Peptide cyclization mediated by metal-free S-arylation: S-protected cysteine sulfoxide as an umpolung of cysteine nucleophile

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Abstract: Covalent linking of side chains provides a method to produce cyclic or stapling peptides that are important in developing peptide-based drugs. A variety of crosslinking formats contribute to fixing the active conformer and prolonging its biological activity under physiological conditions. One format uses the cysteine (Cys) thiol to participate in crosslinking through nucleophilic thiolate anions or thiyl radicals to form thioether and disulfide bonds. Removal of the S-protection from an S-protected Cys derivative generates the thiol which functions as a nucleophile. S-Oxidation of a protected Cys allows the formation of a sulfoxide that operates as an umpolung electrophile. Herein, the applicability of S-*p*-methoxybenzyl Cys sulfoxide (Cys(MBzl)(O)) to the formation of thioether linkage between tryptophan (Trp) and Cys has been investigated. The reaction of peptides containing Cys(MBzl)(O) and Trp with trifluoromethanesulfonic acid (TFMSA) or methanesulfonic acid (MSA) in TFA in the presence of guanidine hydrochloride (Gn·HCl) proceeded to give cyclic or stapling peptides possessing the Cys–Trp thioether linkage. In this reaction, strong acids such as TFMSA or MSA are necessary to activate the sulfoxide. Additionally, Gn·HCl plays a critical role in producing an electrophilic Cys derivative that combines with the indole by aromatic electrophilic substitution. The findings led us to conclude that the less electrophilic Cys(MBzl)(O) serves as an acid-activated umpolung of Cys nucleophile and is useful for S-arylation-mediated peptide cyclization.

Introduction

The nucleophilic thiol in the cysteine (Cys) side chain supports various thiol-specific reactions such as S-alkylation, Michael reaction and disulfide formation. Such groups serve as bridgeheads for the site-specific modification of peptides/proteins.[1] Intramolecular reactions of thiols have led to cyclization or stapling of peptides. An S-protected cysteine, a precursor of cysteine in peptide synthesis, also can work as a nucleophilic sulfide. Oxidation of nucleophilic thiols allows however for umpolung of the reactivity as exemplified by conversion of thiols to disulfides which show electrophilic character, which is also observed in S-protected cysteines. An unexpected S-arylation was reported in 1979 to occur during the acidic deprotection of a protected peptide.[2] Yajima et al. described that *S*-*p*-methoxybenzyl cysteine sulfoxide Cys(MBzl)(O) (**1**) formed during peptide chain elongation was converted to *S*-*p*-methoxyphenyl cysteine (**2**) by acidic deprotection in the presence of anisole. In this conversion, aromatic electrophilic substitution (S_EAr) involving the acid-activated electrophilic sulfoxide occurs as shown in Fig. 1(a).

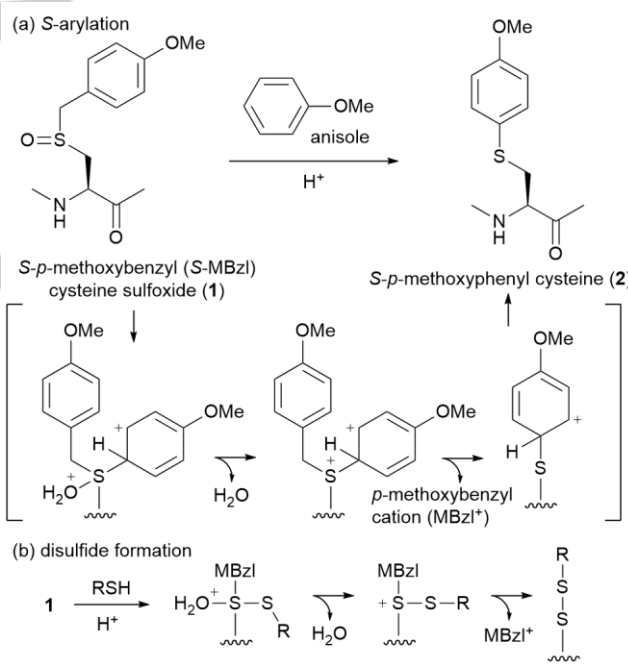


Figure 1. Chemical behaviour of S-p-methoxybenzyl cysteine sulfoxide under acidic conditions. (a) S-Arylation. (b) Disulfide formation, MBzl = p-methoxybenzyl.

Recently, such an S-arylation, featuring sulfenylation using sulfoxides, of the C-H bonds of arenes under metal-free conditions[3] has gained popularity as a means of access to S-aryl compounds distinct from transition metal-mediated processes.[4] Use of sulfoxides in the formation of disulfides in peptides was also achieved (Fig. 1(b)).[5]

For 50 years, toxic bicyclic octa- and heptapeptides such as Amatoxin and Phallotoxin, from the green death cap mushroom *Amantia Phalloides*, have received attention as synthetic targets,[6] and more recently as toxic payloads in antibody-drug conjugates.[7] In addition to their lactam bridge, these bicyclic peptides have a characteristic tryptathionine unit **3** connecting tryptophan and cysteine side chains *via* a thioether (Fig. 2). Synthetic routes to such bicyclic peptides use one of two methods to produce the tryptathionine unit.[8] One uses the Savige-Fontana reaction, which involves nucleophilic attack of the cysteine thiol on the hydroxypyrollo[2,3-*b*]indole (Hpi) (**4**)[9,10] (Fig. 2, route A,), while the other employs electrophilic substitution of indole by sulfenyl halide species such as (*S*-chlorocysteine (**5**)[11] or *S*-iodocysteine (**6**)[12] derived from cysteine or cystine (Fig. 2, route B).

