248. Iodine Oxidation of *S*-Trityl- and *S*-Acetamidomethyl-cysteine-peptides Containing Tryptophan: Conditions Leading to the Formation of Tryptophan-2-thioethers[1])

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Dedicated to Professor *R. Schwyzer* on the occasion of his 60th birthday

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Summary

Cystine peptides are conveniently prepared from *S*-acetamidomethyl- or *S*-trityl-protected cysteine derivatives by direct oxidation with iodine. Since the reaction proceeds through the formation of sulfenyl iodides, these highly reactive groups may substitute the indole ring of tryptophan residues, resulting in the formation of 2-thioethers. During the synthesis of the peptide hormone somatostatin, we investigated this possible side reaction. By-products of the tryptophan-2-thioether type can be produced under conditions which lead to a marked retardation of the disulfide bond formation. The largest amount of these compounds were formed when the oxidation was carried out in 90% aqueous trifluoroethanol.

In model peptides in which tryptophan and cysteine residues were separated by 1 to 4 glycine residues, the ring size of the resulting thioether exerted a strong influence on the yield: in peptides with 1 and 2 glycines, only dimeric disulfides were formed. Incorporation of 3 and 4 glycine residues gave thioethers in yields of about 40% and 70% respectively. Conversely, under normal conditions of iodine oxidation, when disulfides are rapidly formed from the *S*-acetamidomethyl- or *S*-trityl-cysteine residues, tryptophan-2-thioethers are produced only in insignificant amounts or not at all.

The oxidation by iodine of *S*-trityl- and *S*-acetamidomethyl-cysteine peptides has been found to be an efficient method for the formation of cystine derivatives [2]. The reaction is assumed to proceed by way of the highly active sulfenyl iodides [2]. The less reactive sulfenyl chlorides have been utilized by *Wieland et al.* in the synthesis of tryptophan-2-thioethers [3]. *Scoffone et al.* [4] developed a method for the specific modification of peptides by reaction of 2-nitrophenylsulfenyl chloride with the side chains of tryptophan residues.

[1]) Abbreviations are according to the IUPAC-IUB Commission on Biochemical Nomenclature [1]. Further abbreviations used: Acm = acetamidomethyl; HOBt = 1-hydroxybenzotriazole; TLC. = thin-layer chromatogram; DMF = dimethylformamide; Hx = hexane.

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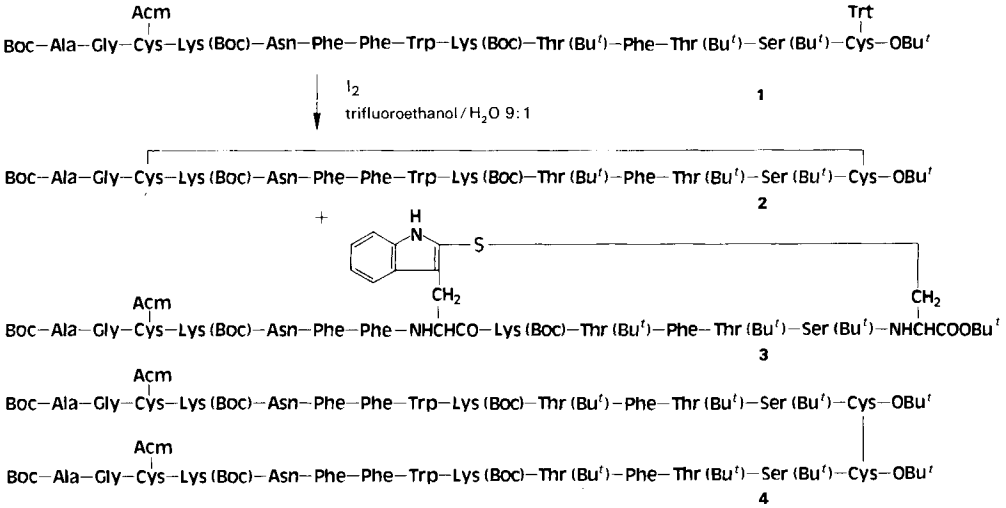
In view of these reactions, tryptophan-2-thioethers might possibly be formed as by-products in the synthesis of cystine peptides by iodine oxidation of protected cysteine derivatives containing tryptophan. We therefore made a systematic search for by-products of this type during the synthesis of the cystine-containing tetradecapeptide somatostatin.

