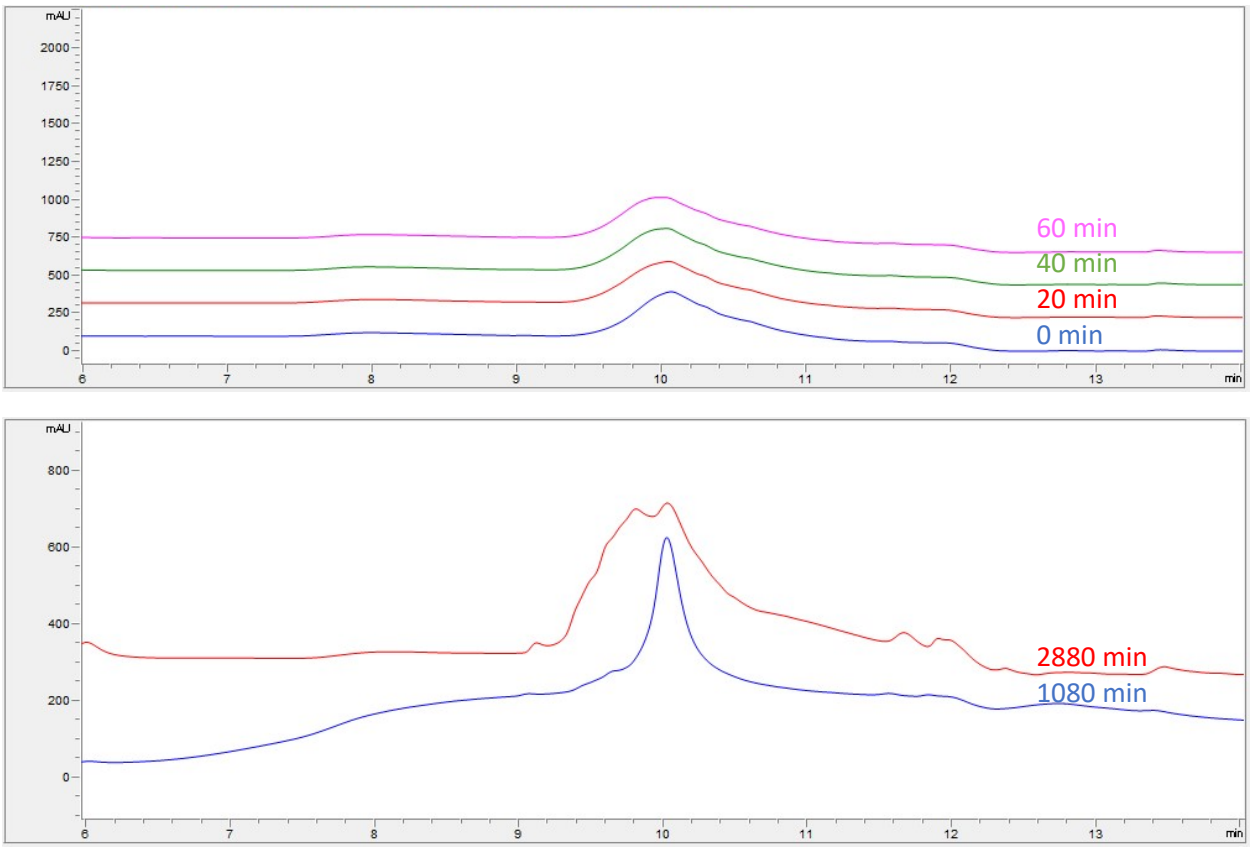
S32. Predicted disulfide linkages and trypsin fragments of ***Tr*CART-2**.



