Supporting Information

Resin-supported cyclic telluride as a heterogeneous promoter of disulfide formation under solid–liquid biphasic conditions

Yuya Nishizawa,[a†] Yuri Satoh,[a†] Osamu Kanie,[b] and Kenta Arai[*a,c]

[†]These authors contributed equally to this study.

* Corresponding author (k-arai4470@tokai-u.jp)

[a] Department of Chemistry, School of Science, Tokai University, Kitakaname, Hiratsuka-shi, Kanagawa 259-1292, Japan

[b] Department of Bioengineering, School of Engineering Kitakaname, Hiratsuka-shi, Kanagawa 259-1292, Japan

[c] Institute of Advanced Biosciences, Tokai University, Kitakaname, Hiratsuka-shi, Kanagawa 259-1292, Japan

Contents

1. Experimental

*Synthesis of oxytocin (**10**)*

The standard Fmoc-SPPS protocol using dicyclohexylcarbodiimide (DCC) as a condensing reagent was employed. Fmoc-Rink amide resin (109 mg, 0.05 mmol) was swelled with DMF for 16 h at 4 °C. After removing DMF, the resin was treated with 20% piperidine/DMF for 5 min with vortex mixing. After the deprotection reaction was further repeated with fresh 20% piperidine/DMF for 15 min, the resin was fully washed with DMF (×5). Fmoc-Gly-OBt, which was prepared by mixing Fmoc-Gly-OH (64.9 mg, 0.2 mmol), 0.5 M HOBt/DMF (300 μL), and 0.5 M DCC/DMF (300 μL) for 30 min, was added to the resin. The mixture was vortexed for 60 min at 50 °C. After the coupling, the resin was washed with 50% MeOH/DCM (×3). The reaction progression was monitored by a Kaiser test. The unreacted amino groups were acetylated by using 10% Ac_2O and 5% DIEA in DMF for 5 min. Applying a similar protocol, the peptide chain was elongated on the resin, and the N-terminal Fmoc group was finally deprotected by 20% piperidine/DMF to yield H-Cys(Trt)-Tyr(*t*-Bu)-Ile-Gln(Trt)-Asn(Trt)-Cys(Trt)-Pro-Leu-Gly-NH-resin (187 mg). The obtained resin was fully washed with 50% MeOH/DCM (×1) and DCM (×3) and dried in vacuo. A portion of the obtained resin (100 mg, 27 μmol) was treated with a TFA cocktail (trifluoroacetic acid (TFA) : H_2O : triisopropylsilane (TIS): 1,2-ethanedithiol (EDT) = 94 : 2.5 : 1.0 : 2.5, v/v/v/v, 4 mL), and the mixture was stirred for 3 h at room temperature. After the removal of TFA by N_2 stream, the deprotected peptide was precipitated with Et_2O, washed with Et_2O (×3) and dried in vacuo. The resulting crude peptide was purified by using HPLC, which was equipped a 3 mL sample solution loop and a RP-column (ODS-HL φ10 × 250 mm [GL science, Tokyo, Japan]), at a flow rate of 4.7 mL/min. After injecting the sample solution, the ratio of eluent B was increased linearly from 15% to 35% in 0–20 min. The corrected fraction containing the target peptide (**10**) was lyophilized to yield **10** as a white powder (6.4 μmol, 24%). MALDI-TOF-MS (m/z) found: 1009.67, calcd for $[M+H]^+$: 1009.46. Peptide **10** was divided into small portions in micro-centrifuge tubes (2.0 mL capacity) so as to be 100 nmol per a tube and lyophilized. The resulting product was then used to SS-formation experiment using **RSTe** as described in Experimental (See the main text).

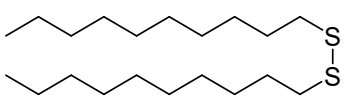
*Preparation of reduced CX-397 (**12**)*

An excess amount of DTT^{red} (18 mg) was added to a powder of CX397 (8 mg) dissolved in 100 mM Tris-HCl buffer solution containing 1 mM EDTA and 4 M guanidinium thiocyanate as a denaturant at pH 8.0 (600 μL). After incubation at 25 °C for 60 min, the resulting fully reduced CX397 (**12**) was purified by passing through a column packed with Sephadex G25 resin, which was equilibrated with 0.1 M acetic acid. The collected fraction of R^{CX397} was lyophilized to give a white

powder of **12**. The powder of **12** was redissolved in aqueous 0.1% TFA solution (4 mL), and the quantity of the protein in the solution was estimated by UV absorbance at 275 nm based on the molar extinction coefficient (ε = 2960 M^{-1} cm^{-1} [S1]). Compound **12** was divided into small portions in micro-centrifuge tubes (2.0 mL capacity) so as to be 80 nmol per tube and lyophilized. The resulting product was then used to SS-formation experiment using **RSTe** as described in Experimental (See the main text).

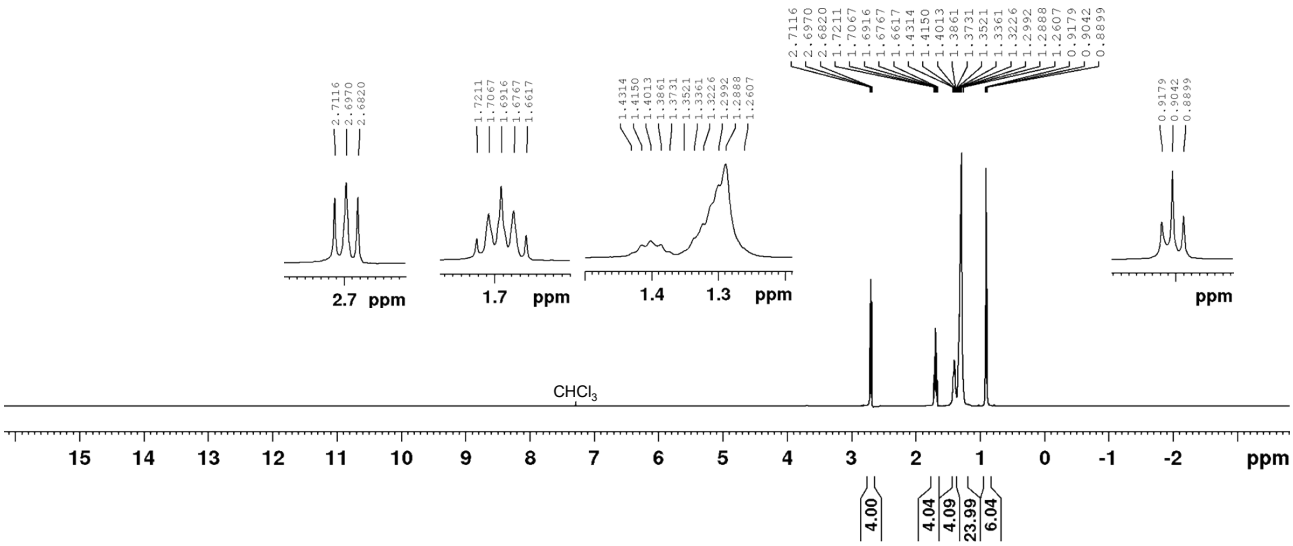
2. NMR spectra of disulfide compounds

9a: *1,2-Didecyl disulfide*

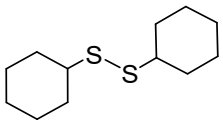


9a

Using thiol **8a** (102.5 mg, 0.59 mmol) and **RSTe** (2.95 μmol [0.5 mol%]), disulfide **9a** was obtained as a colorless oil. Yield: 102 mg (quant.) using $CHCl_3$ as a solvent, respectively; ^{1}H NMR ($CDCl_3$): δ = 2.70 (t, *J* = 7.3 Hz, 4H), 1.72–1.66 (m, 4H), 1.43–1.37 (m, 4H), 1.35–1.26 (m, 24H), 0.90 ppm (t, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$): δ = 39.23, 31.91, 29.58, 29.54, 29.33, 29.27, 29.25, 28.55, 22.69, 14.11 ppm. Spectroscopic data is in accordance with our previous report.[S2]

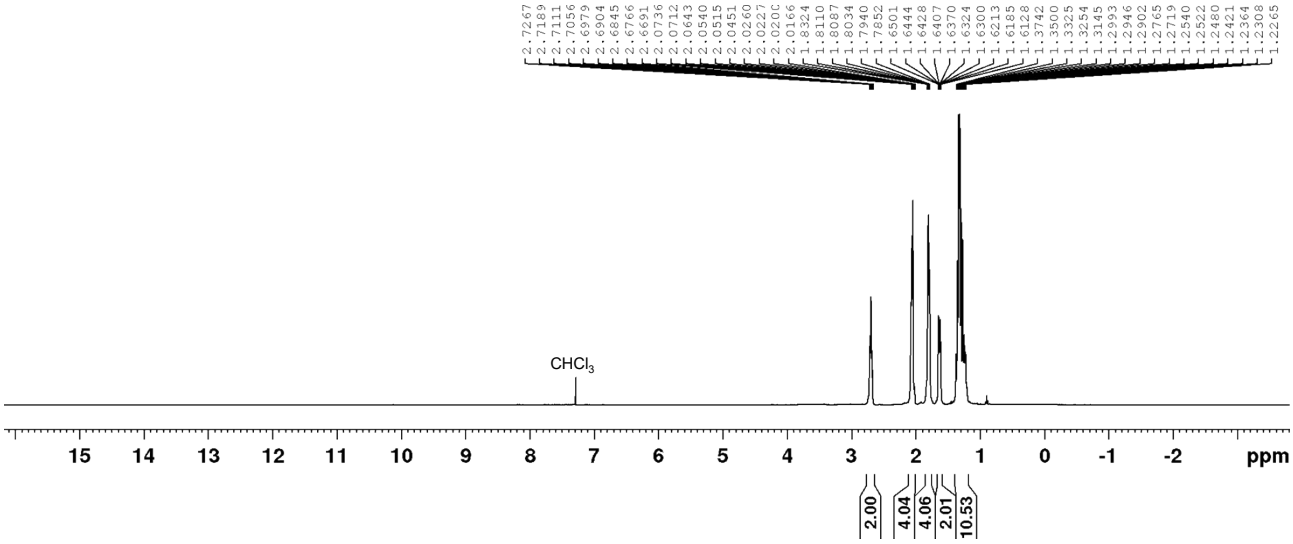


9b: *1,2-Dicyclohexyl disulfide*

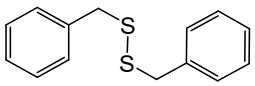


9b

Using thiol **8b** (62.7 mg, 0.54 mmol) and **RSTe** (2.70 μmol [0.5 mol%]), disulfide **9a** was obtained as a colorless oil. Yield: 60.5 mg (97%); ^{1}H NMR ($CDCl_3$): δ = 2.73–2.67 (m, 2H), 2.07–2.02 (m, 4H), 1.83–1.79 (m, 4H), 1.65–1.61 (m, 2H), 1.37–1.20 ppm (m, 10H). Spectroscopic data is in accordance with our previous report.[S2]

