SYNTHESIS AND APPLICATION OF S-TERT.BUTYL-SULFONIUM PEPTIDES

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INTRODUCTION

Acidolytic deprotection of Boc-methionine peptides leads to the formation of S-tert.-butylsulfonium peptides as by-products [1,2,3]. We want to demonstrate the usefulness of these sulfonium intermediates for the purification of substance P analogs. Different procedures of S-tert.butylation of methionine peptides as well as carba-analogs of oxytocin and the stabilities of the resulting sulfonium peptides were studied.
In a previous communication we reported, that S-tert.-butylsulfonium intermediates have been used in the iodination of tyrosine peptides to prevent S-oxydation [4].

RESULTS AND DISCUSSION

S-tert.Butylation. Different procedures for S-tert.-butylation of methionine peptides are summarized in Table 1. A quantitative S-alkylation occurs using hydrogenfluoride (even in presence of a 100-fold excess of anisole) as well as in TFMSA. In MSA high yields were obtained too, but in contrast to TFMSA it is a bad solvent for some peptides. Unprotected peptides can be S-alkylated when Boc-amino acids or acetic acid tert.-butylester are added as tert.butyl donors.
The alkylation of the cyclic thioether bridges of dCOT-1 and dCOT-6 was incomplete, in contrast to the S-tert.-butylation of the methionine peptides.

Table 1
tert.Butylation of methionine peptides and carba analogs of oxytocin by different procedures.

Starting material	Method	% S-Bu^t
Boc-Arg(NO_2)-Pro-Lys(Boc)-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (protected SP)	HF/anisole	100
Boc-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	HF	>95
Boc-S-Ala-Trp-Met-Asp-Phe-NH_2	HF	>95
Boc-Tyr(Bu^t)-Gly-Gly-Phe-Met-OH	HF or TFMSA	100
H-Tyr-Gly-Gly-Phe-Met-OH	HF/Boc-Gly or TFMSA/Boc-Gly	100
H-Gly-Met-NH_2	TFMSA/CH_3COOBu^t	> 95
	MSA/Boc-Gly	90
Boc-Gly-Met-NH_2	20% MSA in methanol	20
CH_2-CO-Tyr-Ile-Gln-Asn-NH-CH-CO-Pro-Leu-Gly-NH_2 CH_2-CH_2——S-CH_2 (dCOT-1)	HF/Boc-Ile	~ 50
CH_2-CO-Tyr-Ile-Gln-Asn-NH-CH-CO-Pro-Leu-Gly-NH_2 CH_2-S——CH_2-CH_2 (dCOT-6)	HF/Boc-Ile	~ 50

TFMSA = trifluoromethane sulfonic acid, MSA = methane sulfonic acid. The tert.butylations were performed at 0°C for 10 min. (with the exception of protected SP) and the products were isolated by precipitation with ether (HF was removed in vaccuo previously).

Quantitative S-tert.butylation has been attainable also by a later addition of methionine peptides to the reaction mixtures of HF, TFMSA or MSA with Boc-amino acids. This demonstrates the formation of relatively stable alkylating intermediates in this systems, e.g. tert.butylfluoride, in HF , and tert.butylesters in TFMSA and MSA, as indicated by preliminary NMR-studies for MSA/Boc-amide; see also [3].

Stability of S-tert.butylsulfonium peptides. The investigation of the stability by means of HPLC shows that the S-Bu^t-peptides are stable at -20°C for 6 month and at 5°C for several days. At higher temperatures they are decomposed regenerating the thioether bridge of methionine (Fig. 1). The activation energy for the decomposition of (S-Bu^t)-substance P was estimated from the

Arrhenius plot as 75 kJ.
Side reactions (e.g. formation of S-Bu^t-homocysteine peptides) were not observed. The desamino carba oxytocins (S-Bu^t)-dCOT-1 and (S-Bu^t)-dCOT-6 are highly unstable (Fig. 1), in contrast to the S-tert.butylsulfonium derivatives of methionine peptides. The carba-1 analog is less stable than the carba-6 analog, suggesting differences in the sterical accessibility of the two sulfur atoms. The stereochemical nonequivalence is also underlined by the different oxydation kinetics [5] and by the behaviour of these analogs in HPLC (Table 2): As expected the (S-Bu^t)-dCOT-1 behaves more hydrophilic, like other sulfonium-peptides. The (S-Bu^t)-dCOT-6 behaves more lipophilic than the unmodified peptide, suggesting that the positively charged sulfur atom should be covered by the tert.butyl group.

Table 2
HPLC-capacity factors of substance P- and oxytocin analogs with modified thioether bridges.

	unmodified	S-Bu^t	S-Me	S-O	S O_2
substance P	11,8 (2,19)	- (0,69)	- (0,63)	4,37 (0,81)	6,37
dCOT-1	3,22	2,63	-	2,27	-
dCOT-6	2,56	2,93	-	2,16	-

Column RP-18 15x0,6cm, flow rate 1,5 ml/min., UV detection at 240 nm, mobile phase methanol 50%/ 0,3% TFA in water 50% (values in parentheses: methanol 60% / 0,3% TFA in water 40%).

