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Thiol catalysis of selenosulfide bond cleavage by a triarylphosphine

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Abstract

The arylthiol 4-mercaptophenylacetic acid (MPAA) is a powerful catalyst of selenosulfide bond reduction by the triarylphosphine 3,3',3''-phosphanetriyltris(benzenesulfonic acid) trisodium salt (TPPTS). Both reagents are water-soluble at neutral pH and are particularly adapted for working with unprotected peptidic substrates. Contrary to trialkylphosphines such as *tris*(2-carboxyethyl)phosphine hydrochloride (TCEP), TPPTS has the advantage of not inducing deselenization reactions. We believe that the work reported here will be of value for those manipulating selenosulfide bonds in peptidic or protein molecules.

Main

The reduction of dichalcogenides by phosphines is a long-standing reaction that has been the subject of several mechanistic studies. The reductive cleavage of disulfide bonds into thiols by phosphines has concentrated most efforts primarily due to the importance of thiols in synthetic organic chemistry and peptide/protein chemistry ($X, Y = S$; Fig. 1a).¹⁻⁴ In-depth studies from several authors scrutinized the reaction of various disulfides with triphenylphosphine ($R_3 = Ph$, Fig. 1a).⁵⁻⁹ These investigations pointed out the critical role played by water in the reduction process,^{5-7,9} and the higher propensity of aryl disulfides or mixed alkyl aryl disulfides over alkyl disulfides to be cleaved by triphenylphosphine.

Comparatively, alkyl selenosulfides ($R_1, R_2 = Alk, X = S, Y = Se$, Fig. 1a) are reduced with difficulty by triphenylphosphine.¹⁰ In practice, their cleavage require more reducing phosphorus compounds such as trialkylphosphines, among which *tris*(2-carboxyethyl)phosphine hydrochloride (TCEP, Fig. 1b) is particularly popular for reactions conducted in aqueous media. However, a limitation of using trialkylphosphines for the reduction of alkyl selenosulfides is their propensity to deselenize the alkyl selenol component.¹¹⁻¹⁴

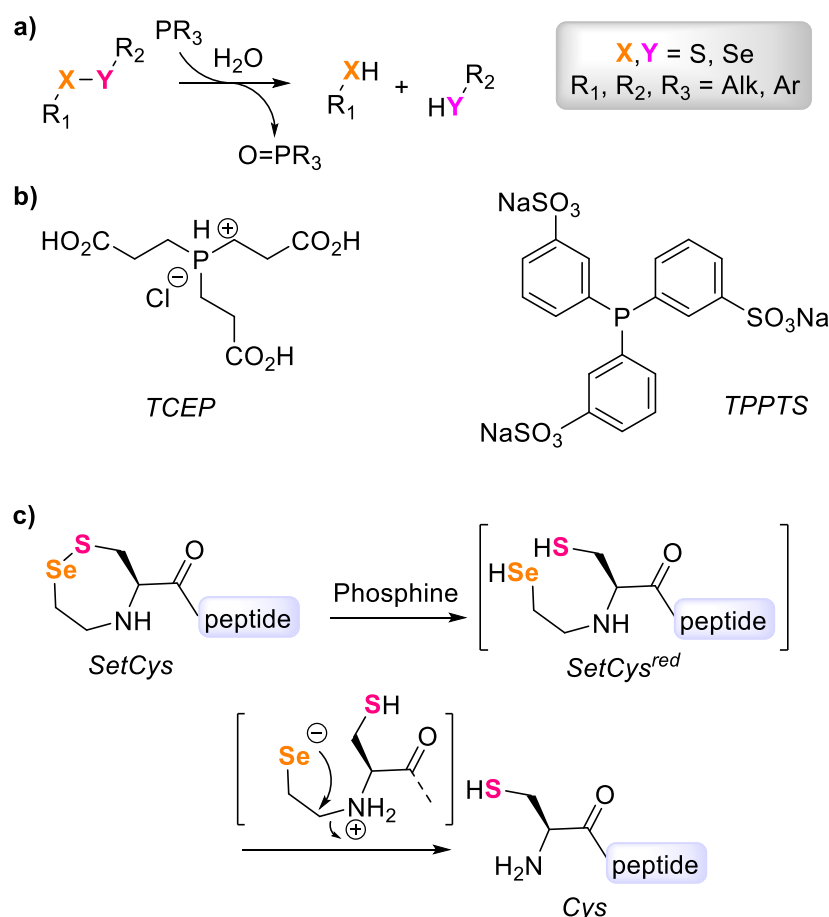


Figure 1. a) Reduction of dichalcogenides by phosphines. b) Water-soluble phosphines studied in this work. c) Conversion of SetCys into Cys upon reduction by a phosphine.