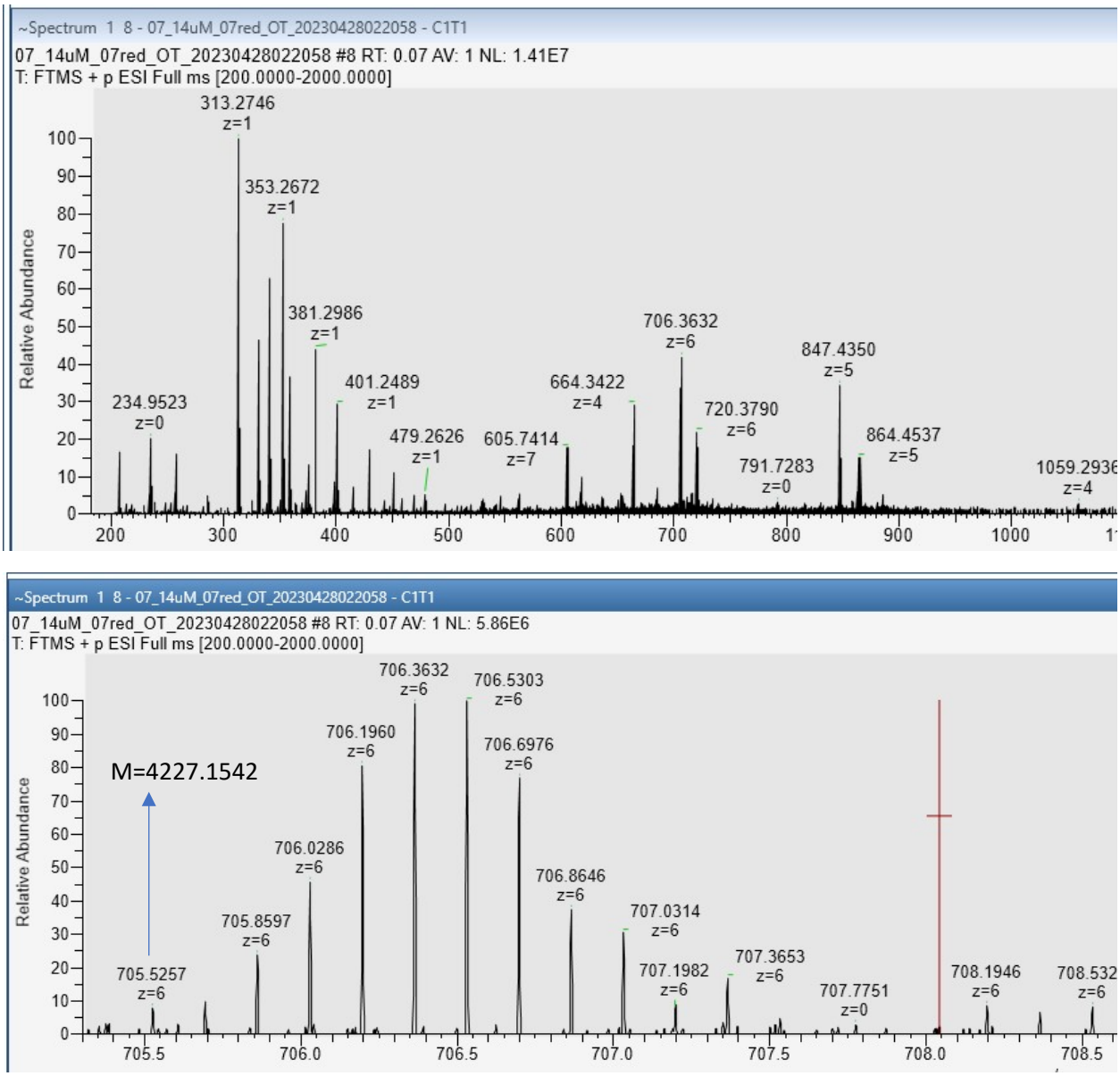
S33. HRMS of oxidized *Tr*CART-2 obtained from reaction with Cbi for 1 hour and digested showing the detection of expected ionized trypsin fragments. The smaller fragments were not observed.



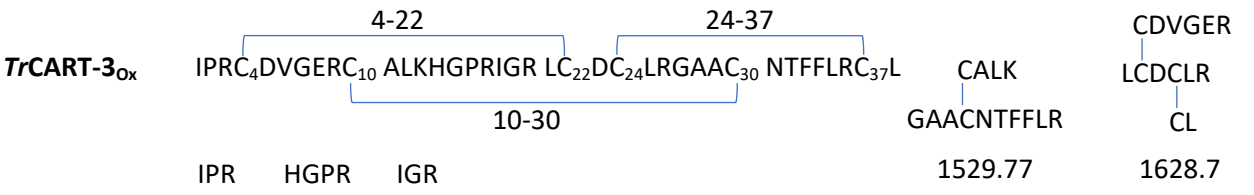
S34. HPLC purity trace (220 nm) of reduced ***Tr*CART-3** showing 94% purity at an elution time of 9.946 min on method C.