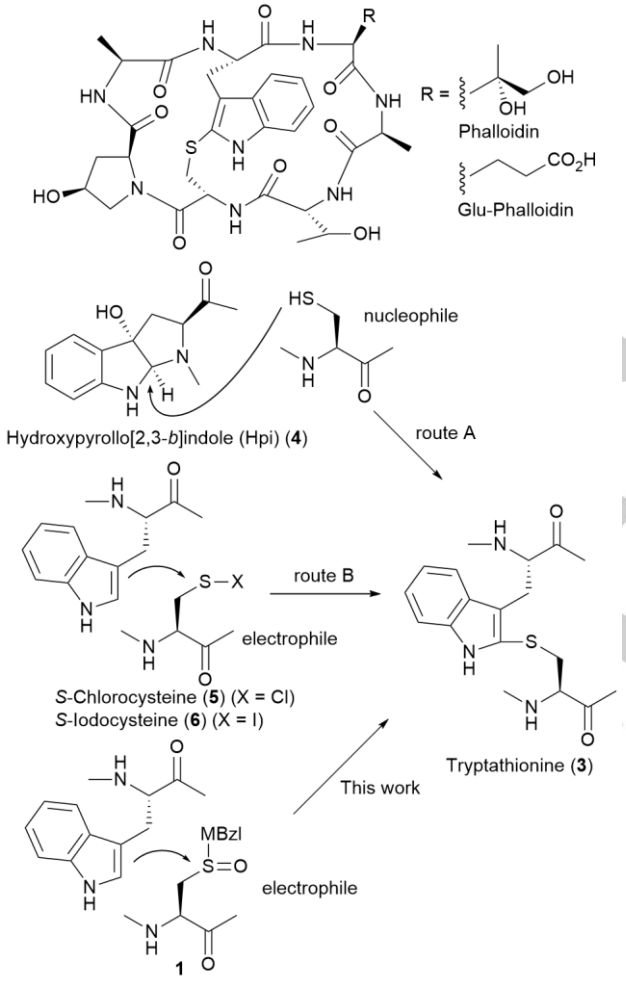


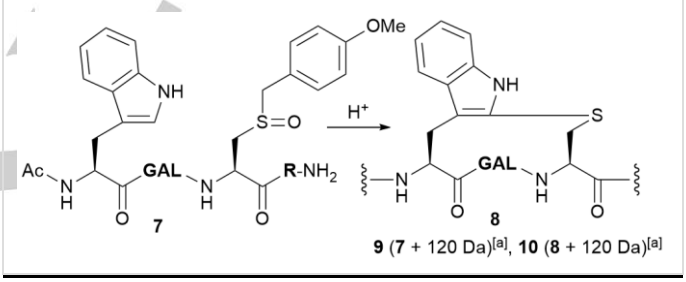
Figure 2. Representative structure of tryptathionine-containing bicyclic peptides (e.g. Phallooidin). Conventional (routes A and B) and novel access to the tryptathionine moiety.

We hypothesized that S-protected cysteine sulfoxides such as **1** operate as an electrophile which can be activated by acids to attack the indole ring, thus providing a novel access to the tryptathionine core found in Amatoxin derivatives (Fig. 2). Recent applications of stapling technology[13] to establish helical structures *via* covalent linking of side chains prompted us to evaluate the S-arylation method which can afford a tryptathionine linkage, as a new stapling format for peptide-based drugs. Here, we discuss the use of **1** in the construction of the tryptathionine linkage. This could lead to practical applications such as the syntheses of tryptathionine-containing peptides, including Amatoxin derivatives and α-helical peptides as drug leads.

Results and Discussion

Initially, the peptide Ac-Trp-**GAL**-Cys(MBzl)(O)-**R**-NH_2 (**7**) with the Trp and Cys residues in a *i* and *i* + 4 relationship was selected as a model peptide. This spatial relationship has been well established as contributing to the facile linking of the two side chains. An arginine residue was incorporated to enhance the water solubility of peptides, but the use of arginine subsequently led to an interesting result.

Table 1. Optimization of reaction conditions for formation of the thioether linkage of tryptathionine.



entry	acid in TFA (M)	additives (M)	Products (%)
1	TFMSA (1)	–	**8** (30), **8'** (7), **9** (10), **10** (53)
2	–	–	**7** (>95)
3	TFMSA (1)	Gn·HCl (2)	**8** (74), **10** (26)
4	TFMSA (1)	Gn·HCl (3)	**8** (80), **10** (20)
5	TFMSA (1)	Gn·HCl (4)	**8** (>85)
6	–	Gn·HCl (4)	**8** (40), **7** (60)
7	TFMSA (1)	$Gn_2{\cdot}H_2SO_4$ (2)	**7** (>95)
8	TFMSA (1)	$(n\text{Bu})_4$NCl (4)	**8** (30), **7** (70)
9	TFMSA (0.5)	Gn·HCl (4)	**8** (>70)
10	TFMSA (0.2)	Gn·HCl (4)	**8** (53), **7** (27), **10** (20)
11	MSA (3)	Gn·HCl (4)	**8** (>70)
12	MSA (1)	Gn·HCl (4)	**8** (>90)

A peptide **7** (0.5 mM) was treated with acidic reagent system at 4 °C for 3 h. The reaction was diluted with H_2O fivefold and directly analyzed by HPLC. Conversion (%) (or remaining (%)) proportions were determined by HPLC analysis with UV detection at 220 nm and calculated using the equation: percent formation = 100 [(integ. **7**, **8**, **9**, or **10**)/(integ. **7** + **8** + **9** + **10**)], where integ. = integration of peak area of the UV absorption. HPLC monitoring of reactions is shown in Fig. 3 and Fig. S3 in the SI. Thioether linkage at the 2-position of the indole was confirmed by NMR analysis of **8** (Fig. S4 in the SI). [a] Side product **9** or **10** comprises several *p*-methoxybenzyl-alkylated materials of **7**

or **8**, respectively. Precise identification of the alkylated residues has yet to be conducted.