When the protected somatostatin derivative **1** was oxidized in the usual way by iodine in MeOH/DMF the disulfide derivative **2** was isolated after purification in a yield of almost 80% [2]. A by-product **3**, containing the tryptophan-2-thioether grouping, may have been present in the crude reaction mixture, but at the most in a minor amount. However, we expected a higher yield of **3** if the oxidation were

Scheme 1. *Possible products of the iodine oxidation of the protected somatostatin derivative* **1**. *c* (peptide) = 10^{-3} M in trifluoroethanol/H_2O 9:1. Titration with 0.1 M I_2 in CH_2Cl_2 (see text).



performed in a solvent which retarded the reaction of the *S*-acetamidomethyl group with iodine as well as with sulfenyl iodide formed from the *S*-trityl group. One such solvent is trifluoroethanol [2]. By slow addition of iodine in methylene chloride[2]) to a solution of an *S*-trityl-cysteine peptide in 90% aqueous trifluoroethanol the reaction can be carried out as a titration. The end of the reaction is indicated by the persistent yellow color of the excess iodine[3]).

When **1** was treated with iodine in 90% trifluoroethanol *(Scheme 1)*, a hydrophilic by-product was formed in addition to 65% of **2**. This by-product proved to be the tryptophan-2-thioether derivative **3**, so even under conditions known to be unfavorable for disulfide formation, the cystine peptide **2** is still the main product. This could be accounted for as follows. The initial rapid reaction of **1** with iodine in 90% trifluoroethanol is the formation of a sulfenyl iodide from the *S*-trityl group. Owing to the high dilution of **1**, and since sulfenyl iodides react relatively slowly with *S*-trityl groups [2], formation of the dimeric disulfide **4** is suppressed. The

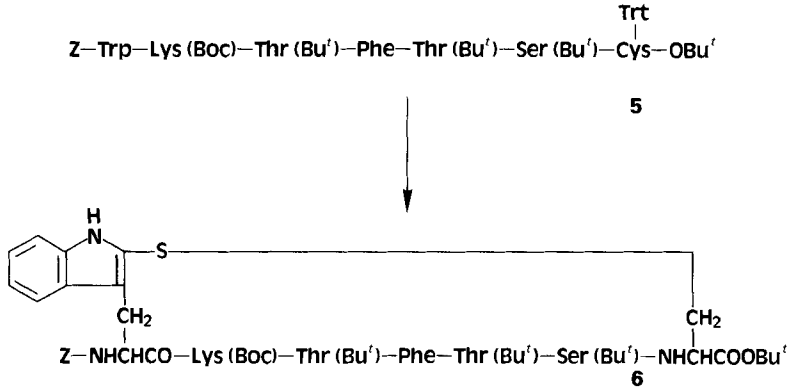
[2]) Iodine is practically insoluble in trifluoroethanol.

[3]) In solvents containing no water, the yellow color remains, even if there is a deficit of iodine. In aqueous acetic acid and aqueous MeOH the color disappears very slowly (*cf.* also [5]).

sulfenyl iodide is forced to react intramolecularly, which it does by forming preferentially **2** and, to lesser extent, the thioether **3**. On the other hand, the two intramolecular pathways would be of no significance if conditions favoring the formation of the disulfide **4** were chosen.

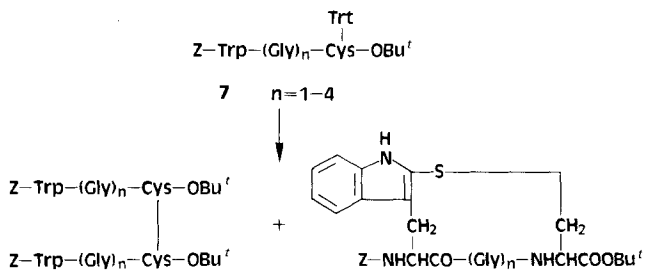
The UV. spectrum of **3** showed a modified absorption in the region of the indole ring characteristic of a tryptophan-2-thioether [6]: $\lambda_{max} = 291$ nm, $\varepsilon = 10{,}800$. The *t*-butyl protecting groups of **3** were removed with trifluoroacetic acid. The ^{1}H-NMR. spectrum of the resultant product contained the methyl singlet (2.07 ppm) of the *S*-acetamidomethyl group, which is missing in somatostatin. For further proof of the formation of a tryptophan-2-thioether, the shorter somatostatin peptide **5** *(Scheme 2)* was treated with iodine under identical conditions.