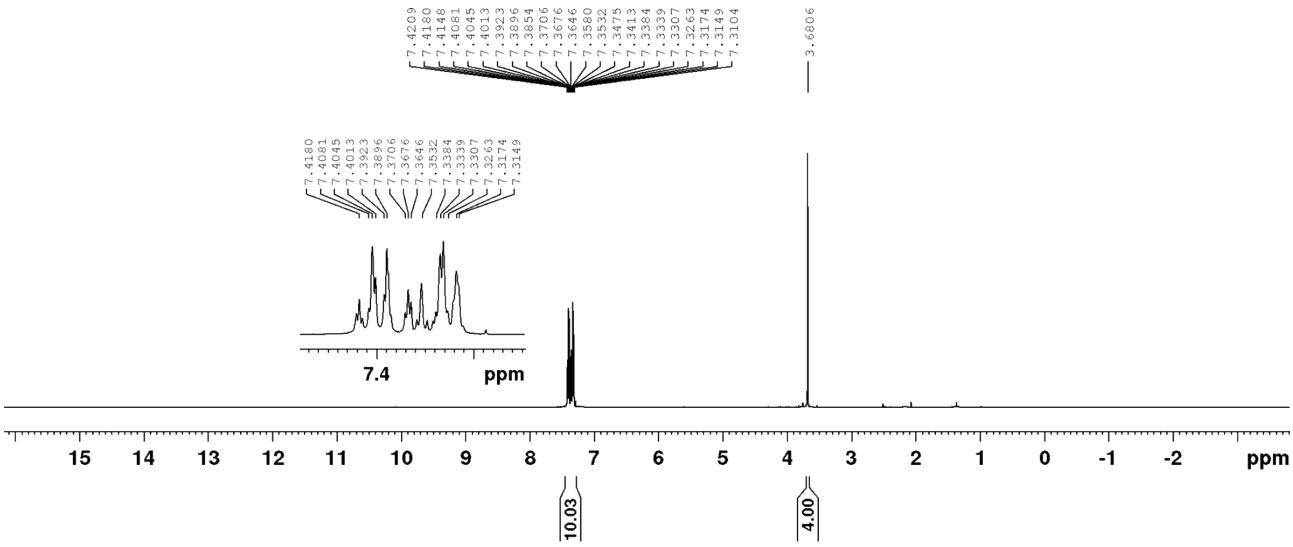


9c: *1,2-Dibenzyl disulfide*

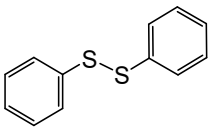


9c

Using thiol **8c** (70.8 mg, 0.57 mmol) and **RSTe** (2.85 μmol [0.5 mol%]), disulfide **9c** was obtained as a white solid. Yield: 66.3 mg (94%); ^{1}H NMR ($CDCl_3$): δ = 7.42–7.31 (m, 10H), 3.68 ppm (s, 4H). Spectroscopic data is in accordance with our previous report.[S2]

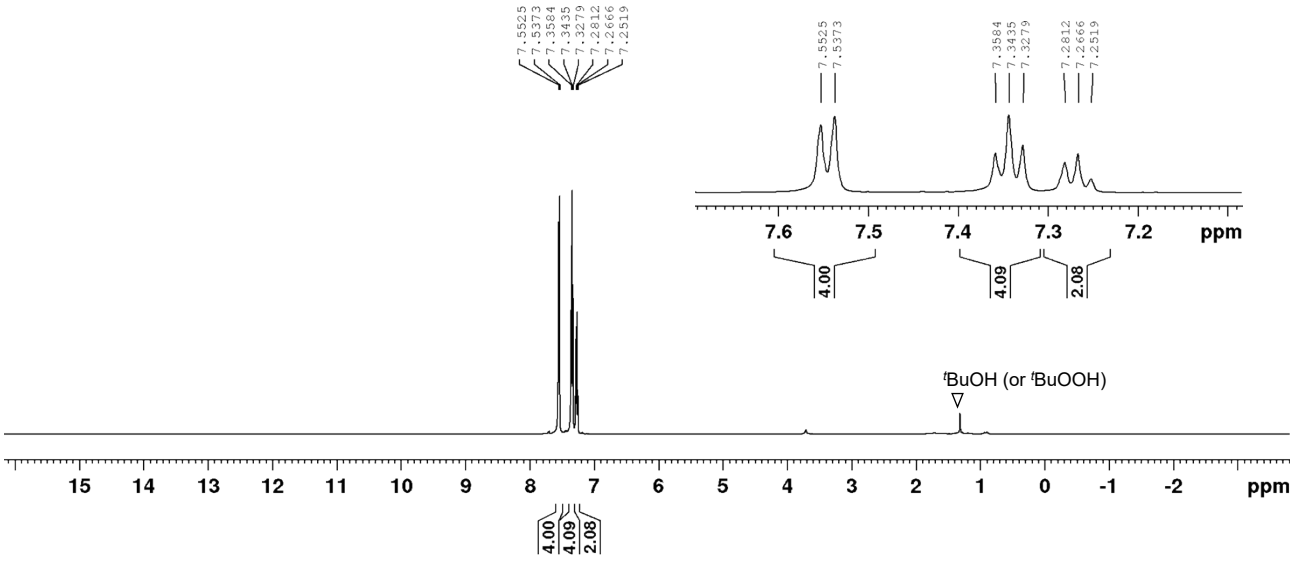


9d: *1,2-Diphenyl disulfide*

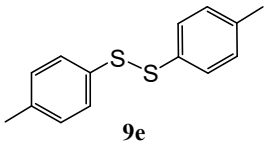


9d

Using thiol **8d** (59.5 mg, 0.54 mmol) and **RSTe** (2.70 μmol [0.5 mol%]), disulfide **9d** was obtained as a white solid. Yield: 59.0 mg (quant.); ^{1}H NMR ($CDCl_3$): δ = 7.54 (d, *J* = 7.6 Hz, 4H), 7.34 (t, *J* = 7.5 Hz, 4H), 7.28–7.25 ppm (m, 2H). Spectroscopic data is in accordance with our previous report.[S2]

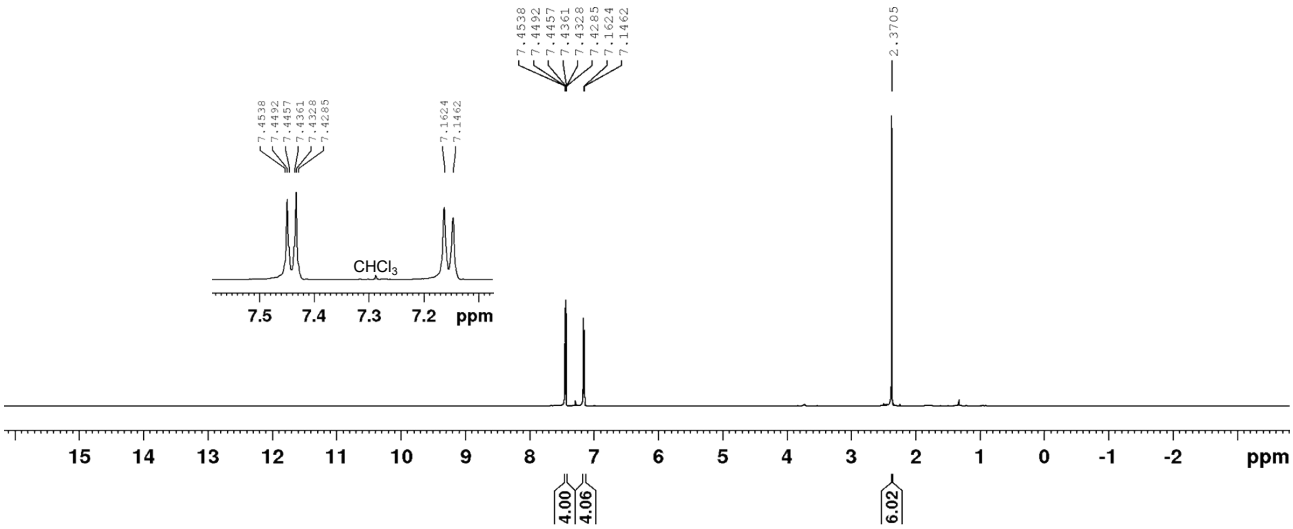


9e: *Bis(4-methylphenyl) disulfide*

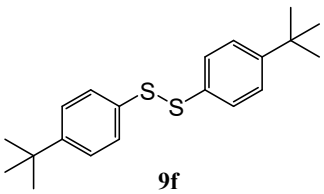


9e

Using thiol **8e** (67.1 mg, 0.54 mmol) and **RSTe** (2.70 μmol [0.5 mol%]), disulfide **9e** was obtained as a white solid. Yield: 66.2 mg (99%); ^{1}H NMR ($CDCl_3$): δ = 7.45–7.43 (m, 4H), 7.15 (d, *J* = 8.1 Hz, 4H), 2.37 ppm (s, 6H). Spectroscopic data is in accordance with our previous report.[S2]