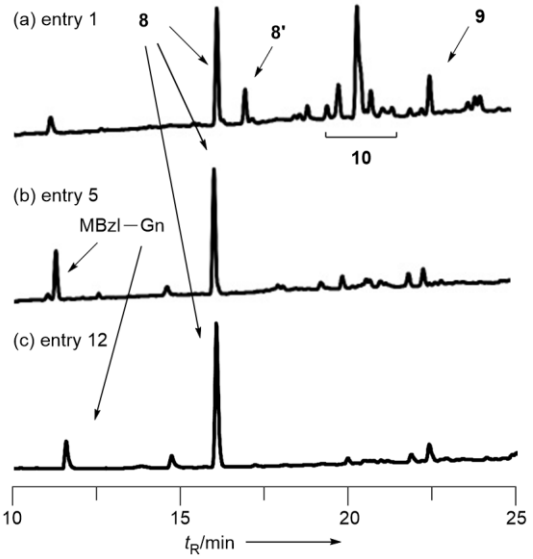


Figure 3. Comparative HPLC analyses of the reactions in Table 1 between the absence ((a) entry 1) and presence ((b) entry 5 and (c) entry 12) of Gn·HCl. MBzl–Gn; Adduct of MBzl cation with guanidine. Analytical HPLC conditions: linear gradient of 0.1% TFA/CH_3CN in 0.1% TFA/H_2O, 5% to 65% over 30 min. UV detection at 220 nm.

The required peptide **7** was obtained by standard Fmoc solid-phase peptide synthesis (SPPS) on Rink amide resin using Fmoc-Cys(MBzl)(O)-OH and subsequent deprotection with TFA in the presence of scavengers. The reagent cocktail consisting of TFA, anisole and H_2O (90:5:5, (v/v)) was used at ambient temperature for 1 h for deprotection of the Cys(MBzl)(O)-containing protected resin.[5c] Chiral center of the sulfoxide afforded a mixture of diastereomer peptides. After HPLC purification, main diastereomer was subjected to the following evaluations. There was a risk of decomposition of the Cys(MBzl)(O) residue in the TFA–anisole or in 20% piperidine/DMF system, but no significant decomposition of **7** was observed (Figs. S1 and S2 in the supporting information (SI)).

Disulfide formation accompanying liberation of the *p*-methoxybenzyl cation, followed by acid-induced activation of the sulfoxide and successive reaction with thiols, requires harsher acidic conditions, such as the use of trifluoromethanesulfonic acid (TFMSA) (Fig. 1(b)).[5,14] Consequently, peptide **7** was Initially subjected to reactions with 1 M TFMSA in TFA in the absence of cation scavengers. As expected, the use of strong acids such as TFMSA led to formation of the tryptathionine linkage but the yield of the desired peptide **8** was only 30% (Table 1, entry 1, Fig. 3(a)), and a product **8'** with the same molecular weight as **8** was also detected. In addition, several side products (**9** and **10**, combined conversion 63%) with a molecular weight 120 more than **7** and **8**, respectively, were formed. These side products would be formed by alkylation by the *p*-methoxybenzyl cation of the indole ring of tryptophan (for **9**), the thioether of tryptathionine (for **10**) or the guanidinium group of arginine (for **9** or **10**). Although the alkylation of Arg seemed less likely, an attempt to prevent the alkylation of the guanidinium group in Arg, revealed that 4 M guanidine hydrochloride (Gn·HCl) in the reaction mixture efficiently suppressed the side reactions. The desirable reaction almost reached completion to afford **8** over 80% conversion yield along with the formation of *p*-methoxybenzyl guanidine (MBzl–Gn) (Table 1, entries 3–5) and Fig. 3(b). The conversion in TFA was also improved by Gn·HCl (Table 1, entries 2 vs 6). These results indicate that Gn·HCl promotes the formation of **8** by suppression of several side reactions.

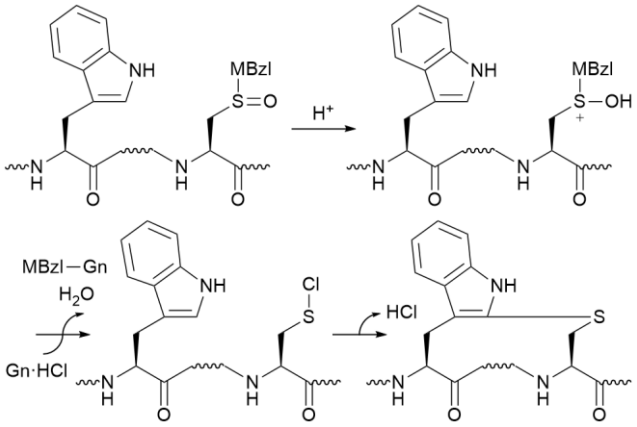


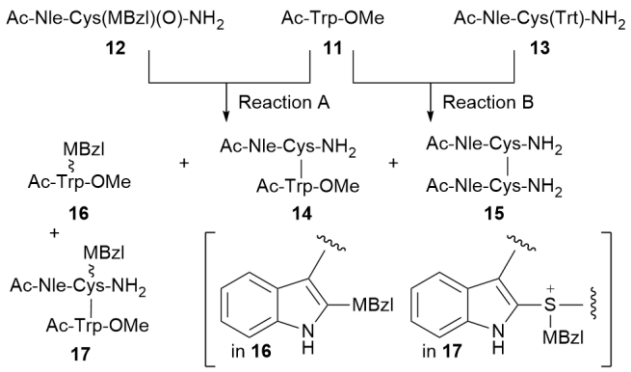
Figure 4. Speculated reaction mechanism for the formation of the thioether linkage in the reaction in the presence of Gn·HCl under acidic conditions.