Scheme 2. *Iodine oxidation of the shortened, protected somatostatin derivative* **5** *to tryptophan-2-thioether* **6**. Conditions as in *Scheme 1.*



Compound **5** cannot form an intramolecular disulfide bond, and the production of the dimeric disulfide is suppressed by the high dilution. The product **6** obtained again exhibited the characteristic UV. spectrum and the structure was additionally confirmed by the mass spectrum.

Scheme 3. *Influence of ring size on the yields of the tryptophan-2-thioethers* **9**. Conditions as in *Scheme 1.*

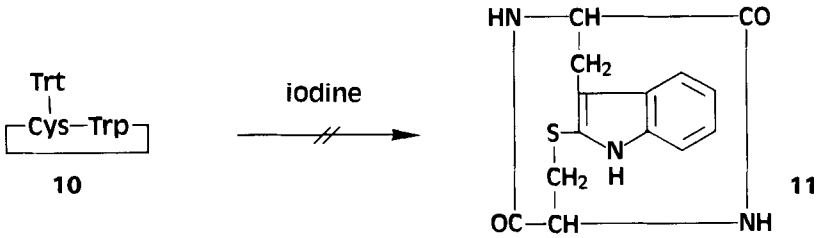


n		
1	*ca.* 80%	not detectable
2	*ca.* 80%	not detectable
3	*ca.* 45%	*ca.* 40%
4	*ca.* 10%	*ca.* 70%

To obtain a better understanding of the conditions favoring the formation of a tryptophan-2-thioether bond, the model peptides **7** were synthsized[4]) and treated with iodine under the standard conditions. The crude products were analyzed by TLC. for the presence of a spot with a slightly higher Rf-value than the dimeric disulfide **8**[5]), and the UV. spectra were examined for a maximum at 290 nm. The yields indicated in *Scheme 3* were obtained.

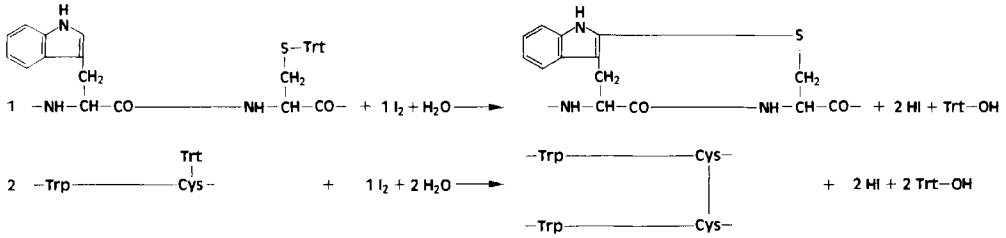
Formation of the tryptophan-2-thioether thus depends very much upon the ring size. Instead of ring formation (with **7**, $n=1$ or 2), the reaction proceeds exclusively, despite the high dilution, to the dimeric disulfide **8**. This explains why we were not able to transform **10** to the bicyclic dipeptide **11** *(Scheme 4)*. However, **11** has been obtained by *Wieland et al.* [8] *via* a different pathway.

Scheme 4



Using the titrimetric method, *i.e.* addition of the iodine solution to the peptide dissolved in 90% trifluoroethanol up to the yellow end point, the amount of iodine consumed is an indication of the final ratio of tryptophan-2-thioether to disulfide. The reaction leading to the tryptophan-2-thioether requires 1 equivalent of iodine for each *S*-trityl group, whereas the same amount of iodine is able to form a disulfide from two *S*-trityl groups *(Scheme 5)*.

