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SELECTIVE OXIDATION OF SULPHUR IN PEPTIDE SYNTHESIS

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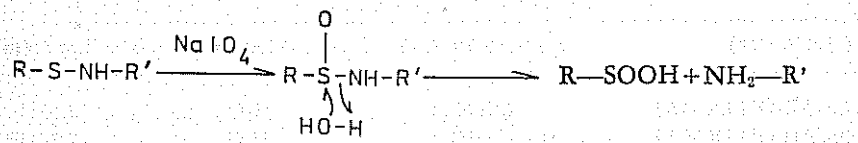
The removal of the Nps group is effected by numerous methods [6] the most common being hydrochloric acid in ether. We examined the possibility of removing this protecting group under mild oxidizing conditions (see Table 1) and studied in detail the kinetics of cleavage with sodium periodate (the course of the reaction can be examined in terms of extinction decrease at 390 nm). The reaction is of the first order with respect both to the substrate and the reagent, and the reaction rate depends on the water content of the reaction medium; this may be connected with periodate dissociation. The activation energy of this reaction is 12.5 kcal/mol (as follows from the dependence of the reaction rate on temperature). In order to obtain an insight into the mechanism of this reaction we prepared o- and p-nitrobenzenesulphenyl amides and found that the cleavage proceeds at a comparable rate in both cases; this eliminates the possibility that the o-nitro group participates in the cleavage. The reaction product is the corresponding sulphinic acid and the following reaction scheme can therefore be proposed:



Oxidizing cleavage can be used also for the removal of the protecting group from Nps-Trp. One eqivalent exactly of m-chloroperoxybenzoic acid, however, must be used; sodium periodate or an excess of the oxidizing reagent leads to tryptophan oxidation.

By contrast, periodate can be employed also for the removal of the Nps group from peptides with sulphur-containing amino acids since sulphur is oxidized to the sulphoxide only (proposed [2] and used e.g., by Irie et al. [1], as well as Izeboud and Beyerman [3], as a from of protection of methionine), which can be reduced during the final stages of synthesis. Periodate can be used even if the peptide contains a disulphide bond since the latter is not (as demonstrated with oxytocin) affected by this reagent.

We used the oxidizing cleavage of the Nps group in the synthesis of deamino-1-carba-oxytocin in which the sulphoxide served to protect the sulphur of S-(γ-

methoxycarbonylpropyl)cysteine. The carboxyl-terminal glycine amide was acylated stepwise by the corresponding active esters. The protecting Nps group (with the exception of Z-tetrapeptide amide) was removed by oxidation, effected up to tripeptides by periodic or m-chloroperoxybenzoic acid and from penta- and hexapeptides by an excess of periodate in water.

Deamino-1-carba-oxytocin sulphoxide obtained by cyclization, was converted into the fully active (1790 I.U./mg) thioether form by reduction by hydrogen bromide and acetone (Iselin [2]) which has been demonstrated not to deteriorate tyrosine in the peptide chain. When the sulphoxide was reduced under the conditions described in the literature by 2-mercaptoethanol [2] or dithiothreitol [5] the reduction proceeded to a low degree only, and a large excess of the reducing agent had to be used for a longer reaction time (see Table 2).

We checked the increase of the solubility of some oxidized peptides with oxytocin sequence (Table 3).

The oxidation of sulphur is a stabilizing factor protecting the group on the sulphur atom against acidolytic cleavage (Table 4). Liquid hydrogen fluoride, however, leads to partial sulphoxide reduction.

We also examined the behaviour of Cys(O)(Bzl) during reduction by sodium in liquid ammonia. The main product of this reaction is cysteinesulphinic acid (which is readily converted into cysteic acid). If the peptide synthetized containing Cys(Bzl) is partly oxidized (it is difficult to demonstrate the presence of S-benzylcysteine sulphoxide in the peptide since the thioether form of this amino acid is regenerated during hydrolysis in 6M HCl), sulphhydryl groups are not quan-