As shown by HPLC analysis (Fig. 3(b)), the guanidium salt trapped the *p*-methoxybenzyl cation. Still, it was unclear whether the guanidium cation or the chloride anion contributes to promotion of the formation of the tryptathionine linkage. Accordingly, the effect of guanidium sulfate ($Gn_2{\cdot}H_2SO_4$) or tetrabutylammonium chloride ($(n\text{Bu})_4\text{NCl}$) on the reaction was examined but the reactions failed to proceed or went to incompletion (Table 1, entries 7 and 8). Based on these results, a speculated reaction mechanism shown in Fig. 4 was developed although other possibilities could not be excluded.

In the mechanism in Fig. 4, the S_EAr reaction occurred as was seen in the formation of the thioether linkage by previously reported protocols using the sulfenyl halide species (**5** or **6**, Fig. 2, route B), derived from cystine-sulfuryl chloride (SO_2Cl_2)[11] or triphenylmethyl cysteine (Cys(Trt))-iodine system.[12] Brønsted acids activate the less electrophilic sulfoxides to the sulfonium cation, which can then be converted irreversibly to the highly electrophilic *S*-chlorocysteine unit which participates in the reaction with the indole system. The step for generating the *S*-chlorocysteine species requires Gn·HCl which is critically relevant to both the scavenging the cation and nucleophilic substitution on the sulfur center. Finally, the S_EAr process goes to completion through the deprotonated aromatization. Critical involvement of Gn·HCl in the sequence of reactions allows the TFA–Gn·HCl system lacking TFMSA to give **8** in 60% yield (Table 1, entry 6). We then attempted to reduce the acidity of the system by decreasing the level of TFMSA or replacing it with methanesulfonic acid (MSA)[2,15] (Table 1, entries 9–12). The reaction with 1 M MSA–4 M Gn·HCl in TFA was regarded as optimum (Table 1, entry 12 and Fig. 3(c)). Reaction of **7** under the optimum conditions afforded **8** in 48% isolated yield after

HPLC purification. Additionally, the optimum conditions also successfully converted the two- or five-residue spacing substrate to the corresponding Trp–Cys thioether products as the main product. However, some dimer products were detected in the case of the two-residue spacing substrate (Fig. S5 in the SI).

Table 2. Intermolecular linking of cysteine and tryptophan derivatives.



entry	reaction[a]	conditions	Products and (substrate)
1	A	1 M MSA–4 M Gn·HCl in TFA	**14, 16, 17**
2	B	I_2 in DMF	**14, 15,** (**11**)
3	A	1 M MSA–100 mM Gn·HI[b] in TFA	**14**, **15**, **16**, (**11**)

[a] Reaction A: The mixture of 11 (1 mM) and 12 (1 mM) in 1 M MSA in TFA in the presence of 4 M Gn·HCl (entry 1) or 100 mM Gn·HI (entry 3) was treated at 4 °C for 3 h. The reaction was diluted with H_2O fivefold and directly analyzed by HPLC. Reaction B: The mixture of 11 (1 mM) and 13 (1 mM) was treated with 10 equiv. of I_2 in DMF at room temperature (rt) for 3 h. An aliquot of the reaction was directly analyzed by HPLC. [b] Due to the minimal solubility of Gn·HI in TFA. Thioether linkage of **14** was verified to be formed at the 2-position of the indole by NMR analysis (Fig. S7 in the SI).

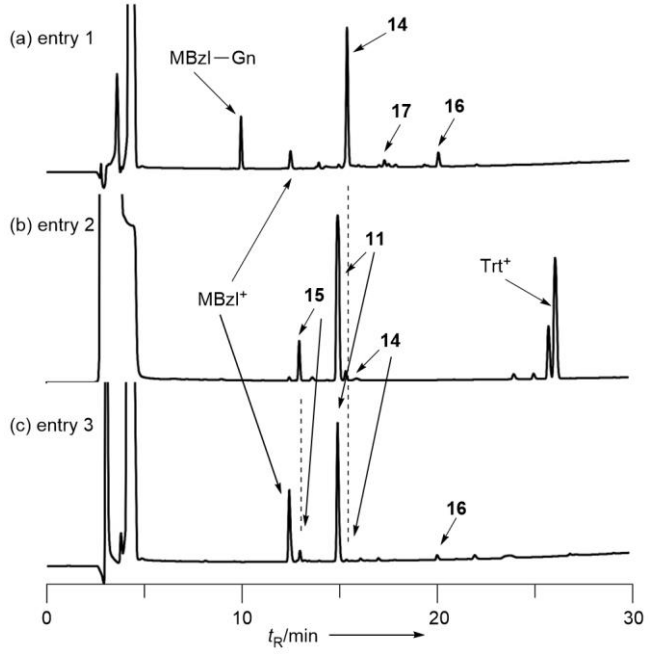


Figure 5. HPLC analysis of reactions in Table 2. (a) entry 1; (b) entry 2; (c) entry 3. Analytical HPLC conditions: linear gradient of 0.1% TFA/CH_3CN in 0.1% TFA/H_2O, 5% to 95% over 30 min. UV detection at 220 nm.