Our interest in the chemistry of selenosulfides comes from the recent discovery that *N*-(2-selenoethyl)cysteine, in the form of its cyclic selenosulfide called SetCys, can be converted into cysteine residue (Cys) in the presence of TCEP (Fig. 1c).¹⁵ The loss of the 2-selenoethyl arm proceeds through the reductive opening of the cyclic selenosulfide bond by the phosphine, which is followed by the spontaneous cleavage of the carbon-nitrogen bond through an intramolecular nucleophilic substitution mechanism. Cysteine surrogates such as SetCys are useful tools for protein chemical synthesis because such chemical devices enable to control the concatenation of peptide segments using the native chemical ligation (NCL¹⁶). NCL is a widely used method in the field of protein chemical synthesis to connect unprotected peptide segments in water by reacting a C-terminal peptide thioester with a N-terminal cysteinyl peptide (Fig. 2a).^{2,17} To achieve synthetic useful rates, NCL is often catalyzed by thiol additives that activate the thioester component of the reaction through thiol-thioester exchange.^{18,19} The gold standard catalyst for promoting NCL is 4-mercaptophenyl acetic acid (MPAA),²⁰ but other thiol additives such as methyl thioglycolate (MTG),²¹ or sodium 2-mercaptoethylsulfonate (MPSNa),²² are also described in some works (Fig. 2b).

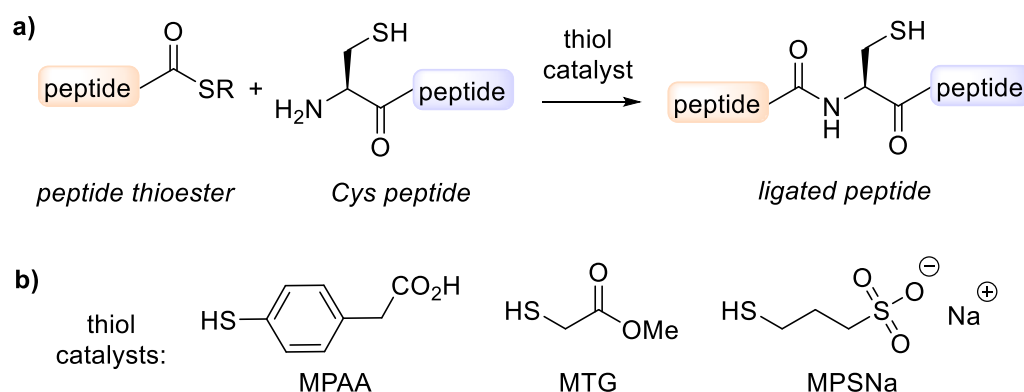


Figure 2. a) NCL reaction. b) Some thiols used as additives during NCL.

Since the conversion of SetCys into Cys can be controlled by the cleavage of the Se-S bond during elaborated peptide assemblies by NCL, it is important to have a full understanding of the SetCys reactivity regarding different types of reductants that might be present in the reaction mixture. We therefore investigated the propensity of SetCys to be converted into Cys in the presence of TCEP or TPPTS (3,3',3''-phosphanetriyltris(benzenesulfonic acid) trisodium salt), two water soluble phosphines that differ markedly by their reductive power (Fig. 1b). Considering the large excesses of thiol additive usually needed for catalyzing NCL, we also examined if their presence has any role in the SetCys conversion into Cys. The studied thiols, MPPA, MTG and MPSNa, cover by their pK_a the range of thiols used for NCL catalysis.