Table 1. Rate constants of cleavage of Nps group under different conditions

c_{Nps} (.3×10⁻⁴M)	c_{ox} (.3×10⁻⁴M)	Solvent	$k_{25°C}$ (min⁻¹)
1 (Nps-Ala-OH.DCHA)	1 ($NaIO_4$)	H_2O	2.1×10⁻⁴ [b]
1 (Nps-Ala-OH.DCHA)	10 ($NaIO_4$)	H_2O	2.3×10⁻³
1 (Nps-Ala-OH.DCHA)	100 ($NaIO_4$)	H_2O	2.3×10⁻²
10 (Nps-Ala-OH.DCHA)	10 ($NaIO_4$)	H_2O/MeOH 9:1	1.6×10⁻³ [b]
10 (Nps-Ala-OH.DCHA)	100 ($NaIO_4$)	H_2O/MeOH 9:1	1.9×10⁻²
1 (Nps-Ala-OH.DCHA)	100 ($NaIO_4$)	H_2O/MeOH 1:1	2.1×10⁻³
1 (Nps-Ala-OH.DCHA)	10 ($NaIO_4$)	H_2O/DMF 9:1	1.0×10⁻³
1 (Nps-Ala-OH.DCHA)	10 ($NaIO_4$)	H_2O/DMF 1:1	3.4×10⁻⁵
1 (Nps-Ala-OH.DCHA)	100 ($Bu_4N^+IO_4^-$)	MeOH	8.3×10⁻⁵
1 (Nps-Ala-OH.DCHA)	100 (H_2O_2)	H_2O	1.0×10⁻⁴
1 (Nps-Ala-OH.DCHA)	10 (m-CPBA[a])	MeOH	1.4×10⁻¹
1 (Nps-Ala-OH.DCHA)	10 (m-CPBA[a])	MeOH/H_2O 9:1	1.7×10⁻¹
1 (Nps-Ala-OH.DCHA)	10 (m-CPBA[a])	MeOH/H_2O 1:1	5.2×10⁻¹
1 (o-NO_2-C_6H_4-SNH_2)	10 ($NaIO_4$)	MeOH/H_2O 1:1	1.2×10⁻³
1 (p-NO_2-C_6H_4-SNH_2)	10 ($NaIO_4$)	MeOH/H_2O 1:1	3.2×10⁻³

[a] m-CPBA stands for m-chloroperoxybenzoic acid; [b] the reaction is roughly of the first order during the first moments only, on the whole reaction is of the second order.

Table 2. Reduction of deamino-1-carba-oxytocin sulphoxide

Reagent	Reaction time (h)	% SO (according to TLC)	% S (according to TLC)	Uterotonic activity (I.U./mg)
HBr-acetone	0.1	0	100	1790
Dithiothreitol (10 eqv.)	24	85	15	
2-Mercaptoethanol (5 eqv.)	24	90	10	150
2-Mercaptoethanol (25 eqv.)	24	80	20	
2-Mercaptoethanol (25 eqv.)	72	50	50	
2-Mercaptoethanol (25 eqv.)	240	15	85	

titatively generated during the reduction; this could explain the observed low yields of cyclization of peptides containing a disulphide bond and also the difficulties encountered during their purification. The behaviour of S-methylcysteine, S-(γ-methoxycarbonylpropyl)cysteine and methionine sulphoxides is similar; in all cases sulphinic acid, a compound with a free sulphhydryl group, are formed and in some cases even a product not containing sulphur [1] (Ala from Cys(O)(Me), Abu from Met(O)).

Table 3. Concentration of saturated solutions of certain peptides at 25°C

Nps-Asn-Cys(C_3H_6COOMe)-Pro-Leu-Gly-NH_2
$c_{max(H_2O)} = 4.6\times10^{-4}$M sulphoxide 1.5×10^{-3}M

Nps-Gln-Asn-Cys(C_3H_6COOMe)-Pro-Leu-Gly-NH_2
$c_{max(H_2O)} = 2.1\times10^{-3}$M sulphoxide 1.8×10^{-2}M

Z-Cys(Bzl)-Tyr(Bu^t)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2
$c_{max(H_2O/MeOH\ 4:1)} = 4.2\times10^{-5}$M bis-sulphoxide 1.3×10^{-4}M

H-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2
$c_{max(H_2O)} = 9.0\times10^{-4}$M bis-sulphoxide 1.6×10^{-3}M

Table 4. Reaction of cysteine derivatives with liquid hydrogen fluoride in presence of two equivalents of anisol

Compound	Reaction conditions	% Protecting group removed
Cys(Bzl)	60 min, 20°C	100
Cys(O)(Bzl)	30 min, 0°C	0
Cys(O)(Bzl)	120 min, 25°C	~10
Cys(BzlMe$_3$)[a]	30 min, 0°C	100
Cys(O)(BzlMe$_3$)	30 min, 0°C	13

[a] Krojidlo[4].

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