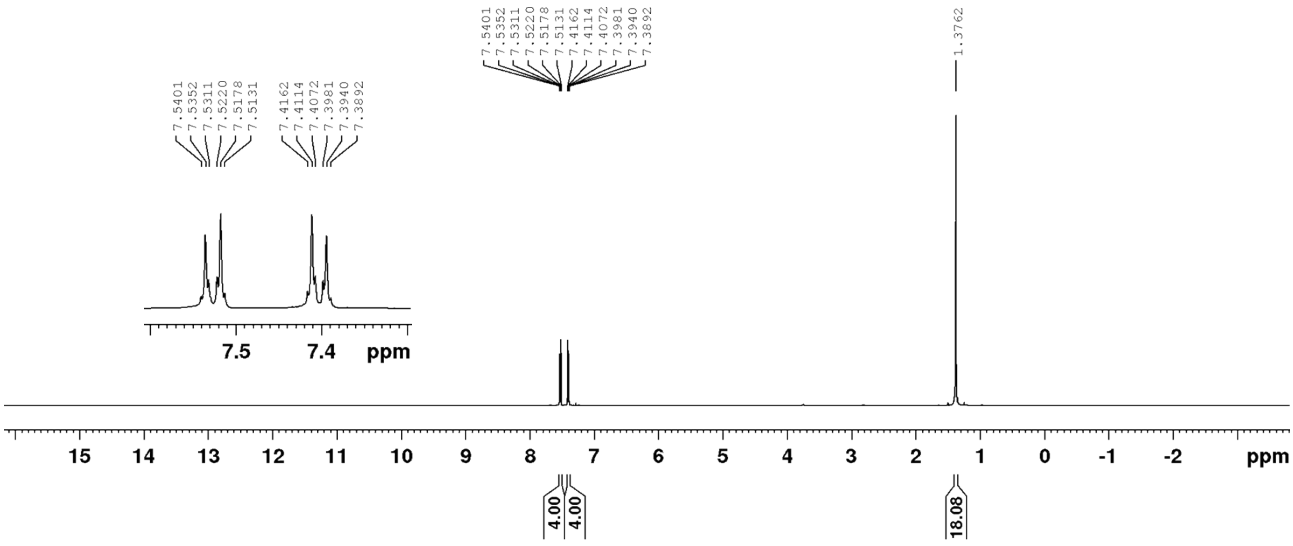


9f: *1,2-Bis(4-(tert-butyl)phenyl) disulfide*

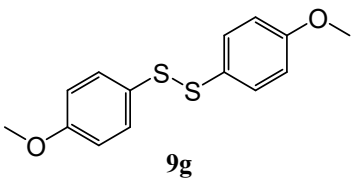


9f

Using thiol **8f** (73.1 mg, 0.44 mmol) and **RSTe** (2.20 μmol [0.5 mol%]), disulfide **9f** was obtained as a white solid. Yield: 73.4 mg (quant.); ^{1}H NMR ($CDCl_3$): δ = 7.54–7.51 (m, 4H), 7.42–7.39 (m, 4H), 1.38 ppm (m, 18H). Spectroscopic data is in accordance with our previous report.[S2]

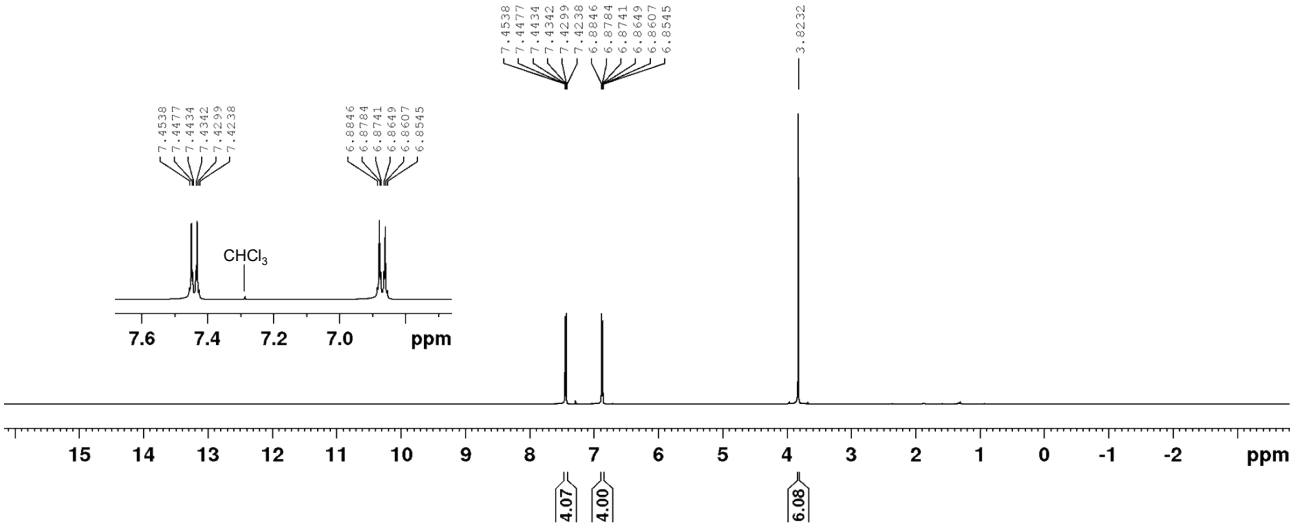


9g: *Bis(4-methoxyphenyl) disulfide*

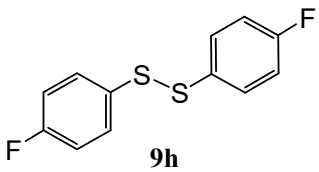


9g

Using thiol **8g** (52.2 mg, 0.37 mmol) and **RSTe** (1.85 μmol [0.5 mol%]), disulfide **9g** was obtained as an off-white solid. Yield: 49.4 mg (96%); ^{1}H NMR ($CDCl_3$): δ = 7.45–7.42 (m, 4H), 6.88–6.85 (m, 4H), 3.82 ppm (s, 6H). Spectroscopic data is in accordance with our previous report.[S2]

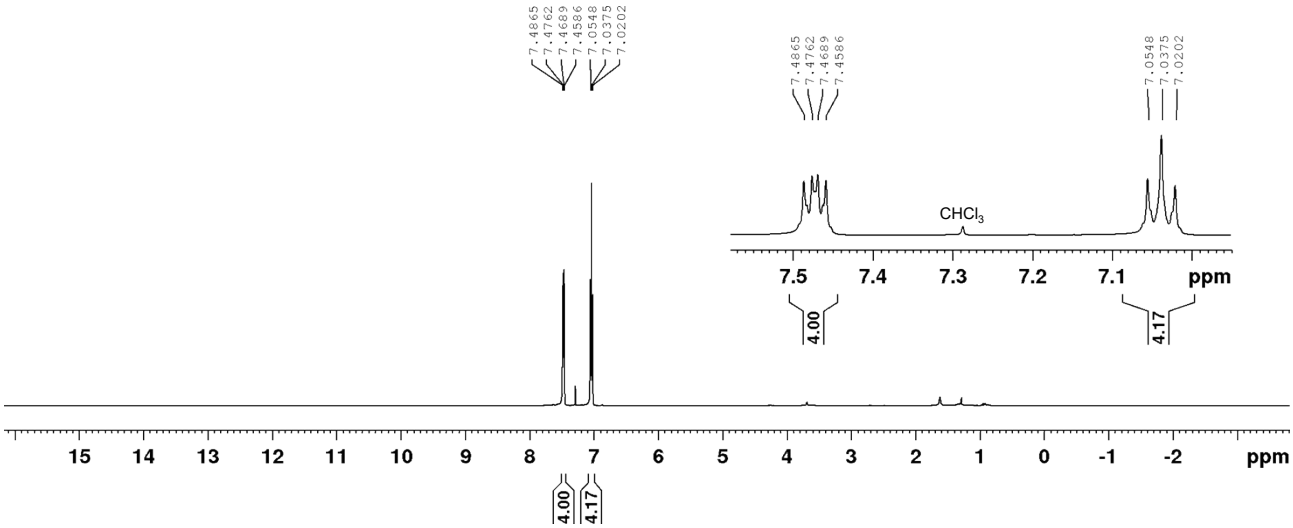


9h: *Bis(4-fluorophenyl) disulfide*

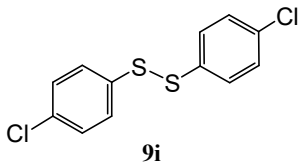


9h

Using thiol **8h** (69.2 mg, 0.54 mmol) and **RSTe** (2.70 μmol [0.5 mol%]), disulfide **9h** was obtained as an off-white solid. Yield: 60.8 mg (89%); ^{1}H NMR ($CDCl_3$): δ = 7.47 (dd, J = 5.15, 8.80 Hz, 4H), 7.04 ppm (t, J = 17.3 Hz, 4H). Spectroscopic data is in accordance with our previous report.[S2]

