TOTAL SYNTHESIS OF HYPOTHALAMIC "SOMATOSTATIN"

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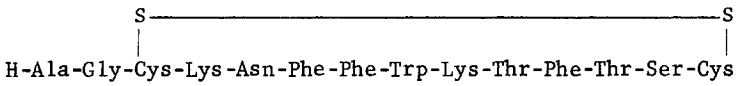
Summary: The synthesis of the tetradecapeptide somatostatin (somatotropin release inhibiting factor, SRIF) by conventional methods of peptide synthesis is described. The synthetic material was found to be biologically active at the pico-gram/ml dose level.

Recently Guillemin's group (1,2) reported the isolation and sequence determination of ovine SRIF which was found to have the primary structure (I).



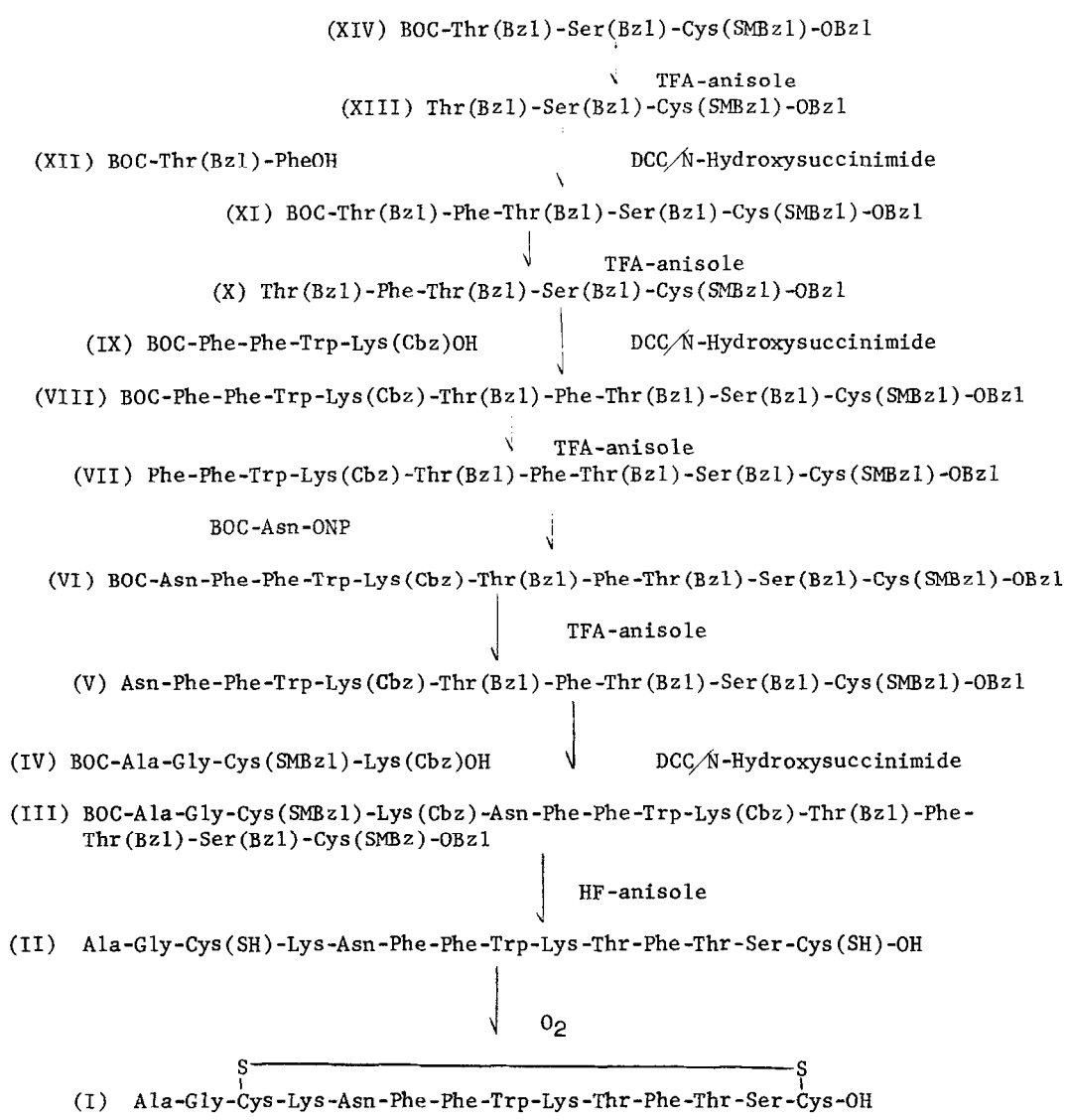
The linear, reduced form, of this tetradecapeptide was synthesized by solid-phase techniques (1) and found to have the biological activity of the native SRIF. The oxidation of the disulfhydryl compound to the cyclic disulfide tetradecapeptide was reported by the same group (3).

The total synthesis of the above sequence (I) by conventional methods would further confirm the deduced structure for the natural compound and in addition make available sufficient quantities for broad biological testing because of its potential significance in clinical medicine (1,4,5).

A fragment condensation approach was adopted for the construction of the tetradecapeptide and the DCC/N-hydroxysuccinimide (6) or DCC/1-hydroxybenzotriazole (7) method was applied for the coupling of the fragments without racemization. Asparagine was added by the p-nitrophenyl ester method (8) in order to avoid dehydration of the side chain amide function to β-cyanoalanine (9).