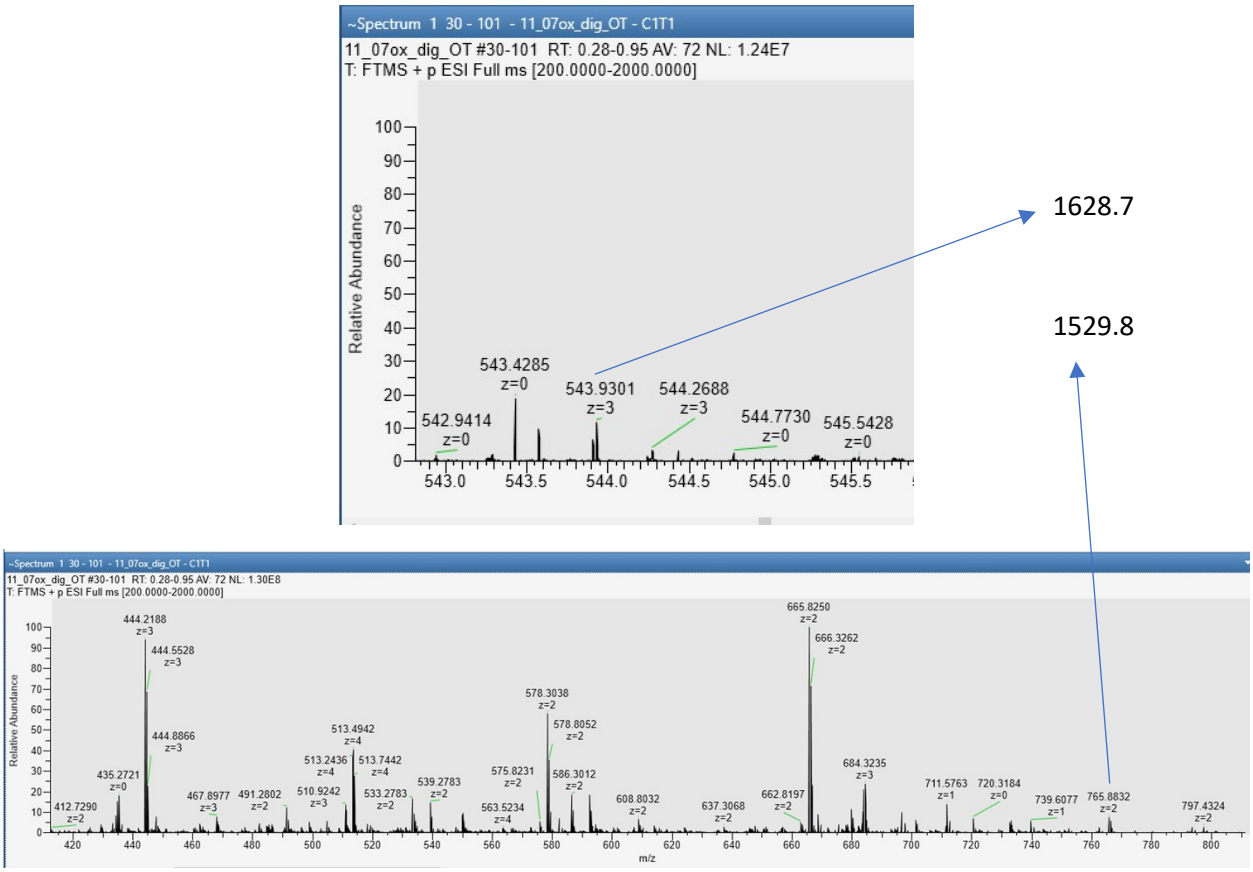
S35. HPLC traces (220 nm) of *Tr*CART-3 reacted with DMSO over the course of 48 hours. From 1 to 18 hours, no change in retention time was observed. At 48 hours, the HPLC profile was observed to change, however mixed products are suggested by method C.



