81. Synthesis of Somatostatin

Preliminary Communication[1])

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Summary. A tetradecapeptide with the proposed structure of growth hormone release inhibiting factor was synthesized by a classical fragment approach.

The isolation and structure elucidation of somatostatin (growth hormone release inhibiting factor) by *Guillemin et al.* [1] has opened new perspectives for the treatment of endocrine dysfunctions involving growth hormone secretion. In order to fully evaluate the clinical potential of this compound, substantial quantities will be necessary, hence the development of an efficient synthesis of somatostatin is mandatory[2]). We report a solution to this problem embodying the following features:

The molecule is assembled from two fragments of equal size, a choice guided by considerations of synthetic expediency. All couplings are performed under conditions known to lead to minimal racemization. The carefully chosen protecting groups assure minimal side reactions during the removal of intermediate α-amino protecting groups. A large number of intermediates are crystalline, hence their purification and characterization is facile.

The cystine bridge is closed under controlled conditions, leading in good yield to the cyclic product. The side chain protecting groups are removed under relatively mild conditions in the last step. Somatostatin of high biological potency in an analytically well defined state is obtained after minimal purification in gram quantities.

Two heptapeptides were synthesized in the following way (*Schemes 1* and *2*): the pentapeptide 3–7 was built up by stepwise elongation from the C-terminal amino acid. Amino acid 4 (lysine) was introduced as the activated nitrophenyl ester and amino acid 5 (asparagine) was introduced as the activated trichlorophenyl ester. All other couplings were achieved with the dicyclohexylcarbodiimide-hydroxybenzotriazole method [4]. The dipeptide 1–2 constructed *via* the dicyclohexylcarbodiimide-hydroxybenzotriazole method was then coupled as the trichlorophenyl ester to the pentapeptide 3–7 leading to the desired heptapeptide 1–7.

The hexapeptide 8–13 was also built up by stepwise elongation from the carboxyl end. Amino acid 11 (phenylalanine) was introduced as the activated trichlorophenyl ester and amino acid 9 (lysine) was introduced as the activated nitrophenyl ester. All other couplings were achieved by the dicyclohexylcarbodiimide-hydroxybenzotriazole method. Azide-coupling of the fragment 8–13 to the C-terminal amino acid 14 led to the desired heptapeptide 8–14.

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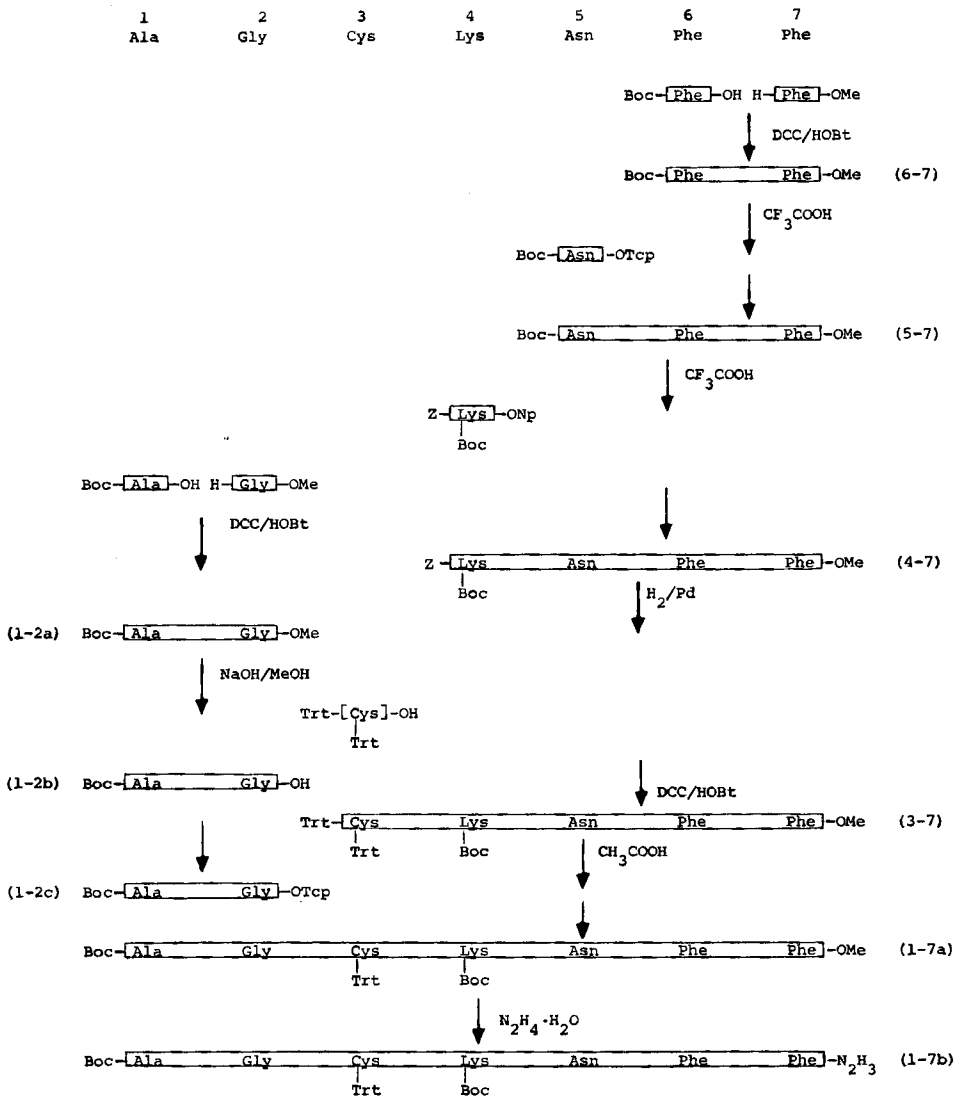
[1]) A complete paper will be submitted to this Journal.