In the conventional Cys(Trt)–iodine system used for the formation of the thioether linkage, Cys(Trt), a nucleophilic S-species is converted into the corresponding *S*-iodocysteine[16a], which is electrophilic and reacts either with indole to form tryptathionine,[16b] or produces a disulfide bond through either homolysis of the S-I bond or nucleophilic attack of the remaining sulfide to the S-I bond. Meanwhile, the MSA–Gn·HCl in the TFA system should generate the *S*-chlorocysteine that is not susceptible to homolytic cleavage of the S-Cl bond and is less electrophilic than *S*-iodocysteine. The result is the preferential S-arylation by cysteine of the tryptophan residues. This was confirmed by intermolecular thioether formation of Ac-Trp-OMe (**11**) with Ac-Nle-Cys(MBzl)(O)-NH_2 (**12**) or with Ac-Nle-Cys(Trt)-NH_2 (**13**) (Table 2 and Fig. 5). Because indole-containing materials show stronger UV absorption than structures lacking indole, the reaction outcome in each case was evaluated by HPLC analysis and the distribution of the products was not quantified. The reaction of **11** (1 mM) with Cys(MBzl)(O) dipeptide **12** (1 mM) in 1 M MSA–4 M Gn·HCl in TFA at 4 °C for 3 h afforded the desired thioether peptide **14** in 47% isolated yield without any detectable amount of the disulfide peptide **15** although the indole-alkylated material **16** and the assumed S-alkylated products **17** were detected (Table 2, entry 1 and Fig. 5(a)). Treatment of a mixture of **11** and Cys(Trt)-dipeptide **13** (1 mM each) with I_2 (10 mM) in DMF gave essentially no thioether **14** but formed the disulfide **15** (Table 2, entry 2 and Fig. 5(b)). The clearly observed difference between the two systems led us to speculate that replacement of Gn·HCl with Gn·HI in the reaction using Cys(MBzl)(O) under acidic conditions should allow the formation of the intermediary *S*-iodocysteine unit, and result in preferential formation of the disulfide **15**. The reaction of **11** and **12** in 1 M MSA in TFA in the presence of 100 mM Gn·HI showed a trend similar to that observed in the I_2-mediated reaction (Table 2, entries 2 vs 3 and Figs. 5(b vs c)). These observations led us to conclude that under acidic conditions in the presence of appropriate guanidinium salts (Gn·HX: X = halogen), Cys(MBzl)(O) functions as a crypto S-halocysteine that can be activated by acid to afford different products depending on the halogen (X). In addition to the fact that the S-Cl species generally functioning as effective sulfenylating reagent of tryptophan,[11,17] data obtained here would also support the speculated mechanism in Fig. 4. Additionally, the application of the conventional SO_2Cl_2-mediated protocol for the preparation of **14** met with failure (Fig. S6 in the SI). Our developed protocol requires no oxidizing reagent to form the S-Cl species presenting in peptide sequences, which is one advantage over the SO_2Cl_2 protocol.

The effects of the *S*-chlorocysteine as a plausible intermediary species on several amino acids (Xaa) were evaluated using model peptides (Ac-**G**-Xaa-**RAL**-Cys(MBzl)(O)-**GAL**-Trp-**RG**-NH_2: Xaa = His (**18a**); Lys (**18b**); Phe (**18c**); Tyr (**18d**); Met (**18e**)). Here, Xaa, Cys(MBzl)(O) and Trp appeared in the *i* – 4, *i* and *i* + 4 spacing, respectively, in the sequence thus minimizing the impact of distance between residues on the reaction involving the *S*-chlorocysteine. There was concern about the possible involvement of the phenol side chain of Tyr in the S_EAr reaction, but reactions with the exception of that involving the

Met-containing peptide **18e** proceeded efficiently to give desired peptides with a thioether linkage (**19a-19d**) without any significant side products (Fig. 6(a) for **18d** and Fig. S8 in the SI for **18a**-**18c**). The indole-specific thioether formation that was observed is probably due to the electron rich nature of the indole ring which is greater than that of phenol. The presence of the tryptathionine unit in **19d** was confirmed both by condensation of Ac-**GYRA**-OH (**20**) with tryptathionine-containing cyclic peptide (tryptathionine form of H-**L**-Cys-**GAL**-Trp-**RG**-NH_2 (**21**)) and by the peptide mapping using chymotrypsin (Figs. S9 and S10 in the SI). Reaction of **18e** at 20 °C afforded a mixture of several components including the desired **19e** (Fig. 6(b)). Raising the reaction temperature to 37 °C, however, dramatically improved the outcomes and gave **19e** as the major product (Fig. 6(c)). With this unexpected result, we hypothesized that the disulfanium cation, resulting from the attack of the Met sulfide to the *S*-chlorocysteine, would remain less reactive at 20 °C, but would become reactive to the indole to result in the formation of **19e** at 37 °C.

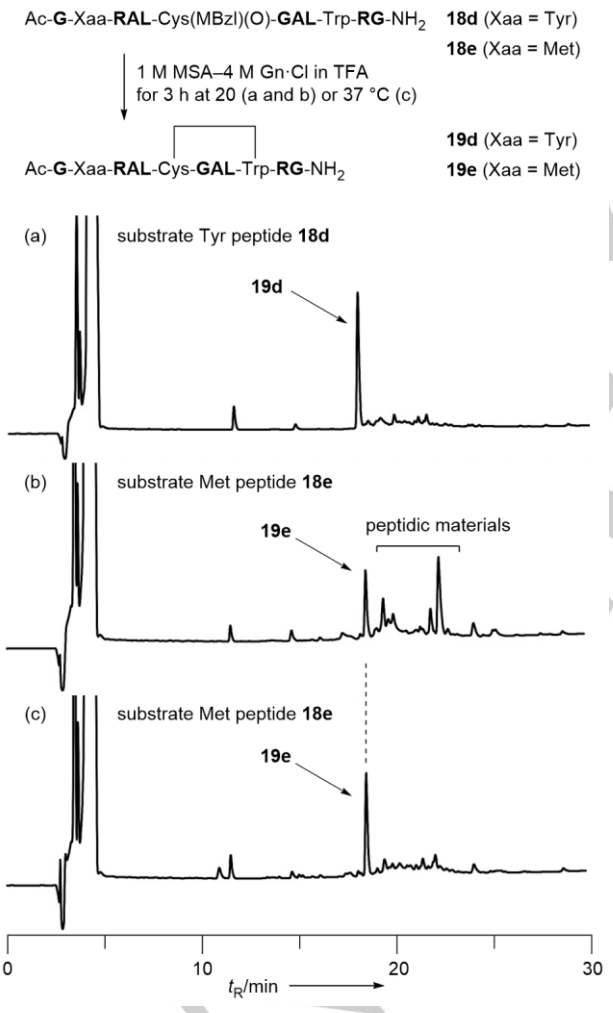
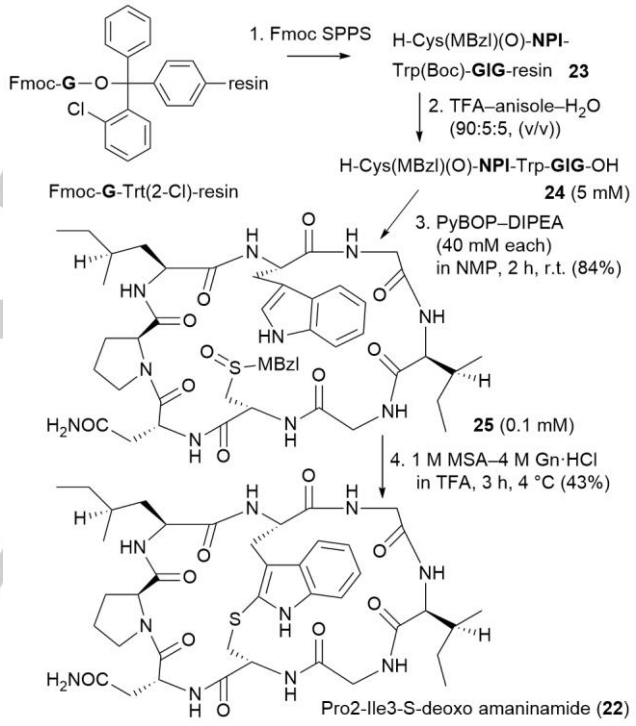