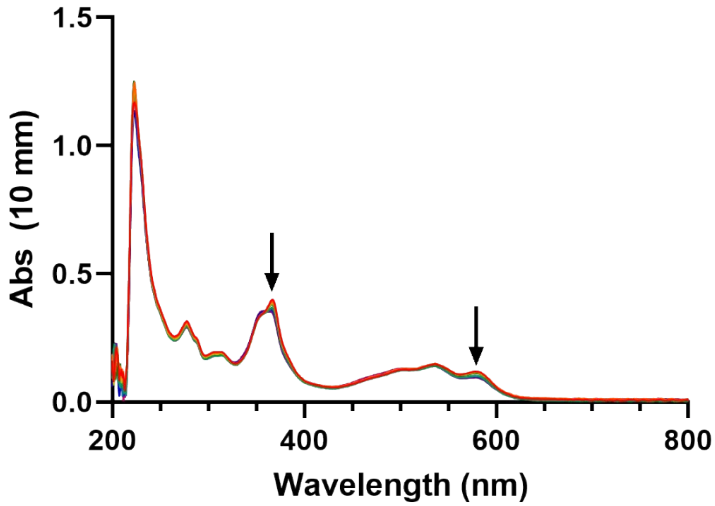
S36. HRMS of reduced *Tr*CART-3 full scan (top) and zoomed (bottom) with the appropriate mass of 4227.1542 (m/z 705.5257, z=6).



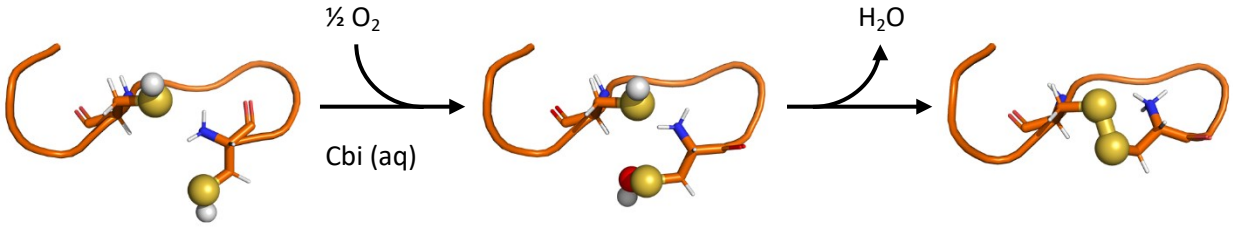
S37. Predicted disulfide linkages and trypsin fragments of *Tr*CART-3.



S38. HRMS of oxidized *Tr*CART-3 obtained from reaction with Cbi for 1 hour and digested showing the detection of expected ionized trypsin fragments.