SetCys peptide **1** was used as a model compound for studying the kinetics of SetCys conversion into Cys (Fig. 3a). The results as function of the nature and the concentration of phosphines and thiol additives used are gathered in Fig. 3b-f. All the experiments were performed at pH 6.0 since previous investigations showed that the rate of the intramolecular substitution process leading to the loss of the 2-selenoethyl arm is at its highest at this pH value.¹⁵ Furthermore, sodium ascorbate (100 mM) was systematically added in all reaction mixtures. Sodium ascorbate is a powerful radical scavenger that is mandatory when TCEP is used as the reductant to inhibit the deselenization of SetCys residue,¹⁵ even in the presence of large excesses of MPPA.²³ Interestingly, we found that sodium ascorbate is no longer required when TPPTS is used as the reductant (see Supplementary Information). Nevertheless, sodium ascorbate was used in all experiments to enable comparison of kinetic data. Note also that some lots of TPPTS used in this study were found to contain some traces of formaldehyde. Since TPPTS was used in large excess in our experiments, its contamination by formaldehyde resulted in the formation of a thiazolidine byproduct by reaction with Cys peptide **2** (~ 5-10%). The quality of TPPTS relative to its contamination by formaldehyde can be easily checked as described in the experimental section (see Fig. 6) and Supplementary Information.