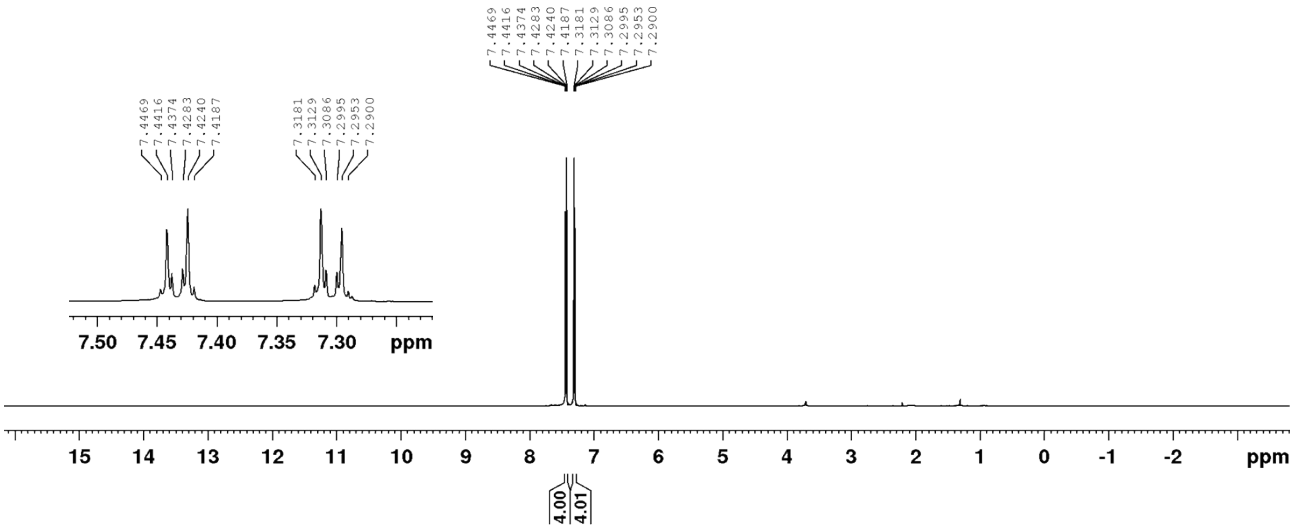


9i: *Bis(4-chlorophenyl) disulfide*

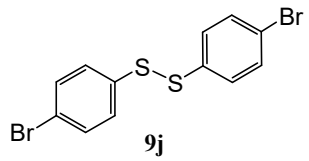


9i

Using thiol **8i** (79.0 mg, 0.54 mmol) and **RSTe** (2.70 μmol [0.5 mol%]), disulfide **9i** was obtained as a white solid. Yield: 68.8 mg (89%); ^{1}H NMR ($CDCl_3$): δ = 7.44–7.42 (m, 4H), 7.32–7.29 ppm (m, 4H). Spectroscopic data is in accordance with our previous report.[S2]

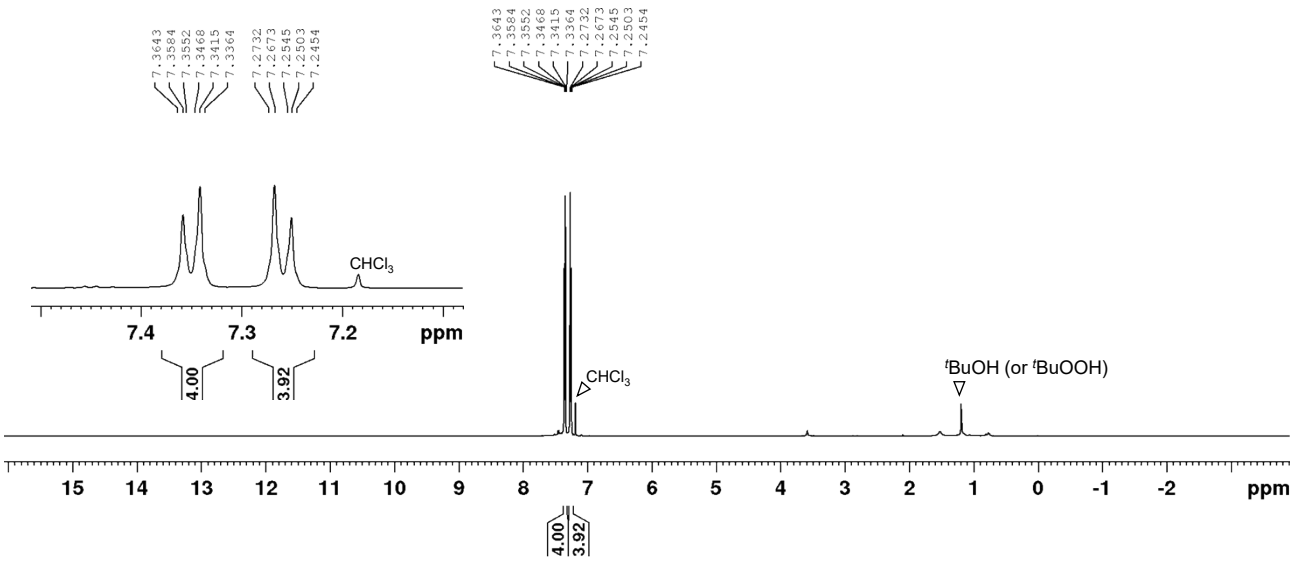


9j: *Bis(4-bromophenyl) disulfide*

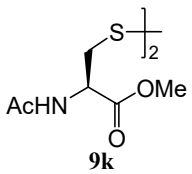


9j

Using thiol **8j** (103.8 mg, 0.55 mmol) and **RSTe** (2.75 μmol [0.5 mol%]), disulfide **9j** was obtained as a white solid. Yield: 88.9 mg (86%); ^{1}H NMR ($CDCl_3$): δ = 7.36–7.34 (m, 4H), 7.27–7.25 ppm (m, 4H). Spectroscopic data is in accordance with our previous report.[S2]

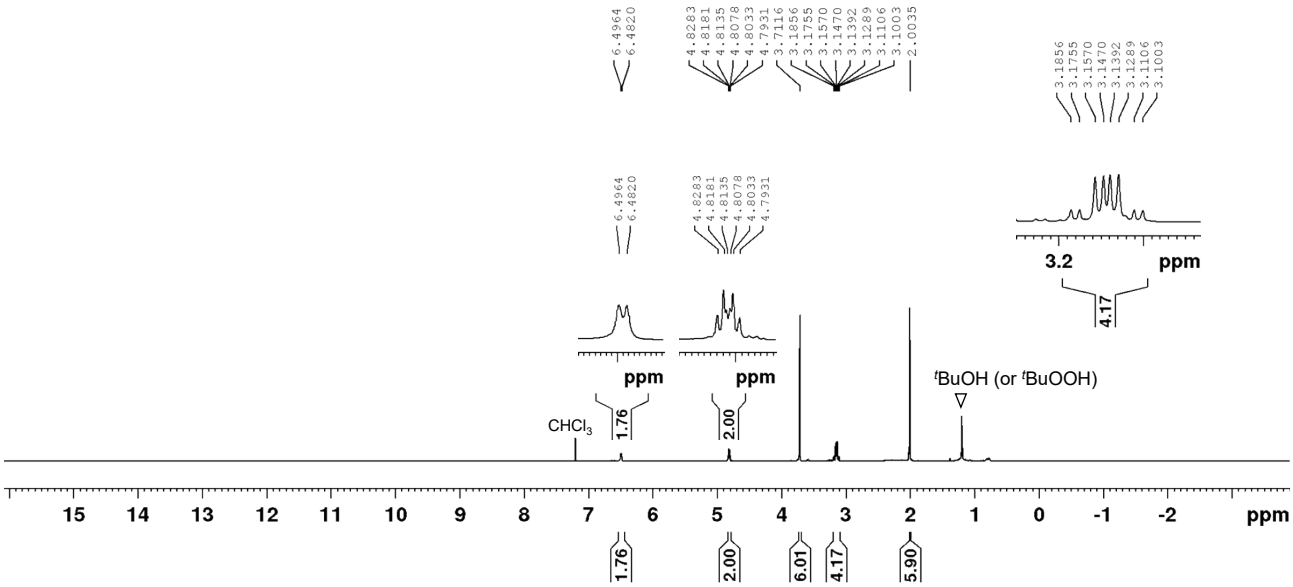


9k: *N-Acetyl-L-cystine methyl ester*

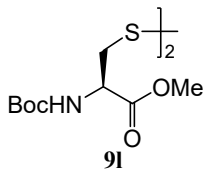


9k

Using thiol **8k** (5.6 mg, 0.032 mmol) and **RSTe** (0.32 μmol [1.0 mol%]), disulfide **9k** was obtained as a white solid. Yield: 5.3 mg (95%); ^{1}H NMR ($CDCl_3$): δ = 6.49 (d, *J* = 7.2 Hz, 2H), 4.82–4.79 (m, 2H), 3.71 (s, 6H), 3.18–3.10 (m, 4H), 2.00 ppm (s, 6H). Spectroscopic data is in accordance with our previous report.[S2]