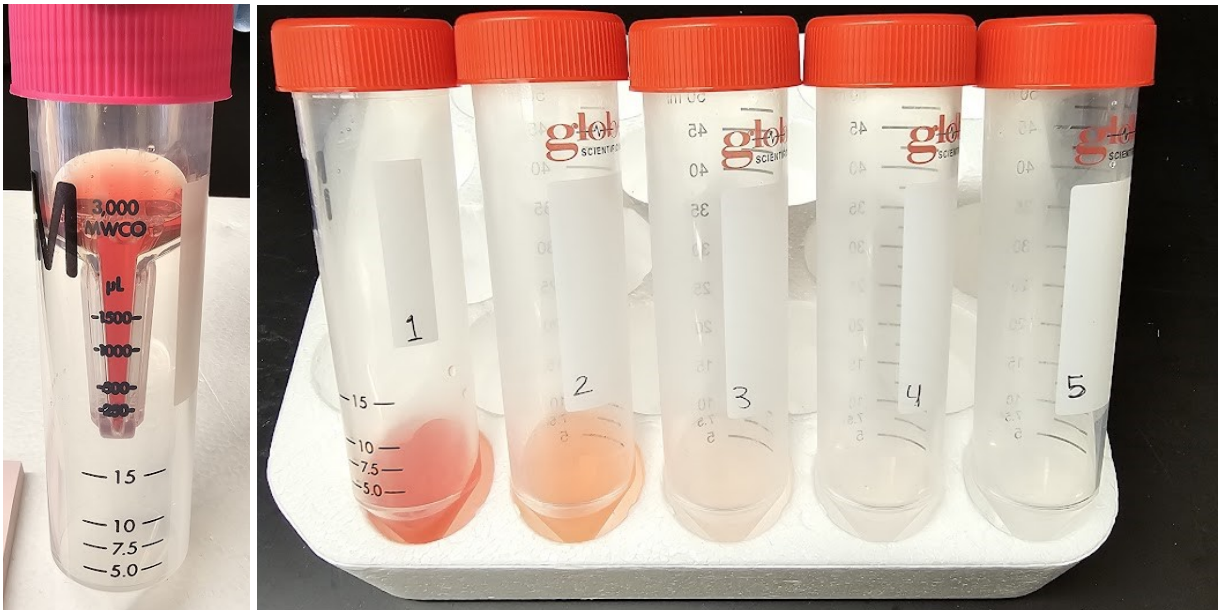
S39. Electronic absorbance spectral changes recorded during Cbi (0.038 mM) reaction with **OT** (0.038 mM) in 2 mL of 20 mM HEPES (pH=7) at room temperature in a quartz cuvette equipped with a stir bar. Spectra were recorded at a cycle time of 20 min for 3 hours. Absorbance at 367 and 580 decreased (ΔAbs = 0.053 and 0.023, respectively).



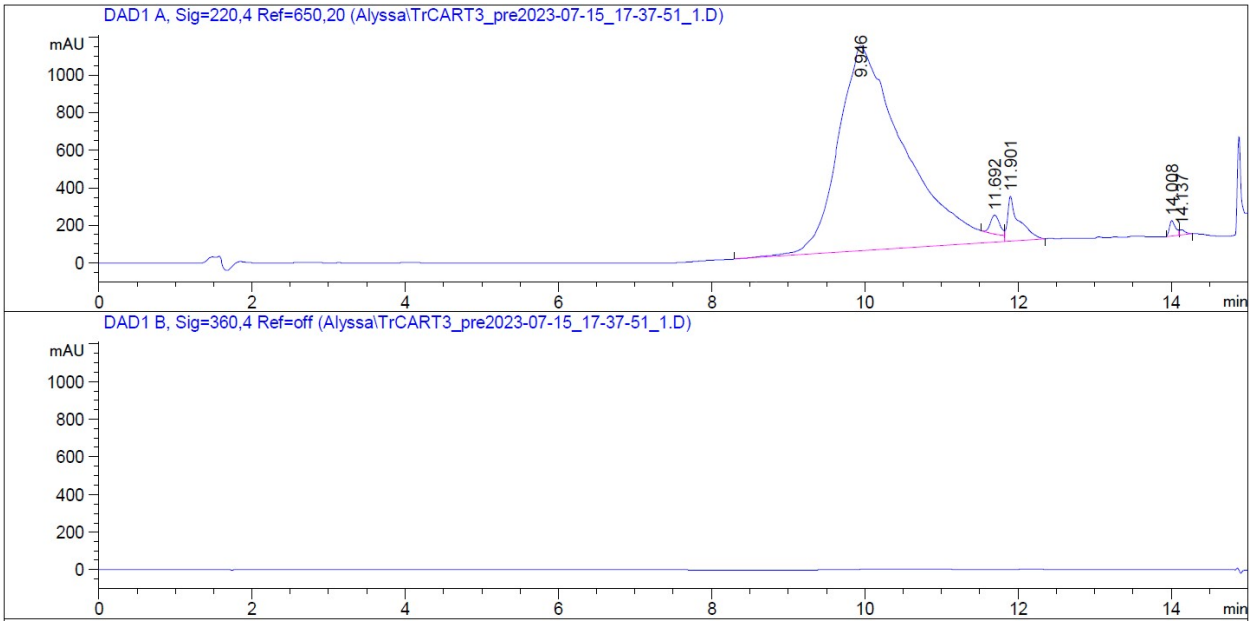
Scheme S1. Proposed mechanism of air-oxidation of cysteine residues to a cystine bond facilitated by Cbi.