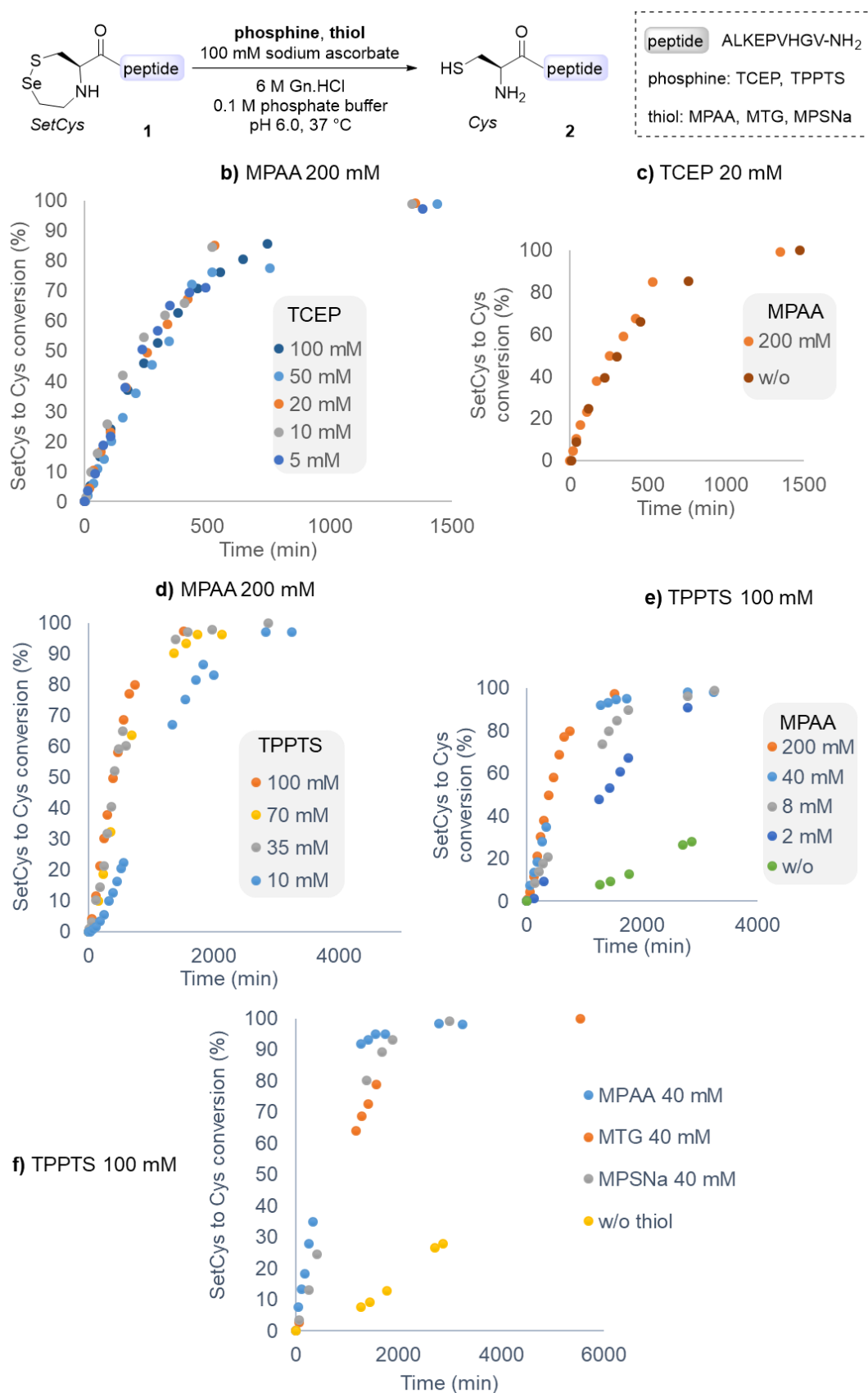


Figure 3. Rate of SetCys conversion into Cys in the presence of varying concentrations of phosphine (TCEP, TPPTS) and thiol (MPAA, MTG, MPSNa). The reactions were monitored at 215 nm by HPLC and/or UPLC-MS.

In the presence of 200 mM MPAA, the rate of SetCys to Cys conversion is independent of TCEP concentration (Fig. 3b). Conversely, at a fixed TCEP concentration of 20 mM, no difference in the rate of SetCys conversion into Cys is observed whether MPAA is present or not (Fig. 3c). In addition, in the presence of TCEP/MPAA, the rate of SetCys conversion into Cys is independent of peptide concentration (see Supplementary Information). Taken together, these data strongly support the mechanism proposed in Fig. 4 path a. According to this mechanism, the reduction of the cyclic selenosulfide by TCEP (STEP 1) is a fast process and the rate of SetCys to Cys conversion dictated by the intramolecular substitution process leading to C-N bond cleavage (STEP 2).

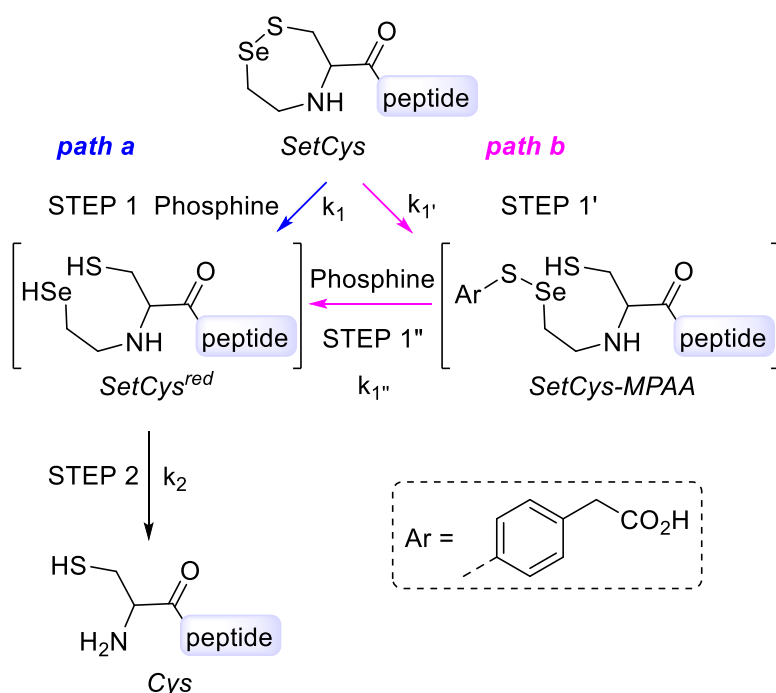


Figure 4. Proposed mechanisms for the conversion of SetCys into Cys upon reaction with a phosphine.