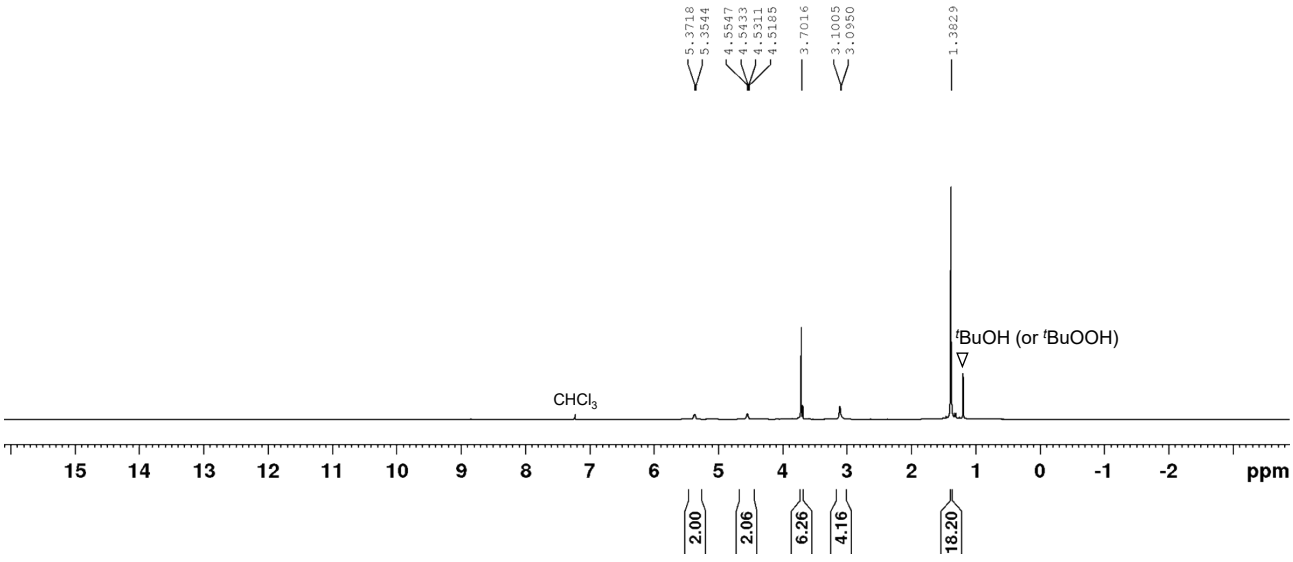


9l: *N-(tert-Butoxycarbonyl)-L-cysteine methyl ester*

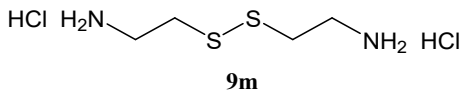


9l

Using thiol **8l** (35.6 mg, 0.15 mmol) and **RSTe** (0.75 μmol [0.5 mol%]), disulfide **9l** was obtained as a white solid. Yield: 35.1 mg (quant.); ^{1}H NMR ($CDCl_3$): δ = 5.36 (d, *J* = 8.7, 2H), δ = 4.54 (q, *J* = 7.2, 2H), 3.70 (s, 6H), 3.10–8.09 (m, 4H), 1.38 (s, 18H) ppm. Spectroscopic data is in accordance with our previous report.[S2]

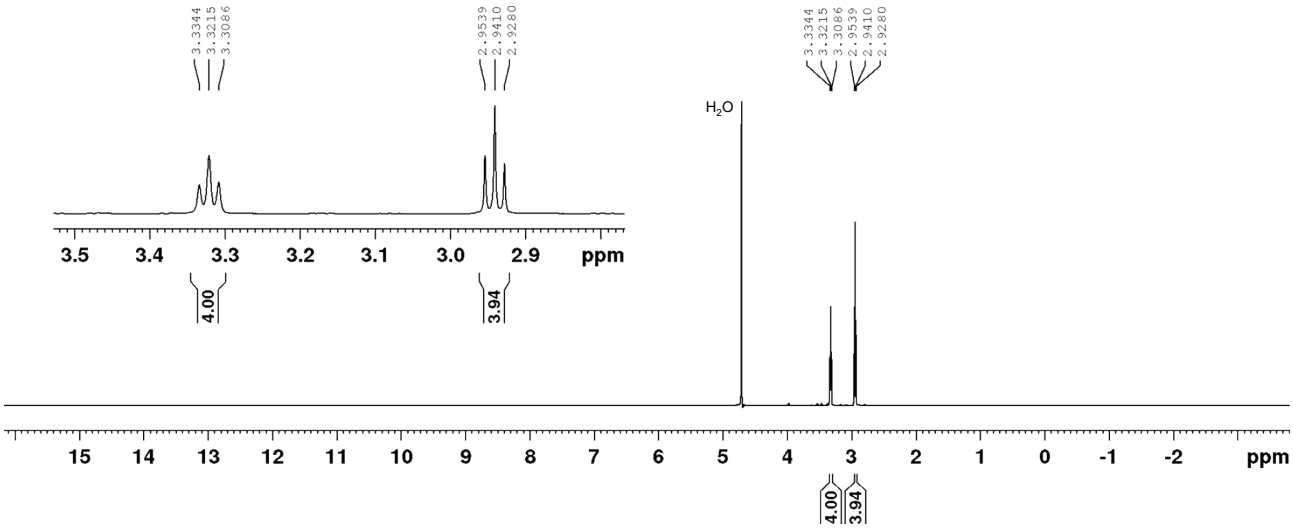


9m: *Cystamine dihydrochloride*

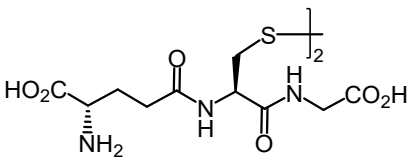


9m

Using thiol **8m** (76.2 mg, 0.67 mmol) and **RSTe** (3.25 μmol [0.5 mol%]), disulfide **9m** was obtained as a white powder. Yield: 75.4 mg (quant); ^{1}H NMR (D_2O): δ = 3.32 (t, *J* = 6.5 Hz, 4H), 2.94 ppm (t, *J* = 6.5 Hz, 4H); ^{13}C NMR (D_2O): δ=37.83, 33.37ppm. Spectroscopic data is in accordance with our previous report.[S2]

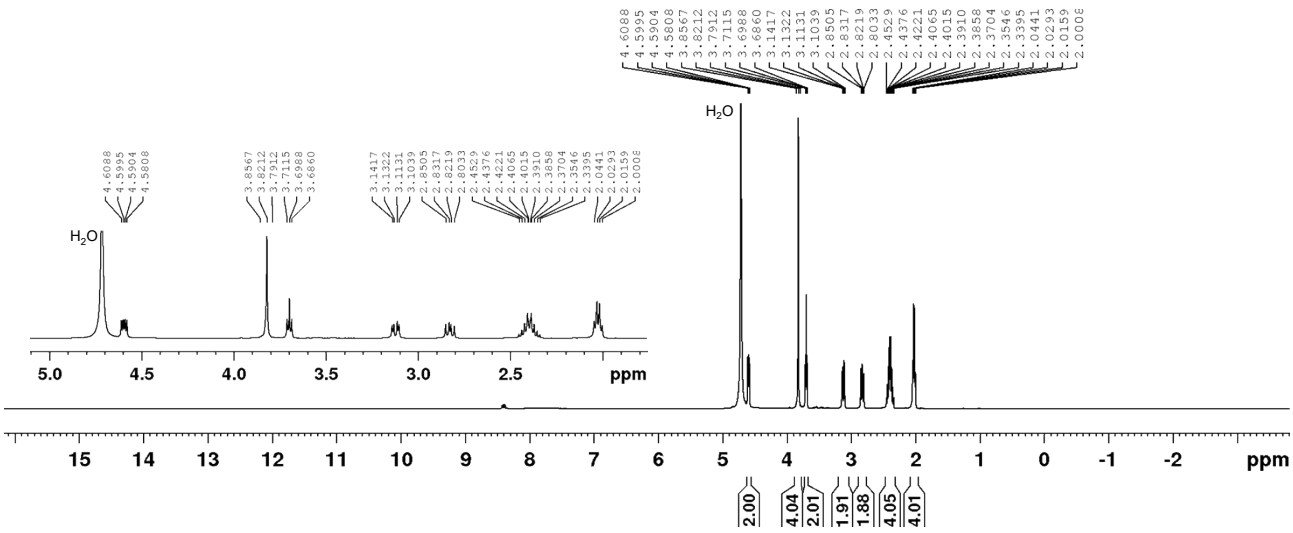


9n: *Oxidized glutathione (GSSG)*

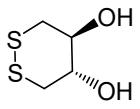


9n

Using thiol **8n** (172.7 mg, 0.56 mmol) and **RSTe** (2.80 μmol [0.5 mol%]), disulfide **9m** was obtained as a white powder. Yield: 172.7 mg (quant.); ^{1}H NMR (D_2O): δ = 4.59 (dd, *J* = 4.65, 9.35 Hz, 2H), 3.86–3.79 (m, 4H), 3.70 ppm (t, *J* = 6.4 Hz, 2H), 3.12 ppm (dd, *J* = 4.8, 14.2 Hz, 2H), 2.83 ppm (dd, *J* = 9.4, 14.2 Hz, 2H), 2.45–2.34 ppm (m, 4H), 2.02 ppm (q, *J* = 8.7 Hz, 4H). Spectroscopic data is in accordance with our previous report.[S2]