***Tr*CART-3 purification via spin column filtration**

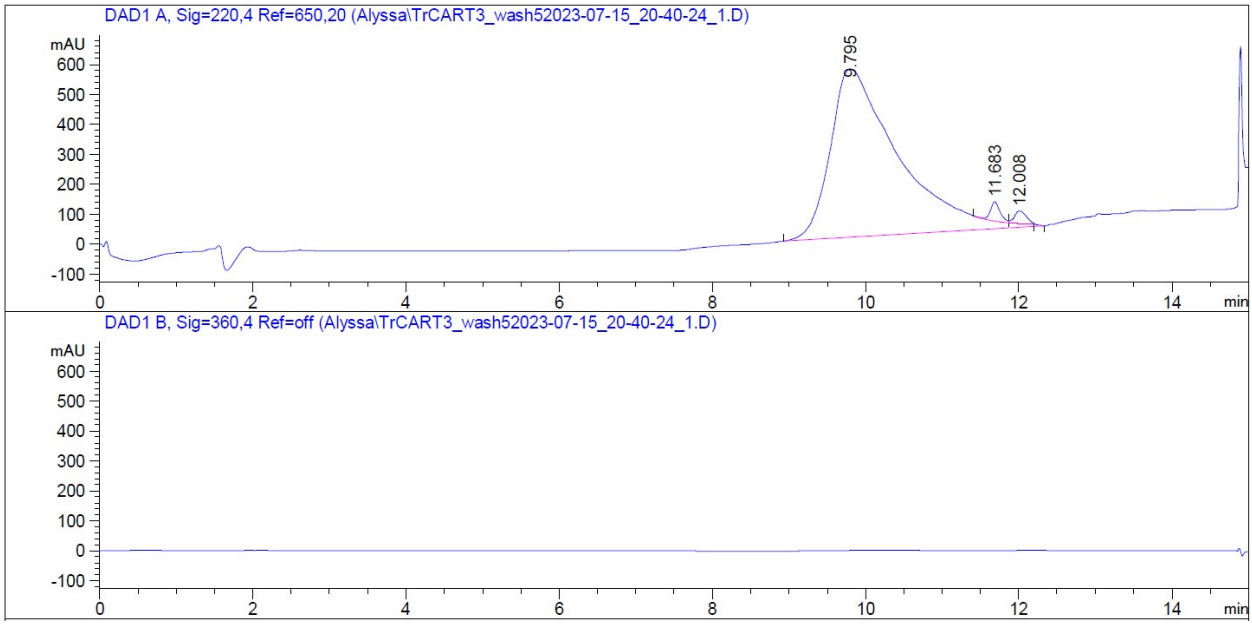
Cbi (1042 g/mol, 2 mg, 0.0019 mmol) and crude ***Tr*CART-3** (4234 g/mol, 8.1 mg, 0.0019 mmol) were combined in 5 mL of 18% ACN in ultrapure water and pipetted into the spin column (3000 MWCO) taking care to not touch the filters. The column was centrifuged at 4000 rpm for 25 min. The filtrate was collected, and an additional 5 mL of solvent was added to the top chamber and gently pipetted up and down to clear the filters. Washing and filtration was repeated until the peptide solution ran clear (5 washes) and an HPLC trace was taken to verify complete filtration of Cbi.



S40. Images of ***Tr*CART-3** purification via spin column filtration. (Left) ***Tr*CART-3** and Cbi (0.0019 mmol each) in 5 mL of 18% ACN in ultrapure water before filtration. (Right) Collected filtrates after centrifugation.



S41. HPLC traces (top, 220 nm; bottom, 360 nm) of ***Tr*CART-3** before mixing with Cbi on method C.



S42. HPLC traces (top, 220 nm; bottom, 360 nm) of ***Tr*CART-3** after mixing with Cbi and 5 rounds of spin column filtration showing no Cbi at the detection wavelength of 360 nm.

1. K. Ó Proinsias, M. Karczewski, A. Zieleniewska and D. Gryko, *J. Org. Chem.*, 2014, **79**, 7752–7757.