[2]) During our investigations several syntheses of somatostatin by the solid phase method [2] and one classical fragment synthesis [3] were reported.

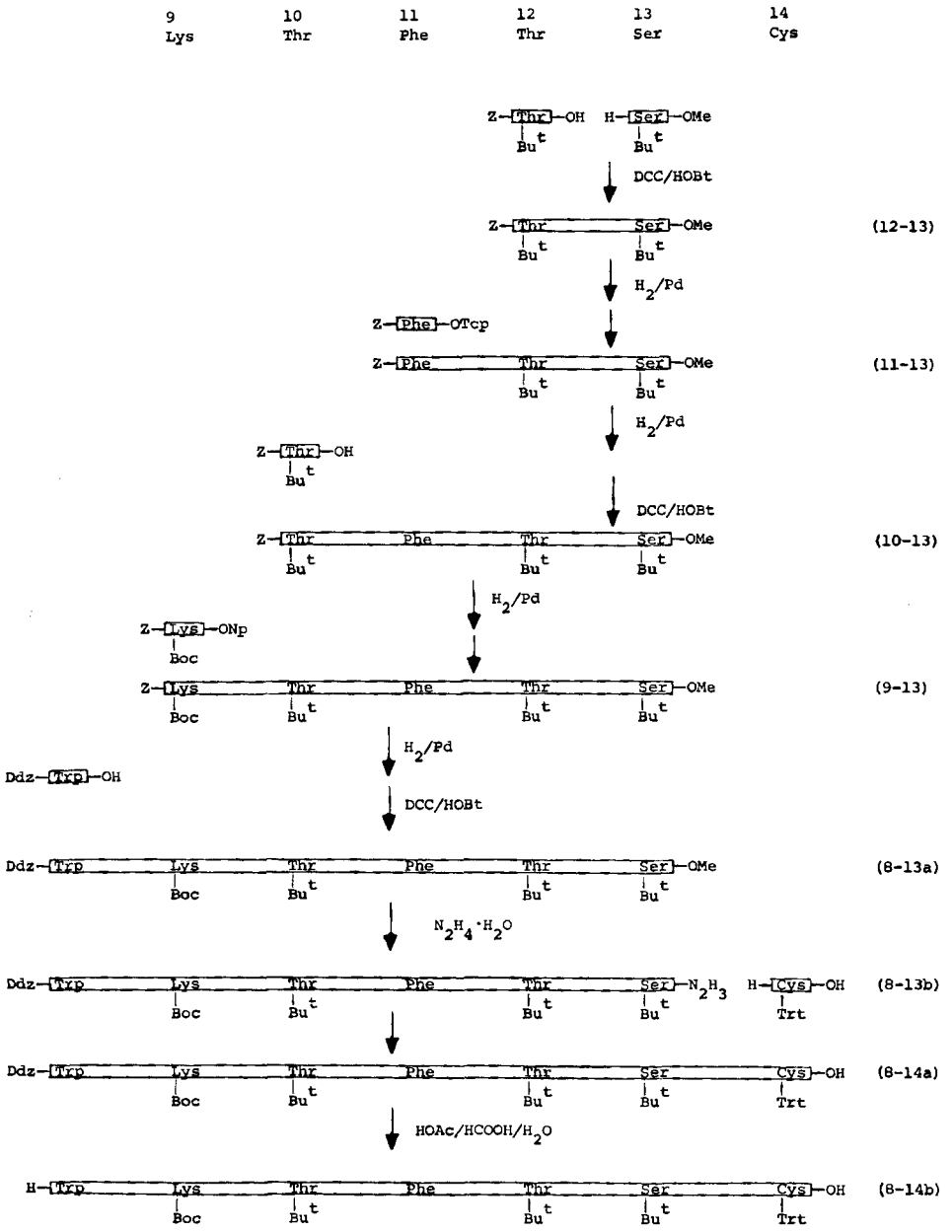
Scheme 1



Abbreviations for both Schemes: Boc, *t*-butyloxycarbonyl; Bu^t, *t*-butyl; DCC, dicyclohexylcarbodiimide; Ddz, α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; Np, *p*-nitrophenyl; Tcp, 2,4,5-trichlorophenyl; Trt, trityl; Z, benzyloxycarbonyl.

The hydroxyl functions of the serine and threonine residues were protected by *t*-butylether formation. The ε-amino group of lysine was blocked with the *t*-butyloxycarbonyl group. The hydrogenolytically removeable benzyloxycarbonyl group was employed for intermediate protection of the α-amino groups, except in fragments containing cysteine. In the latter case, the α,α-dimethyl-3,4-dimethoxybenzyloxycarbonyl residue [5] or the trityl residue were employed for intermediate

Scheme 2



α-amino blocking. These groups were removed by mild acid treatment without affecting the side chain protecting groups. The sulfhydryl groups of the cysteine-units were blocked with trityl [6]. The carboxyl function of the C-terminal amino acid remained unprotected. For the amino acids 5 and 6, the selection of protecting

groups was not critical, and the *t*-butyloxycarbonyl group was employed. Finally, the amine of the N-terminal alanine was protected by the *t*-butyloxycarbonyl group. The synthesis of the fragments is summarized in *Schemes 1* and *2*. Some physical data for the intermediates and their yields are listed in the Table. The coupling of the azide derived from 1–7b with 