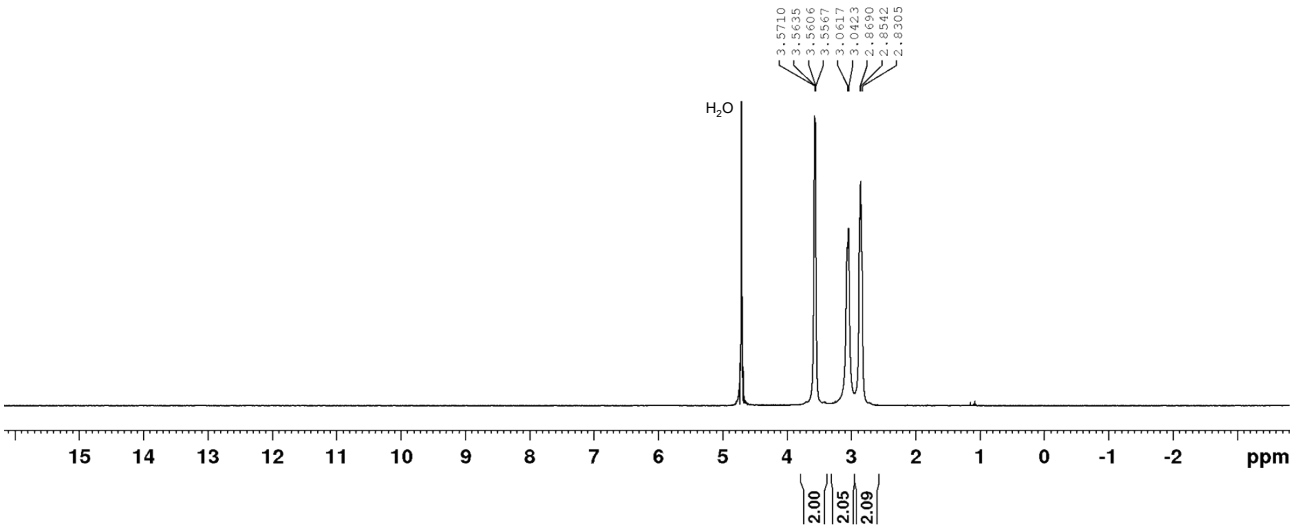


6l: *trans-4,5-Dihydroxy-1,2-dithiane (DTT^{ox})*



9o

Using thiol **8o** (8.3 mg, 0.054 mmol) and **RSTe** (0.27 μmol [5 mol%]), disulfide **9o** was obtained as a white powder. Yield: 8.2 mg (quant.); ^{1}H NMR (D_2O): δ = 3.57–3.56 (m, 2H), 3.06–3.04 (m, 2H), 2.87–2.83 (m, 2H). The obtained spectral data agreed well with that of commercial sample.



3. Supplemental data

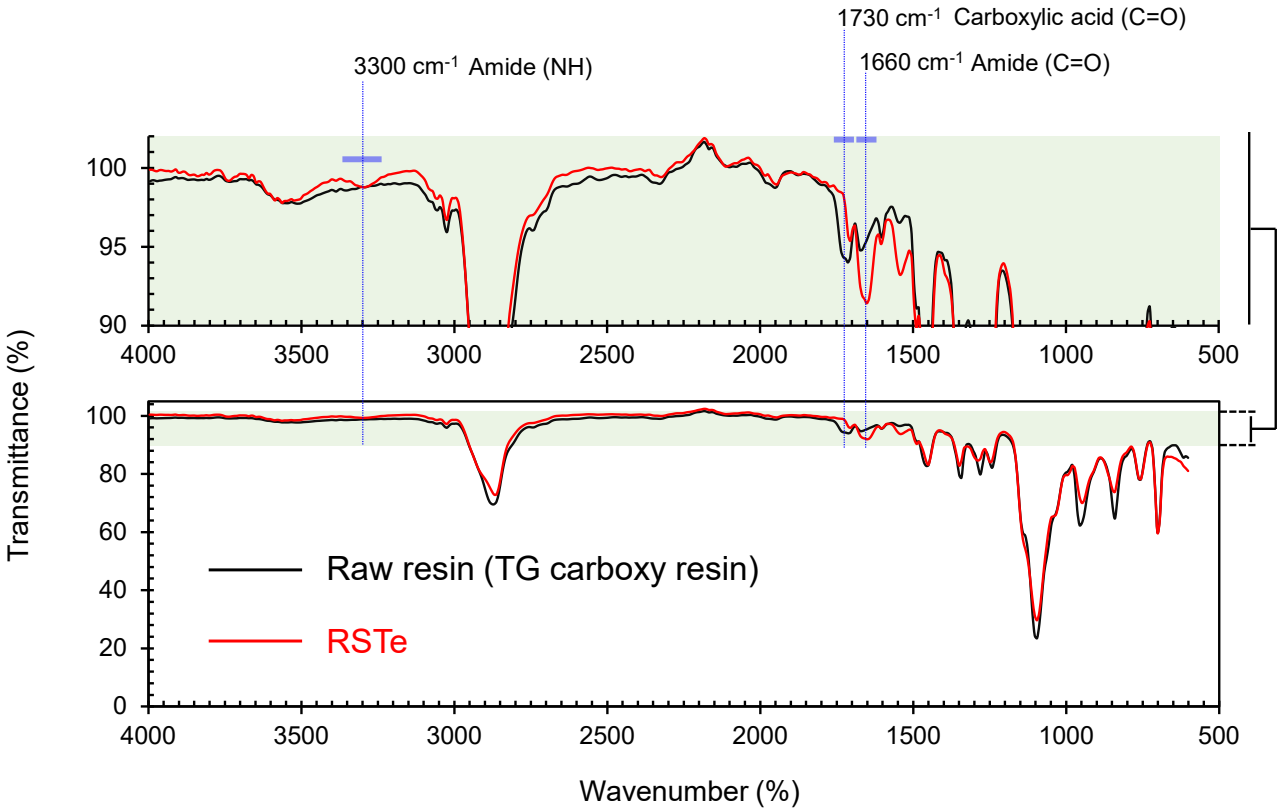


Fig. S1 Attenuated total reflectance (ATR)-fourier transformed infrared spectroscopic (ATR-FTIR) analysis. Black and red lines refer to raw resin (NovaSyn® TG carboxy resin) having no telluride moiety and **RSTe**, respectively.

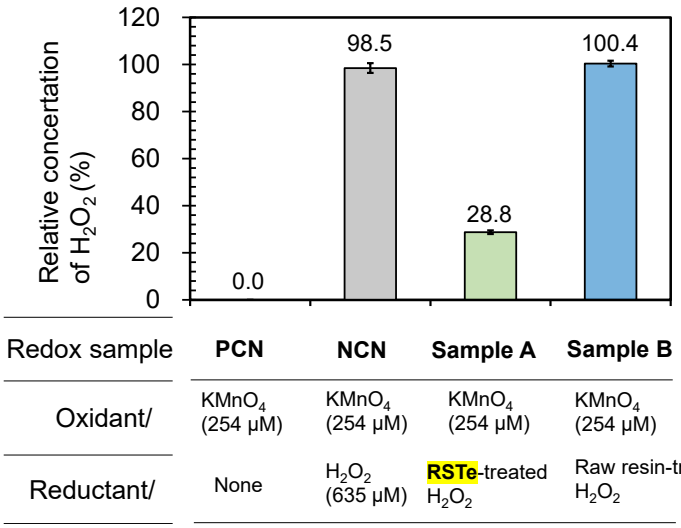
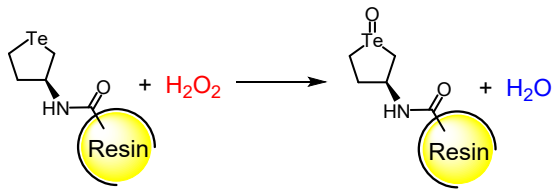


Fig. S2 Quantitative analysis of H_2O_2 in water after treatment with **RSTe** or a raw resin (TG-carboxy ®). The concentration of remained H_2O_2 was estimated by stoichiometric relationship in the redox reaction between H_2O_2 and $KMnO_4$. See ***Note*** for the experimental details described below.

***Note*:** To estimate the modification rate of the resin decorated with telluride **4**, the reduction of H_2O_2 by **RSTe** according to eq. 1 was performed, and the concentration of remained H_2O_2 after the reaction was determined by H_2O_2-$KMnO_4$ redox titration. Initially, H_2O_2 (0.81 μmol) and **RSTe** or raw-resin (TG-carboxy resin®) (0.81 μmol as substituent on the resin) were mixed in 600 μL of water at 37 °C with vigorous mixing for 3 h.



••• (1)

Subsequently, based on eq. 2, H_2O_2 remaining in the reaction solution was oxidized by $KMnO_4$ in the presence of sulfuric acid.

$$2KMnO_4 + 5H_2O_2 + 3H_2SO_4 \rightarrow 2MnSO_4 + K_2SO_4 + 5O_2 + 8H_2O \quad \text{••• (2)}$$

The reaction mixture obtained after the reaction (eq. 1) was centrifuged at 6000 rpm for 30 sec. Then, 400 μL of the supernatant containing up to 540 nmol H_2O_2 was taken up, mixed with 400 μL of aqueous solution containing 216 nmol $KMnO_4$ and 100 μL of 4 M H_2SO_4, and allowed to react for 2 min at room temperature. The concentration of unreacted $KMnO_4$ in the resulting solution was determined on the basis of the absorbance at 525 nm, from which the concentration of unreacted H_2O_2 in the reaction of eq. 1 was estimated.

The result indicated that 71% of H_2O_2 was converted to H_2O during the reaction for eq. 1, suggesting that TG-carboxy resin® was modified with telluride **4** in up to 71%.