Abbreviations: DCC, dicyclohexylcarbodiimide, CBZ, benzyloxycarbonyl, BOC, tert-butyloxycarbonyl, BZL, benzyl, MBZL, p-methoxybenzyl, -ONP, p-nitrophenyl ester, TFA, trifluoroacetic acid, DMF, dimethylformamide

Scheme I



The ε-amino groups of the two lysines were protected by the benzyloxycarbonyl (CBZ) group while the tert-butyloxycarbonyl (BOC) moiety masked the α-amino group of the N-terminal alanine. Benzyl ethers (BZL) and benzyl esters (OBZL) were employed for the protection of the side chains of serine and threonine and the carboxyl group of C-terminal cysteine respectively while the p-methoxy

Table 1.

compound	elementary analysis	$[\alpha]_D^{25}$
BOC-Thr(Bzl)-Ser(Bzl)-Cys(SMBzl)-OBzl (XIV) m.p. 87-90°	$C_{44}H_{52}N_3SO_9$ (798.9) Cald. C 66.15, H 6.56, N 5.26 Found C 66.22, H 6.95, N 5.29	-14.7 (C 0.98, DMF)
BOC-Thr(Bzl)-Phe-Thr(Bzl) Ser(Bzl)-Cys(SMBzl)-OBzl (XI) m.p. 105-107°	$C_{64}H_{75}N_5SO_{12} \cdot H_2O$ (1156.2) Cald. C 66.46, H 6.71, N 6.05 Found C 66.68, H 6.59 N 6.20	-3.6 (C 1, DMF)
BOC-Phe-Phe-Trp-Lys(Cbz)-OH (IX) m.p. 166-168°	$C_{48}H_{56}N_6O_9$ (860.9) Cald. C 66.96, H 6.56, N 9.76 Found C 66.39 H 6.63, N 9.63	-8.91 (C 1, DMF)
BOC-Ala-Gly-Cys(SMBzl)-Lys(Cbz)-OH (IV) m.p. 85-90°	$C_{35}H_{49}N_5SO_{10} \cdot H_2O$ (749.9) Cald. C 56.07, H 6.85, N 9.34 Found C 56.10 H 6.91, N 9.37	-26 (C 1.05, methanol)
BOC-Phe-Phe-Trp-Lys(Cbz)-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Cys(SMBzl)OBzl (VIII) m.p. 200-203°	$C_{107}H_{121}N_{11}SO_{18} \cdot H_2O$ (1899) Cald. C 67.60, H 6.52, N 8.10 Found C 67.31, H 6.60, N 8.46	-5.6 (C 0.5, DMF)
BOC-Asn-Phe-Phe-Trp-Lys(CBz)-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Cys-(SMBzl)-OBzl) (VI) m.p. 220-222°	$C_{111}H_{127}N_{13}SO_{20} \cdot H_2O$ (2013) Cald. C 66.22, H 6.46, N 9.04 Found C 65.96, H 6.40, N 8.97	-11.8 (C 0.99, DMF)
BOC-Ala-Gly-Cys(SMBzl)-Lys(Cbz)-Asn-Phe-Phe-Trp-Lys(Cbz)-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Cys(SMBzl)OBzl (III) m.p. 246-250° dec.	$C_{141}H_{166}N_{18}S_2O_{27}$ (2608.7) Cald. C 64.91, H 6.41, N 9.66, S 2.45 Found C 64.92, H 6,57, N 9.66, S 2.67	-12.8 (C 0.99, DMF)

benzyl group (MBZL) served for the protection of the sulfhydryl side chain functions of the two cysteines.

The removal of the BOC group during the elongation of the peptide chain was carried out by trifluoroacetic acid (TFA) in the presence of excess anisole as a scavenger of the cationic species, while the deprotection of the fully protected tetradecapeptide was accomplished by treatment with anhydrous liquid hydrogen fluoride under controlled conditions (10) and in the presence of anisole.

Table 2. PROPERTIES OF SYNTHETIC SRIF (I)

Amino acid analysis:	Asp (1) 0.92	Ala (1) 0.99
	Thr (2) 1.80	Phe (3) 3.17
	Ser (1) 0.83	Lys (2) 2.26
	Gly (1) 0.83	Cys (2) 1.85*

Trp content was determined from UV spectrum.

$[\alpha]_D^{25}$ -36° ± 1 (C 0.565, 1% AcOH)

* As Cysteic acid.

Table 3.

Solvent system (volume/volume)	R_f*
n-Butanol-gl. acetic acid-water (4:1:1)	0.20
isoPropanol- 1N acetic acid (2:1)	0.66
isoPropanol- 1N ammonium hydroxide (2:1)	0.54
Ethyl acetate-pyridine-water-acetic acid (5:5:3:1)	0.74

*Eastman Chromagram sheet Type K 201R (silica gel)

In scheme I below we summarize the synthetic steps of the procedure. The deprotected linear tetradecapeptide (II) was oxidized by standing for three days in the air and under high dilution (100 mg/l) in an CH_3COONH_4 (0.1 M) buffer solution. The crude product was purified by gel filtration through Sephadex G-25 and elution with 2N-acetic acid then by partition chromatography on Sephadex G-25 in n-butanol-acetic acid-water (4:1:5). Table 1 shows the physical properties of the most important intermediates of the synthesis.

The synthetic tetradecapeptide was homogeneous on TLC in four solvent systems and the linear reduced form was identical with material provided by Dr. R. Guillemin, Salk Institute, La Jolla, California. R_f values are in Table 3.

Our synthetic material was tested for the inhibition of the secretion of somatotropin in monolayer cultures of dispersed rat pituitary cells, the

supernatants of which were assayed by radioimmuno assay, (11) and was active at concentrations $\geq$ 1 nM.

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