We next examined the influence of TPPTS and MPAA concentration on the rate of SetCys to Cys conversion. In the presence of 200 mM MPAA, the rate of SetCys to Cys conversion increased significantly when TPPTS concentration was increased from 10 to 35 mM and then plateaued (Fig. 3d). This result shows that the reduction process involving TPPTS is no longer rate limiting at concentrations greater than 35 mM. If the reaction proceeded as depicted in Fig. 4 path a, the intramolecular substitution process (STEP 2) would dictate the observed rate. However, the half-reaction time for the SetCys to Cys conversion using 100 mM TPPTS and 200 mM MPAA was below that expected on the basis of the TCEP experiments described above ($t_{1/2}$ 6.5 h vs $t_{1/2}$ 4.6 h). This observation led us to examine the role of MPAA in the reaction. Using 100 mM of TPPTS, we found that the rate of the SetCys to Cys conversion is dramatically dependent on MPAA concentration (Fig. 3e). In absence of MPAA (green dots in Fig. 3e), the $t_{1/2}$ is 75.4 h, while it is more than 10-fold higher when MPAA is present at 200 mM (orange dots in Fig. 3e, $t_{1/2}$ 6.5 h). Consequently, another step is rate limiting in the presence of TPPTS that is neither the reduction of the SetCys selenosulfide bond by the phosphine nor the intramolecular substitution process, but that involves MPAA.

To sum-up at this stage, the data accumulated so far show that TPPTS can cleave on its own the SetCys selenosulfide bond and trigger the SetCys to Cys conversion, that is through a mechanism corresponding to path a in Fig. 4 as proposed above for TCEP. However and contrary to TCEP, such a direct reduction of the selenosulfide bridge by TPPTS is very slow. The results point out that MPAA is a powerful catalyst of SetCys reductive opening by the phosphine. Although MTG and MPSNa are also able to accelerate SetCys reduction, these thiol additives proved to be significantly less efficient than MPAA (Fig. 3f). Experimentally, adding as low as 2 mM of MPAA enables to accelerate the rate of the process more than 3-fold (green dots in Fig. 3e $t_{1/2}$ 75.4 h vs dark blue dots $t_{1/2}$ 22.3 h). Apparently, a step involving MPAA but not the phosphine is rate limiting at 100 mM TPPTS.

One plausible explanation for the above results is to consider the nucleophilic cleavage of the SetCys cyclic selenosulfide bond by MPAA thiol leading to an acyclic selenosulfide intermediate, i.e. SetCys-MPAA, as being rate limiting (Fig. 4, path b, STEP 1'). The formed SetCys-MPAA is then reduced by the phosphine TPPTS (STEP 1''), an event that unmask the selenol group and triggers the intramolecular substitution process leading to the production of the Cys peptide (STEP 2). Attempts to detect SetCys-MPAA intermediate formation by LC-MS was unsuccessful. However, previous experiments argue for its formation.¹⁵ Typically, incubating for days a SetCys peptide with 200 mM MPAA in the absence of phosphine results in no apparent reaction. However, evidence for the transient formation of SetCys-MPAA intermediate was obtained when a SetCys peptide was treated with MPAA and a peptide thioester, an experiment that enabled the isolation of a ligation product featuring a SetCys residue at the ligation junction (Fig. 5). The formation of such a compound shows that the cyclic structure of SetCys is opened by reduction with MPAA. The resulting SetCys-MPAA intermediate is then trapped *in situ* by the peptide thioester through an NCL-like reaction before SetCys ring closure. A few experimental and computational works examined the nucleophilic attack of thiolates on dialkyl selenosulfides.²⁴⁻²⁶ These studies revealed that reaction rates of thiolates at selenium are several orders of magnitude higher than those at sulfur. Such results suggest that the MPAA-induced ring-opening of SetCys would rather lead to an alkyl aryl selenosulfide intermediate, as depicted in Fig. 4 and 5.