Figure 6. HPLC evaluation of the reaction of peptides containing amino acids that can react with *S*-chlorocysteine. (a) Reaction of Tyr peptide **18d** at 20 °C. (b) Reaction of Met peptide **18e** at 20 °C. (c) Reaction of **18e** at 37 °C. Analytical HPLC conditions: linear gradient of 0.1% TFA/CH_3CN in 0.1% TFA/H_2O, 5% to 65% over 30 min. UV detection at 220 nm.

The utility of the developed protocol was confirmed by the synthesis of two tryptathionine-containing peptides, Pro2-Ile3-S-deoxo amaninamide **22**[10b] and an estrogen receptor-α (ERα) activity-regulator synthetic peptide (ERAP) analogue.[18] Previously, efficient access to **22** was achieved by an intra-annular Savige-Fontama reaction featuring nucleophilic attack of cysteine on an electrophilic 3a-hydroxypyrrolo[2,3-b]indoline (Hpi) unit.[10c] We also attempted to use a similar intra-annular version of this reaction but with the "umpolung" mode (Fig. 4). The required linear octapeptide (H-Cys(MBzl)(O)-**NPI**-Trp-**GIG**-OH (**24**) was prepared by the construction of the protected peptide resin **23** by standard Fmoc-based SPPS on 2-chloro Trt (Trt(2-Cl)-resin) followed by the release of **24** with TFA–anisole–H_2O (90:5:5, (v/v)).



Scheme 1. Synthesis of Pro2-Ile3-S-deoxo amaninamide (**22**).

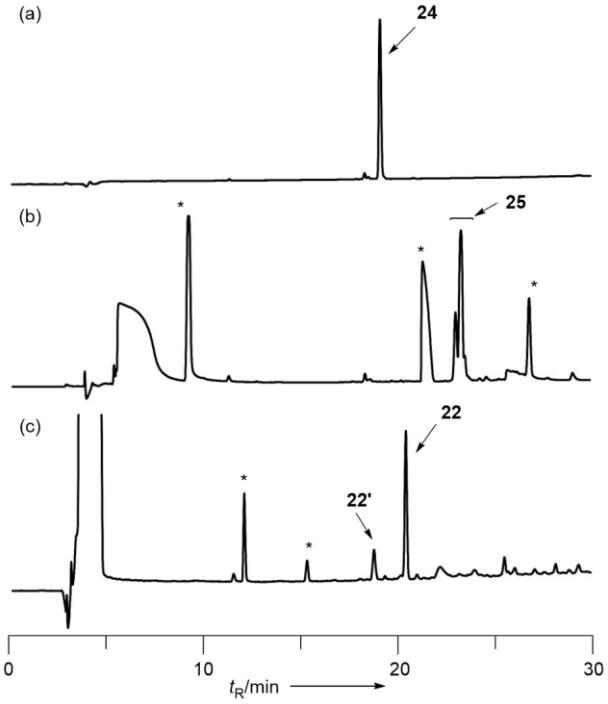


Figure 7. HPLC monitoring of reactions for the synthesis of **22**. (a) Purified linear peptide **24**. (b) Reaction for intramolecular amide formation. (c) Reaction for thioether formation. Analytical HPLC conditions: linear gradient of 0.1% TFA/CH_3CN in 0.1% TFA/H_2O, 5% to 65% over 30 min. UV detection at 220 nm. *Non-peptidic impurities.

Cyclization of **24** (5 mM) in *N*-methylpyrrolidone (NMP) by the action of (benzotriazole-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP)–*N,N*-diisopropylethylamine (DIPEA) (40 mM each) for 2 h at rt afforded a monocyclic peptide (cyclo(-Cys(MBzl)(O)-**NPI**-Trp-**GIG**-) (**25**)) as a mixture of the sulfoxide-derived diastereomers in 84% isolated yield after HPLC purification. Reaction of this precursor **25** (0.1 mM) in 1 M MSA–4 M Gn·HCl in TFA reached completion within 3 h at rt to afford the desired compound **22** in 43% isolated yield after HPLC purification. The NMR spectrum of the isolated material was identical to the published spectra and exhibited a large negative Cotton effect at ~230 nm, which is consistent with the spectra of the desired material (Figs. S11 and S12 in the SI).[10b] In addition to **22**, a material **22'** with the same mass as **22** was also formed as a minor product whose origin remained to be disclosed.