Scheme 5



The hexapeptide **7** ($n=4$) giving the highest yield of the tryptophan-2-thioether derivative in 90% trifluoroethanol was used as a model for the comparison of other solvents with respect to thioether formation. The experimental procedures[6]) and results are summarized in *Table 1*.

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[4]) Peptides **7** were synthesized by routine methods, see [7]: Z-Trp-$(Gly)_n$-OH + H-Cys(Trt)-OBu^t + HOBt + DCCI → **7**.

[5]) The dimeric disulfides **8** were produced by addition of a 0.1 M solution of the *S*-trityl peptide **7** in DMF to 1.25 equiv. of a 0.1 M solution of iodine in CH_2Cl_2, followed by a reaction time of 5 min.

[6]) Owing to the slower reaction in these solvents, the titrimetric method of *Scheme 1* could not be applied here. Instead, an excess of iodine and a reaction time of 5 min were chosen.

Table 1. *Composition of the mixture obtained from the iodine oxidation of* **7** *($n=4$) in different solvents*[a])

Solvents	**8**	**9**
$CHCl_3$	*ca.* 40%	< 10%
MeOH	*ca.* 10%	*ca.* 30%
$AcOH/H_2O$ 9:1	*ca.* 10%	*ca.* 50%

[a]) c (peptide) $= 10^{-3}$ M; c (iodine) $= 2.2 \cdot 10^{-3}$ M; reaction time: 5 min; temp. 20–25°. Yields estimated by TLC.

In the three solvents investigated, considerably less tryptophan-2-thioether **9** was formed than in 90% trifluoroethanol. Besides **9** and the dimeric disulfide **8**, substantial amounts of by-products were present, which were not further investigated.

Our experiments demonstrate that conditions may be found in which tryptophan-2-thioethers are formed from *S*-trityl- or *S*-acetamidomethyl groups and the side chains of tryptophan residues by reaction with iodine. A strong influence of the ring size is evident in the case of intramolecular pathways. However, the formation of thioethers is not of significance during preparation of cystine peptides by iodine oxidation of *S*-trityl- or *S*-acetamidomethyl derivatives. It will always be possible to choose suitable *S*-protecting groups and solvents, leading to conditions which produce the desired cystine compound in a high yield.

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Experimental Part

General. – Melting points (m.p.) were determined in open capillaries and are uncorrected. For the preparation of protected amino acids and standard procedures in peptide synthesis, see [7]. TLC. was carried out on *Merck* silica gel plates. The spots were revealed with UV. and a modified *Reindel-Hoppe* reagent.

General procedure for the preparation of indole-2-thioethers. A 0.1 M solution of iodine in CH_2Cl_2 was added at RT. through a submerged capillary to a well-stirred solution of the *S*-trityl-cysteine peptide (0.2 mmol) in 200 ml of 90% trifluoroethanol (peptide concentration $= 10^{-3}$ M) until a permanent yellow color became visible. Five min later, the solution was decolorized with 20 ml of 0.2 M citrate buffer (pH 5) containing some ascorbic acid. The trifluoroethanol was evaporated and the reaction products isolated and purified as described below.

Isolation of the protected 1–14 somatostatin-tryptophan-2-thioether **3** *(see Scheme 1).* The crude material obtained by the general procedure was precipitated with H_2O, filtered off, and washed free of iodide. According to the TLC., it contained about 65% of the protected cyclic somatostatin disulfide **2**, the presumed tryptophan-2-thioether **3** with a smaller Rf-value, and several by-products. Fractionation by chromatography on silica gel gave 225 mg of protected cyclic somatostatin **2** and 138 mg of impure thioether **3**, eluted with $CHCl_3$/MeOH 95:5 and 9:1, respectively. Compound **3** was further purified by *Craig*-distribution in the solvent system MeOH/$CHCl_3$/CCl_4/aqueous buffer (2.25 l + 0.75 l + 1.3 l + 0.56 l, containing 1 g of ammonium acetate and 34 ml of glacial acetic acid). After 1,500 distribution steps, the contents of the tubes containing pure substance ($K=0.29$) were combined, the solvents evaporated *in vacuo* and the residue lyophilized from *t*-butyl alcohol. The material obtained (29 mg) was characterized as follows: TLC.: 157A, Rf 0.22, blue stain with $FeCl_3$ in conc. sulfuric acid, typical of phalloidin [9]. (Protected cyclic somatostatin: Rf 0.34, no color with $FeCl_3$-reagent.) – UV.