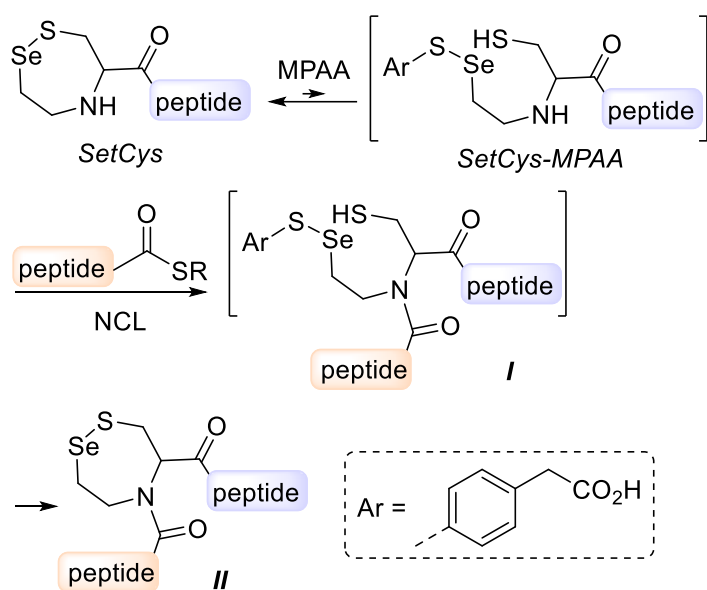


Figure 5. The reaction of a SetCys peptide with MPAA in the presence of a peptide thioester yields ligation product II.

In conclusion, the thiophenol derivative MPAA is a powerful catalyst of SetCys selenosulfide bond reduction by the water-soluble triarylphosphine TPPTS. TPPTS is a mild reductant compared to TCEP that has the advantage of not inducing deselenization reactions when combined with MPAA. We believe that the work reported here will be of value for those manipulating selenosulfide bonds in peptidic or protein molecules.

EXPERIMENTAL SECTION

Synthesis of model SetCys peptide 1. Peptide **1** was synthesized as previously reported in the literature.¹⁵

Conversion of SetCys peptide 1 into Cys peptide 2. General procedure illustrated with 200 mM MPAA and 100 mM TPPTS. The reaction is performed under nitrogen atmosphere. To a solution of Gn·HCl (287 mg) in 0.1 M, pH 7.0 sodium phosphate buffer (300 μ L) were added sodium ascorbate (9.90 mg, 50.0 μ mol, 100 mM), TPPTS (28.4 mg, 50.0 μ mol, 100 mM) and MPAA (16.8 mg, 100 μ mol, 200 mM) and the pH of the mixture was adjusted to 6.0 by adding 6 M NaOH. SetCys peptide **1** (0.48 mg, 0.32 μ mol, 1.00 mM) was then dissolved in the above solution (320 μ L) and the reaction mixture was heated at 37 °C. The conversion of SetCys into Cys was monitored by HPLC at 215 nm. For each analysis, an 8 μ L aliquot was taken from the reaction mixture and quenched by adding 10% AcOH in water (100 μ L). The sample was then extracted with Et₂O to remove MPAA prior to analysis by reversed-phase HPLC or UPLC-MS.

TPPTS quality control

A straightforward method to check for the presence of formaldehyde in TPPTS (or any other reagent used for converting SetCys into Cys) is to incubate Cys peptide **3** with TPPTS (70 mM) at pH 6.0 and 37 °C and quantify the amount of thiazolidine byproduct **4** formed overnight by UPLC-MS (Fig. 6, see also Supplementary Information).²⁷ No formaldehyde could be detected in Gn·HCl, MPAA and sodium ascorbate.

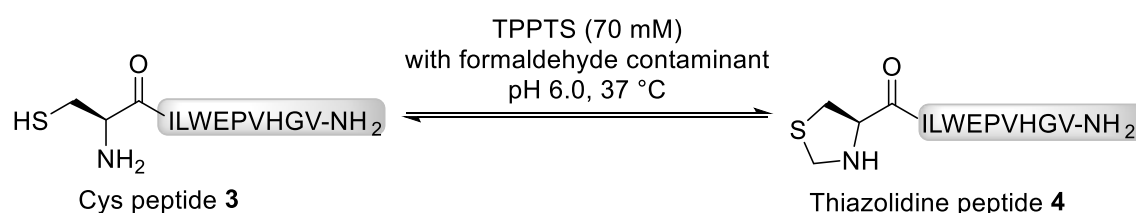


Figure 6. The presence of formaldehyde in TPPTS can be assayed through the propensity of Cys peptides to form stable thiazolidine products upon reaction with aldehydes. The thiazolidine product **4** is detected by UPLC-MS analysis (peptide **3** [M+H]⁺ calcd av. 1152.4, peptide **4** [M+H]⁺ calcd av. 1164.4).

ASSOCIATED CONTENT

Supplementary procedures and analysis data (pdf).

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NOTES

The authors declare no competing financial interest.

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