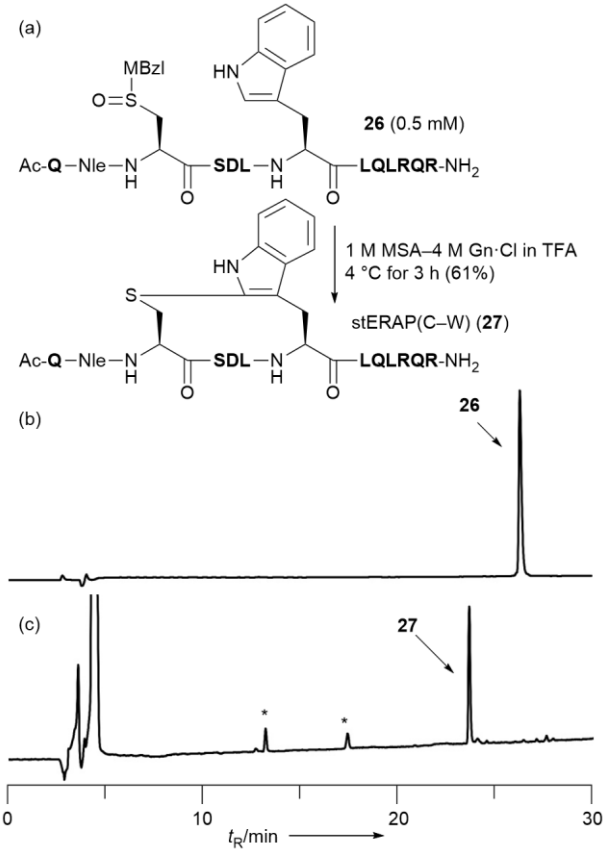


Figure 8. (a) Formation of the thioether of stERAP(C–W) (**27**). HPLC monitoring of the reaction; (b) Purified linear peptide **26**. (c) Crude reaction mixture for the synthesis of **27**. Analytical HPLC conditions: linear gradient of 0.1% TFA/CH_3CN in 0.1% TFA/H_2O, 5% to 65% over 30 min. UV detection at 220 nm. *Non-peptidic impurities.

The developed protocol was applied to the stapling of the α-helical ERAP analogues that inhibit the estrogen-induced cancer cell growth. The Trp–Cys thioether linkage was substituted for the bis-amide bridge in the original stapled ERAP peptide (Ac-**QM**-X-**SDL**-X-**LQLRQR**-NH_2 (original stERAP, where X = stapling residue, and the γ-carboxyl groups of Glu are connected by the 1,4-diaminobutyl linker, see structure in Fig. 10(c)) which exhibits long-lasting *in vivo* anti-tumour activity in breast cancer xenografts in mice.[18b] The required precursor peptide (Ac-**Q**Nle-Cys(MBzl)(O)-**SDL**-Trp-**LQLRQR**-NH_2 (**26**)) was produced with the standard Fmoc SPPS followed by deprotection of the protected peptide resin with TFA–anisole–H_2O (90:5:5, (v/v)). It was subjected to the reaction with 1 M MSA–4 M Gn·HCl in TFA for 3 h at 4 °C (Fig. 8(a)). The attempted reaction proceeded almost quantitatively to afford the desired thioether peptide (stERAP(C–W) (**27**)) in 61% isolated yield after HPLC purification (Figs. 8(b) and 8(c)). The thioether peptide **27** obtained in this way exhibited a UV-Vis absorption characteristic to the tryptathionine (Fig. S13 in the SI) and negative Cotton effect at ~208 nm, but the double minimum at 208 and 222 nm, indicative of an α-helix was not observed due to the large positive Cotton effect the tryptathionine unit at 210–240 nm (Fig. 9). Consequently, the molar ellipticity θ at 208 nm was used to estimate the α-helicity of **27**, **28**, the linear cognate with replacement of the Cys with Ala, and the original stERAP.[19] The thioether-stabilized peptide **27** showed α-helicity comparable to

that of original stERAP (60%) whereas the α-helicity of **28** remained at 36%.

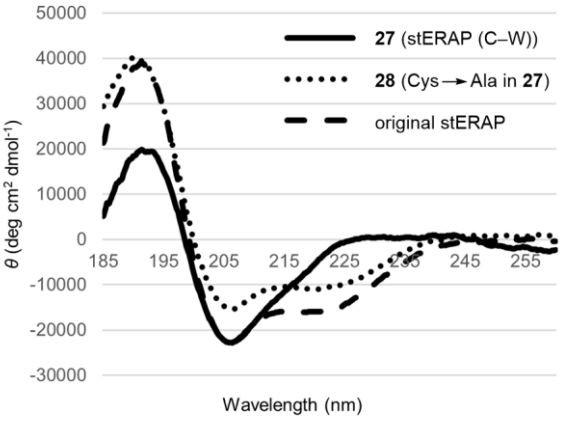


Figure 9. CD spectra for ERAP derivatives (**27**, **28** and original stERAP). CD conditions: 0.05 mg/mL peptide, 25 °C, pH 7.0, 50% trifluoroethanol (TFE) in 10 mM Na phosphate buffer.

Further elucidation of the secondary structure of **27** was achieved by comparative NMR measurement of **27** and the original stERAP. NMR spectrum of **27** showed NMR signals derived from an inter-residual backbone amide proton–amide proton ($^1H^N$-$^1H^N$) nuclear Overhauser effect (NOE) between (i) Ser4 and Trp7, (ii) Ser4 and Leu8 and (iii) Asp5 and Leu8 (Fig. 10(a)). The $^1H^N$-$^1H^N$ NOE signals observed between *i* and *i* + 3 or *i* + 4 amino acid residues are strong indication of the formation of a helical structure. In addition, the result of chemical shift index (CSI) analysis[20] based on the chemical shift values of ^{13}Cα, ^{13}Cβ, ^{13}Co, ^{15}N, $^1H^N$, and ^{1}Hα indicated that the region encompassing Asp5 and Gln12 of **27** adopts an α-helical structure (Fig. 10(b)). Similarly, NOE measurements and CSI analysis of original stERAP indicated that the same region of stERAP (Asp5–Gln12) also forms α-helical structure (Fig. 10(c) and Fig. S14 in the SI).