(in MeOH): $\varepsilon_{291} = 10{,}800$ (protected cyclic somatostatin: $\varepsilon_{281} = 5{,}600$). – ^{1}H-NMR. (360 MHz in D_2O, after cleavage of all *t*-butyl protecting groups with trifluoroacetic acid): 2.07 ppm (*s*, CH_3 of the Acm-group).

Isolation of the protected 8–14 somatostatin-tryptophan-2-thioether **6** *(see Scheme 2).* The crude material obtained by the general procedure was extracted with AcOEt and purified by chromatography on silica gel. Yield 160 mg (60%). TLC.: $CHCl_3$/2-propanol 95:5, Rf 0.19. – UV. (in MeOH): $\varepsilon_{292} = 11900$. – FD-MS.: 1328 ($M^+$).

Isolation of the tryptophan-2-thioethers **9** $n = 3{,}4$ *(see Scheme 3).* The crude material obtained by the general procedure was extracted with $CHCl_3$ and the organic phase washed, dried, and evaporated. The residue was dissolved in $CHCl_3$/MeOH 1:1 and filtered through a column (100×2 cm) of *Sephadex* LH 20. The rapidly eluted dimeric disulfide **8** and the later eluted thioether **9** were isolated separately. Compound **9** was crystallized from the solvent indicated in *Table 2.*

Table 2. *Physical data of* **7** ($n = 1$–4) *and* **9** ($n = 3$–4).

	Elemental composition[a])	Mol-wt. (calc.)	M.p. (uncorr.)	Solvent for crystallization	$[\alpha]_{365}^{20}$ [b])	Rf[c])	UV. (λ_{max} (ε), MeOH)
7							
$n=1$	$C_{47}H_{48}N_4O_6S \cdot \frac{1}{2} H_2O$	806	151–152	90% MeOH	−11°	0.65	271 (6400), 279 (6250), 289 (5320)
$n=2$	$C_{49}H_{51}N_5O_7S \cdot \frac{1}{2} H_2O$	863.1	134[d])	EtOAc/Hx	+11°	0.45	270 (6200), 278 (6070), 288 (5160)
$n=3$	$C_{51}H_{54}N_6O_8S \cdot \frac{1}{2} H_2O$	920.1	206[d])	CH_3CN	+16°	0.35	271 (6400), 279 (6300), 289 (5350)
$n=4$	$C_{53}H_{57}N_7O_9S \cdot 1\ H_2O$	986.1	165[d])	EtOH	+16°	0.23	271 (6300), 279 (6200), 289 (5300)
9							
$n=3$[e])	$C_{32}H_{38}N_6O_8S \cdot \frac{1}{2} H_2O$	675.8	>250[d])	CH_3CN/H_2O	−415°	0.22	289 (10650)
$n=4$[e])	$C_{34}H_{41}N_7O_9S \cdot 2\ H_2O$	759.8	238[d])	$MeOH/H_2O$	−104°	0.10	290 (13150)

[a]) Microanalytical data agree with the elementary composition indicated. [b]) $c = 1$, DMF. [c]) System 157A (= $CHCl_3$/MeOH/glacial AcOH/H_2O 90:10:0.5:1). [d]) With decomposition. [e]) The structures postulated for the compounds were corroborated by MS.

Experiments for the formation of the tryptophan-2-thioether **9**, $n = 4$ *in different solvents.* To 0.1 mmol **7** ($n = 4$) in 100 ml solvent, 2.2 ml of a 0.1M solution of iodine in CH_2Cl_2 was added within 1 min. The yellow solution was stirred for 5 min at RT. then the reaction was stopped by addition of 10 ml of a 0.2M citrate buffer (pH 5), containing 50 mg of ascorbic acid. The products were isolated by chromatography on *Sephadex* LH 20 as described for **9**. The yields indicated in *Table 1* were estimated by dilution series on TLC.

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