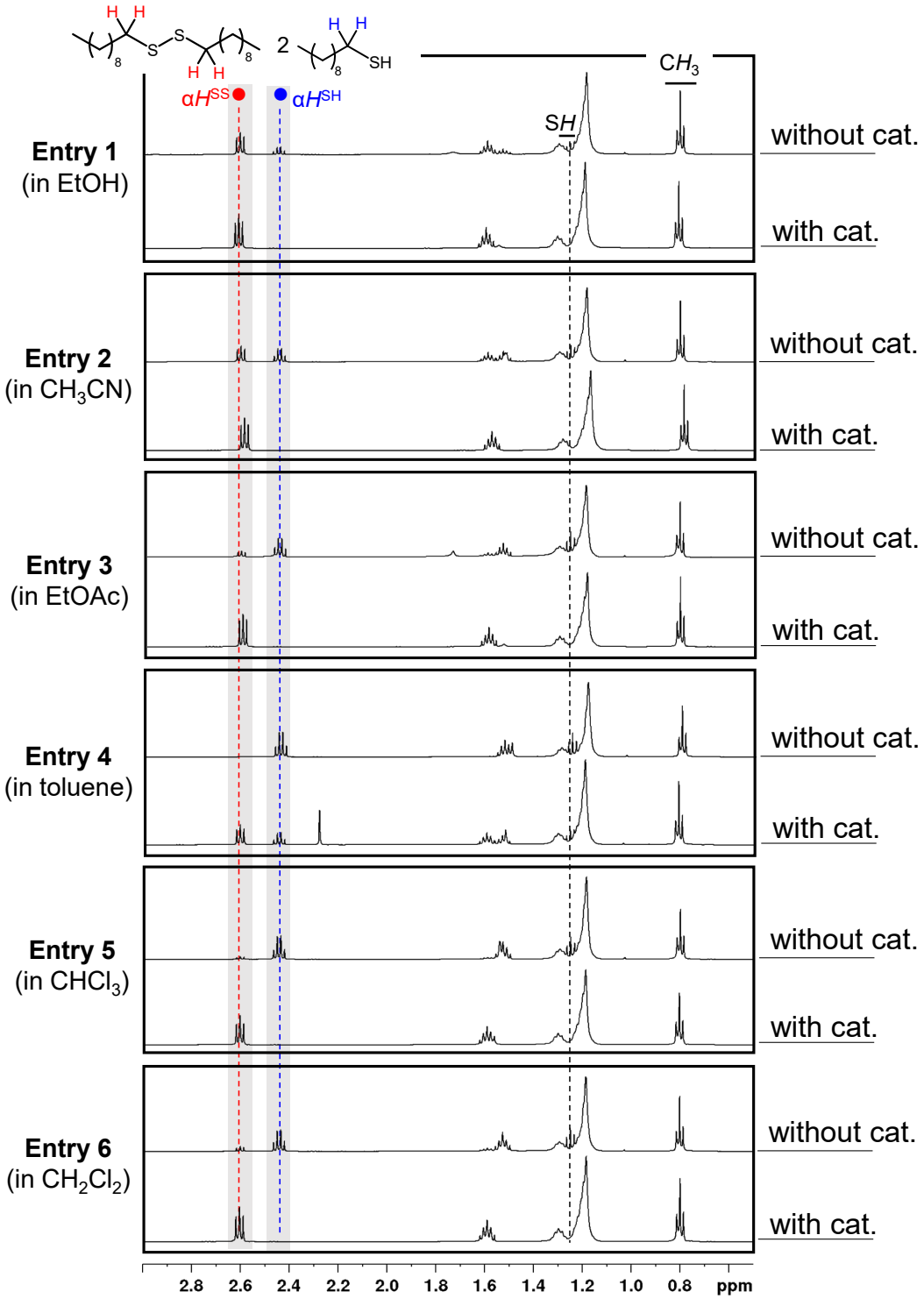
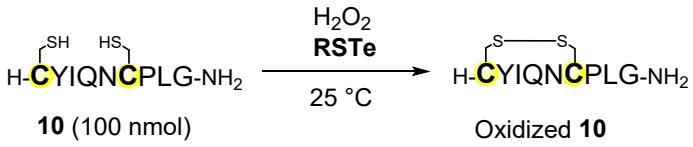


Fig. S3 ^{1}H NMR spectra obtained from catalytic oxidation of 1-decanethiol (**8a**) under various conditions. Entry numbers correspond to those of Table 1 in the main text.

Table S1 Optimization of reaction conditions for SS-formation of oxytocin.



Entry	**RSTe** (nmol)	H_2O_2 (nmol)	Reaction time (h)	Solvent (Vol. = 1 mL)	Products		
					7 (%)[a]	Oxidized **7** (%)[a]	Byproduct [Dimer] (%)[a]
1	100	100	1	0.1%TFA/H_2O	48	50	2
2	100	150	23	MeCN:H_2O [1:3 v:v]	0	96	4
3	100	150	4	0.1%TFA/H_2O	26	74	0
4	100	190	4.5	0.1% TFA/H_2O	4	92	4
5	100	210	2	0.1%TFA/H_2O	0	96	4
6	150	210	1	0.1%TFA/H_2O	0	91	9
7	None	210	2	0.1%TFA/H_2O	99	1	0

[a] Ratio estimated from sum of the peak areas for products and the starting material (**10**) on the HPLC chromatogram.

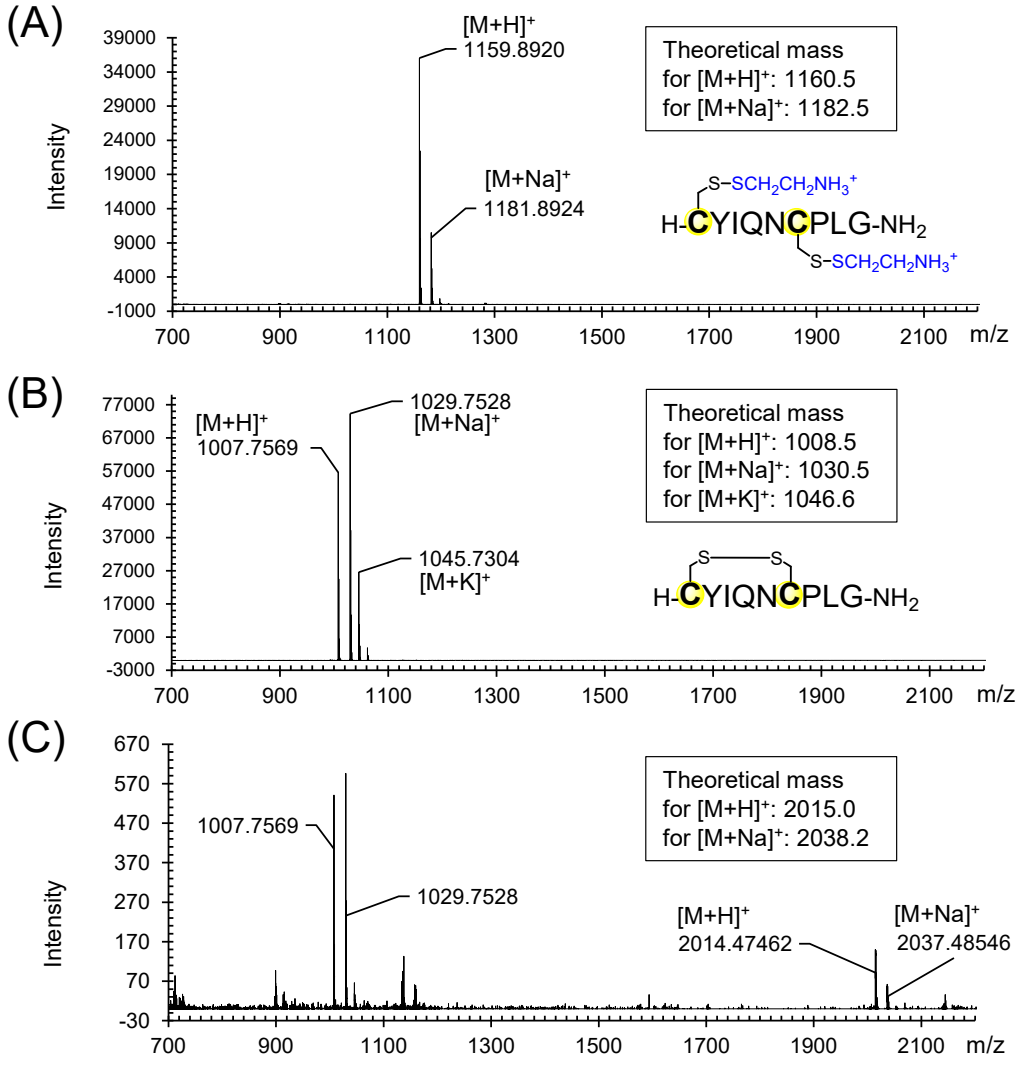


Fig. S4 MALDI-TOF-MS chromatograms of AEMTS-blocked **10** (**A**), oxidized **10** (**B**), and dimerized state via SS-bonds (**C**). Each product was isolated from HPLC (Fig. 6B).