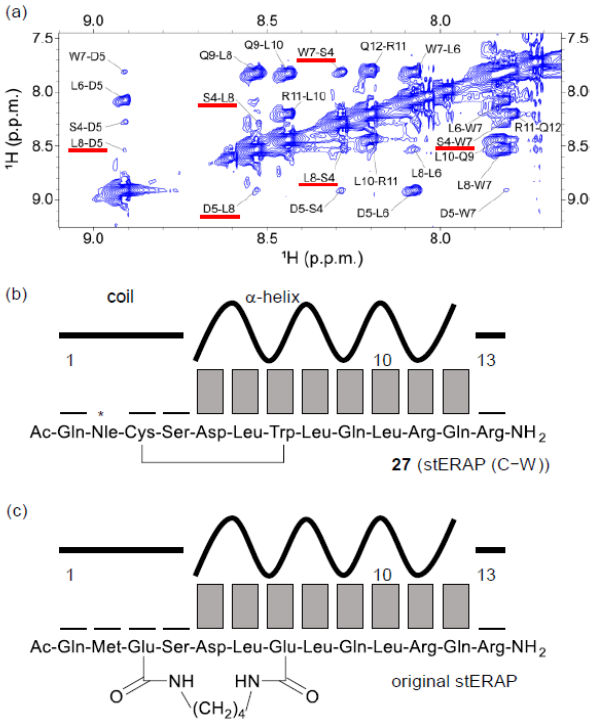


Figure 10. (a) Two-dimensional NMR spectrum and the nuclear Overhauser effect (NOE) between intramolecular amide protons (2D $^1H^N$-$^1H^N$ NOE) of **27**. The red underlining indicates $^1H^N$-$^1H^N$ NOE signals between *i* and *i* + 3 or *i* + 4 amino acid residues. NMR conditions: 3.33 mg/mL peptide, 25°C, pH 7.0, 50% TFE in 10 mM sodium phosphate buffer. (b) Secondary structures of **27** analysed by chemical shift index (CSI) of NMR spectra. (c) Secondary structure of original stERAP. The underbars and shaded-boxes, between the secondary structure diagram and amino acid sequence in panels (b) and (c), are CSI plots indicating random coil and α-helix, respectively. *The unnatural amino acid (Nle) was not included in the CSI analysis. CSI analyses were performed by using program CSI 3.0 based on chemical shift values of ^{13}Cα, ^{13}Cβ, ^{13}Co, ^{15}N, $^1H^N$, and ^{1}Hα of each peptide.

Conclusion

The use of the Arg-containing model peptide **7** provided a significant clue to the optimization of the reaction forming the thioether between Cys(MBzl)(O) and Trp under acidic conditions. Gn·HCl added to the reaction proved not only to function as a scavenger, suppressing various alkylation side reactions induced by the *p*-methoxybenzyl cation, but also to promote the S-arylation, forming the tryptathionine linkage. Such facilitation is related to the generation of *S*-chlorocysteine species followed by an S_EAr reaction with the indole ring. Oxidative umpolung of the nucleophilic sulfide of Cys(MBzl) leads to the sulfoxide of Cys(MBzl)(O) which exhibits less electrophilic character than the *S*-chlorocysteine, and allows for the straightforward preparation of Cys(MBzl)(O)-containing peptides by standard Fmoc SPPS followed by an acidic deprotection protocol using TFA. The Cys(MBzl)(O) is activated to the corresponding sulfonium cation through protonation of the sulfoxide in TFA in the presence of strong acids such as TFMSA and MSA. Subsequently, the coexisting Gn·HCl in the reaction facilitates conversion of the resulting sulfonium cation to the more electrophilic *S*-chlorocysteine structure through nucleophilic attack of chloride anion and concomitant trapping of the cation with guanidine. Here, worthy of note is that the formation of the S-Cl species in

peptides requires no oxidizing reagent. Finally, the S-arylation is completed by the S_EAr reaction of the resulting *S*-chlorocysteine with the indole of Trp. A newly developed linking reaction between the cysteine sulfoxide as a cysteine umpolung and Trp was successfully applied to the peptide cyclization for preparation of Pro2-Ile3-S-deoxo amaninamide (**22**) and stERAP(C–W) (**27**).

((**Note**: Please place comprehensive details in the Supporting Information.))

Acknowledgements

This research was supported in part by The Canon Foundation and by AMED (20ak0101141s0101) (for A. O.). D. K. is grateful for a JSPS fellowship (21J23098). This work was performed in part under the Collaborative Research Program of Institute for Protein Research, Osaka University, CRa-21-02.

Keywords: sulfoxide • S-arylation • peptide cyclization • stapling • tryptathionine

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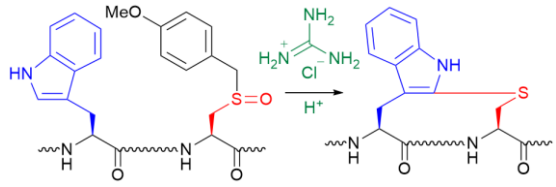
Entry for the Table of Contents



Acid-mediated activation of an S-protected cysteine sulfoxide allows for the cyclization of peptides *via* metal-free C-H sulfenylation of arenes. The less electrophilic S-*p*-methoxybenzyl cysteine serves as an acid-activated umpolung of nucleophilic Cys in the presence of guanidine hydrochloride and is useful for S-arylation-mediated peptide cyclization.