8–14b under carefully controlled conditions [7] led, in 73% yield, to the amorphous protected linear tetradecapeptide having the correct amino acid analysis. Oxidation of this compound with iodine in methanol [8] followed by a short treatment with conc. hydrochloric acid at 0°C under nitrogen afforded crude somatostatin. It was purified by chromatography on carboxymethyl cellulose followed by lyophilization of the fractions containing pure somatostatin. Relyophilization from 1N acetic acid afforded a preparation of *somatostatin* in 43% yield which was homogeneous by electrophoresis and thin layer chromatography.

Some Data for the Intermediates

Fragment[a])	Yield in %	Solvent of Crystallization	M.p.	$[\alpha]_D^{22°}$ (c = 1, in $HCON(CH_3)_2$)
6– 7	85	EtOAc/petrol ether	123–124°	– 13.4°
5– 7	83	MeOH	194–195°	– 34.0°
4– 7	78	MeOH	210° (dec.)	– 31.0°
3– 7	77	EtOAc/petrol ether	180–181°	+ 17.9°
1– 2a	82		Oil	
1– 2b	74	EtOAc/petrol ether	120°	– 9.6°
1– 2c	62	EtOAc/petrol ether	109–111°	– 18.7°
1– 7a	80	MeOH	200–204°	– 20.7°
1– 7b	70	MeOH	214° (dec.)	– 17.3°
12–13	72		Oil	
11–13	77	Ether/hexane	101–103°	+ 9.4°
10–13	76	Ether/hexane	110–113°	+ 27.5°
9–13	85	Ether/petrol ether	81– 85°	+ 16.9°
8–13a	65	Ether/petrol ether	112–114°	+ 10.1°
8–13b	99	MeOH	189–191°	+ 15.2°
8–14a	87	MeOH/ether	135–138°	+ 12.9°

[a]) All crystalline intermediates gave satisfactory elemental analyses. The deprotected fragment 8–14b was characterized by its amino acid analysis. Its NMR. spectrum shows the presence of all *t*-butyloxy derived protecting groups and the absence of the α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl group.