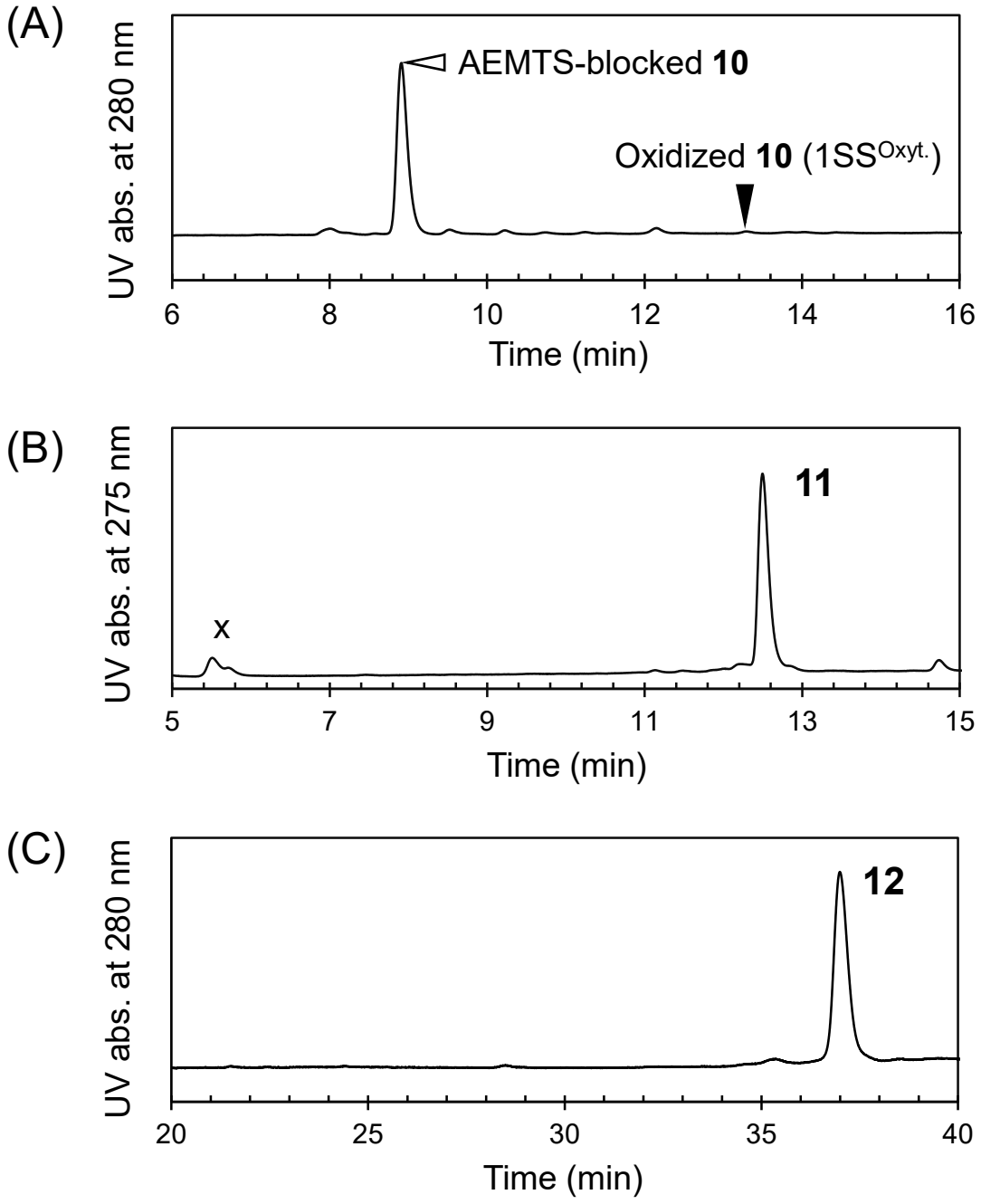


Fig. S5 HPLC chromatograms obtained from SS-formation experiments of peptides **10** (**A**), **11** (**B**) and **12** (**C**) in the *absence* of **RSTe**. Reaction conditions: For (**A**), **10** (100 nmol), H_2O_2 (200 nmol), and raw resin (210 nmol) were mixed in aqueous 0.1 % TFA solution (0.9 mL), and the sample was incubated with agitation at 27 °C for 2 h. For (**B**), **11** (80 nmol), H_2O_2 (320 nmol), and raw resin (160 nmol) were mixed in aqueous 0.1 % TFA solution (1.0 mL), and the sample was incubated with agitation at 27 °C for 2 h. The symbol x represents byproducts resulting from undesired oxidation of side chains rather than SS-formation between Cys residues. For (**C**), **12** (80 nmol), H_2O_2 (480 nmol), and raw resin (240 nmol) were mixed in aqueous 0.1 % TFA solution (1.0 mL), and the sample was incubated with agitation at 27 °C for 2 h.

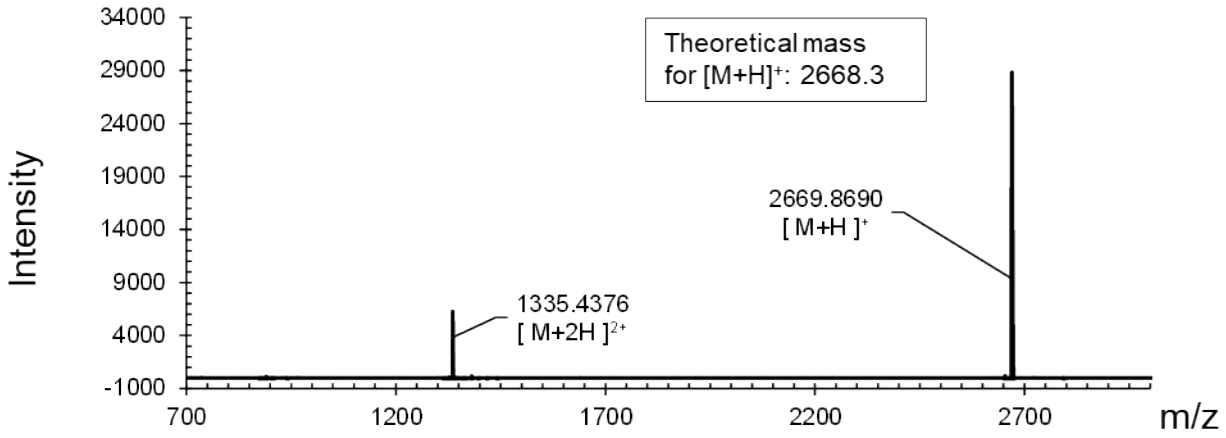


Fig. S6 MALDI-TOF-MS chromatogram of fully oxidized **11** ($2SS^{RlxA}$). $2SS^{RlxA}$ was isolated from HPLC (Fig. 6D).

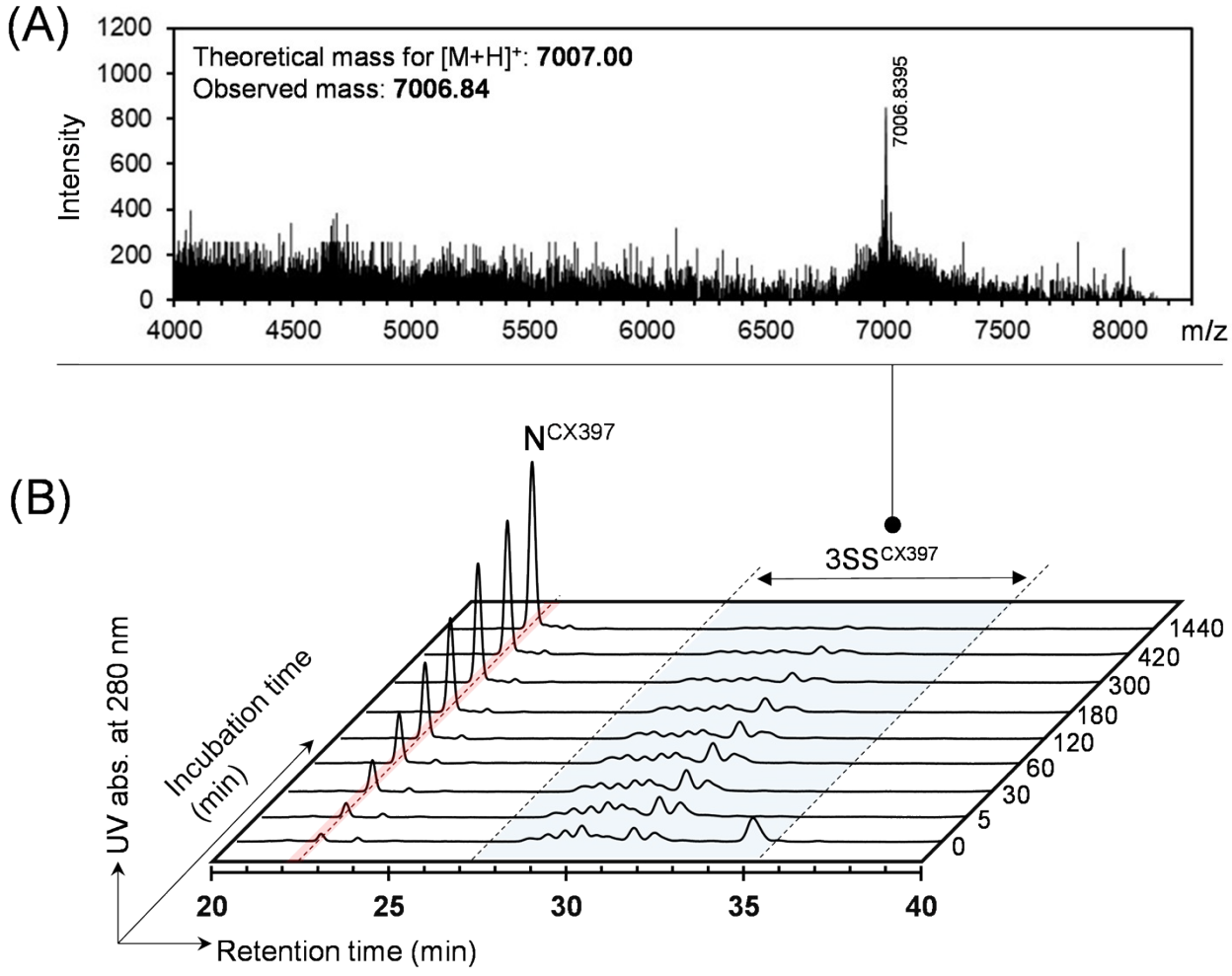


Fig. S7 SS-isomerization experiment of $3SS^{CX397}$ with GSH. (**A**) MALDI-TOF-MS chromatogram of fully oxidized **12** ($3SS^{CX397}$). $3SS^{CX397}$ was isolated from HPLC. (**B**) HPLC chromatograms of samples obtained from SS-isomerization experiment of $3SS^{CX397}$. Reaction conditions were $[3SS^{CX397}]_0$ = 80 μM and $[GSH]_0$ = 0.15 mM in 100 mM Tris-HCl buffer solution containing 1 mM EDTA at pH 8.0 and 25 °C.

References

S1. K. Arai, K. Dedachi and M. Iwaoka, *Chem. Eur. J.*, **2011**, *17*, 481–485.

S2. K. Arai, Y. Osaka, M. Haneda and Y. Sato, *Catal. Sci. Technol.*, **2019**, *9*, 3647–3655.