Purification of substance P peptides via its S-tert. butylsulfonium derivatives. Difficulties occured in the purification of substance P using ion exchange chromatography (broad distribution; Fig. 3, dotted line) due to a strong association as revealed by CD-measurements (Fig. 2) and light scattering experiments [6]. Introduction of polar groups in the C-terminal part of substance P results in SP-analogs with a strongly reduced association behaviour: The O-values of S-Tyr-SP and of the S-methylsulfonium derivative of substance P (11-[Met^+(Me)]-SP, synthesized

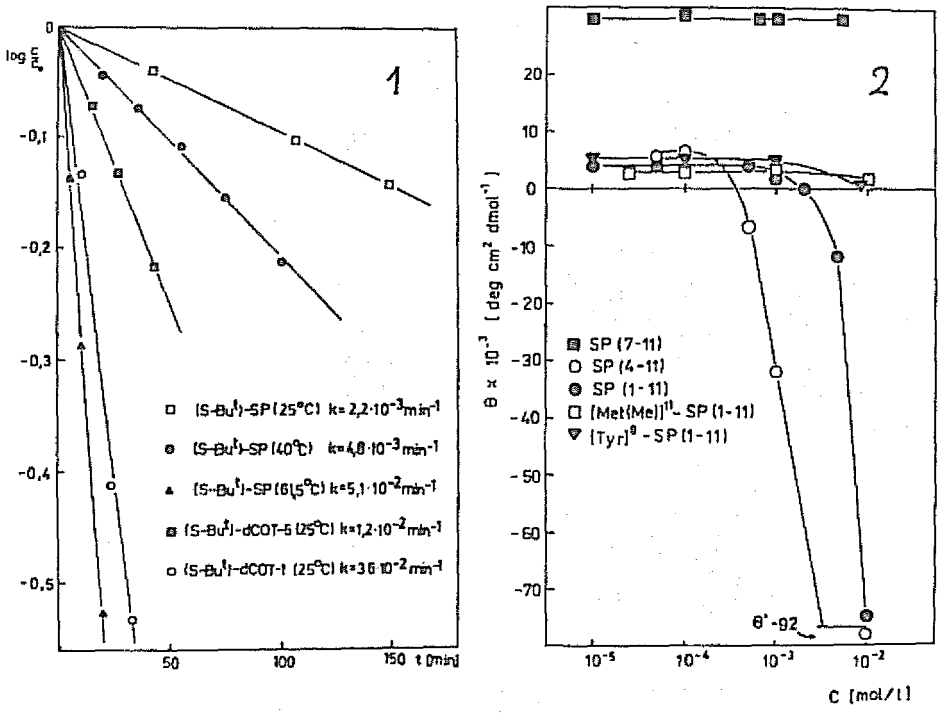

Fig. 1. Decomposition of S-Bu^t-sulfonium derivatives of SP, dCOT-1 and dCOT-6 in water (2 mg/100 µl), measured by HPLC.

Fig. 2. Dependence of CD (molar ellipticity Θ) on concentration c at 220 nm in water, pH 3.0

according to the S-methylation of glucagon [7]) are nearly independent on peptide concentration (Fig. 2). This results prompted us to use the S-tert.butylsulfonium derivative of substance P, formed during HF-deprotection, for the purification step as an intermediate with a diminished association tendency. In contrast to SP itself the sulfonium derivative is eluted without complications (Fig. 3) and gives pure substance P after heating. The procedure has been applied to purify SP in gram amounts as well as SP (4-11), SP (5-11), p-hydroxyphenylacetyl-Arg^1-SP and 8-Tyr(J_2)-SP, all analogs with association behaviour.

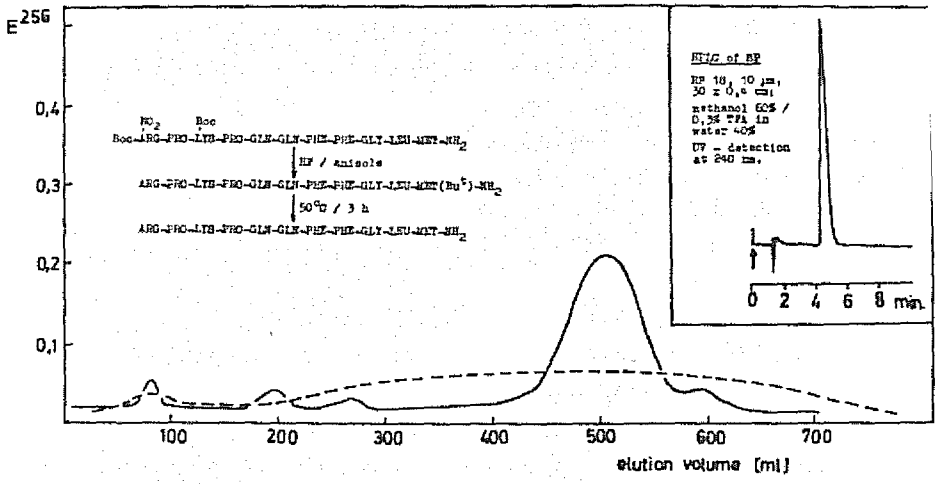

Fig. 3. Purification of substance P via its S-Bu^t-sulfonium derivative on CM-cellulose in ammonium acetate pH 6.0 (0.01 - 0.5 M), SP (- - - -), (S-Bu^t)-SP (_____), at 4°C.

CONCLUSIONS

S-tert.butylsulfonium derivatives of methionine peptides can be synthesized quantitatively, handled at low temperature and reconverted into the methionine peptides without side reactions. The sulfonium intermediates were used successfully in the purification of substance P peptides to minimize association. The tert.butylation of the cyclic thioethers dCOT-1 and dCOT-6 demonstrates the stereochemical nonequivalence of the sulfur atoms in these oxytocin analogs.

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