Analytical data: UV. (MeOH): λ_{max} 289 (6340), 282 (6990), 274 (6550) nm (ε). – $[\alpha]_D^{22°}$ = – 34.5° (c = 1, 1% AcOH) (corrected for water and acetic acid content).

$C_{76}H_{104}N_{18}O_{19}S_2 \cdot 7CH_3COOH \cdot 5H_2O$ (2147) Calc. C 50.34 H 6.62 N 11.74%
Found ,, 50.14 ,, 6.34 ,, 12.86%

Calc. CH_3COOH 19.5 H_2O 4.2%
Found ,, (GLC.) 19.0 ,, (*Karl Fischer*) 4.4%

Amino acid analysis: Ala 0.97 (1); Gly 1 (1); Cys 1.80 (2); Lys 2.15 (2); Asp 0.97 (1); Phe 3.0 (3); Thr 1.91 (2); Ser 0.80 (1).

The biological activity of somatostatin prepared by this method will be reported in separate papers [9].

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82. Isomerisierungen und Umlagerungen in bicyclischen Systemen *via* Cyclopropan-carbaldehyd-enamine

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Summary. The pyrrolidino-aminal (**4**) of bicyclo[3.1.0]hex-2-ene-6-*endo*-carbaldehyde (**3**) underwent a facile (80°), mildly acid catalyzed isomerization to the corresponding *exo*-aminal (**6**), which was characterized by hydrolysis to bicyclo[3.1.0]hex-2-ene-6-*exo*-carbaldehyde (**7**).

At higher temperatures (140°), the two aminals **4** and **6** were converted smoothly to a 1:1 mixture of *syn*- and *anti*-4-(pyrrolidino-methylidene)-bicyclo[3.1.0]hex-2-ene (*syn*- and *anti*-7-pyrrolidino-homofulvene[1]) **8** and **9**). The structures of **8** and **9** were derived from spectral data. This corrects a previous interpretation by *Cook et al.*

The *endo* → *exo*-aminal isomerization (**4** → **6**) is considered to occur via the enamine (**5**) of bicyclo[3.1.0]hex-2-ene-6-carbaldehyde (**3** or **7**), with which the aminals (**4** and **6**) are in equilibrium. The same enamine (**5**), a methylidene cyclopropane derivative, is thought to be the intermediate in the aminal (**4** or **6**) → amino-homofulvene (**8** and **9**) conversion, which, therefore, belongs to the vinyl-methylidene-cyclopropane rearrangement type.

A cationic mechanism for the *endo* → *exo*-aminal isomerization is excluded by the discrepancy in this reaction of the pyrrolidino-aminals (**13** and **15**) of 6-*exo*-methyl-bicyclo[3.1.0]hex-2-ene-6-*endo*-carbaldehyde (**12**) and of bicyclo[3.1.0]hexane-6-*endo*-carbaldehyde (**14**). While the former aminal (**13**) is stable even under acid catalysis and at higher temperatures, the latter (**15**) isomerizes readily to the *exo*-aminal **16**.

The three *endo*-aldehydes **3**, **12** and **14** were prepared by the alkali catalyzed rearrangement of the three chlorohydrins 7-*endo*-chloro-bicyclo[3.2.0]hept-2-en-6-*endo*-ol (**20 ac**), 7-*endo*-chloro-7-*exo*-methyl-bicyclo[3.2.0]hept-2-en-6-*endo*-ol (**20 ad**) and 7-*endo*-chloro-bicyclo[3.2.0]heptan-6-*endo*-ol (**20 bc**) according to the method of *Brook*.

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[1]) The prefix 'homo' before a trivial name signifies in general that the system (chain or ring) has been enlarged by one carbon atom. The special case of double bond to cyclopropane modification has received individual attention in connection with Winstein's homoconjugation concept. The systematic names of **8** and **9**, according to the IUPAC nomenclature rules, would be 4-(pyrrolidino-methylidene)-bicyclo[3.1.0]hex-2-ene.