Monatshefte für Chemie 120, 1029–1041 (1989)

Monatshefte für Chemie
Chemical Monthly

On the Hypothetical Protein F154 of the TTV1 Virus/*Thermoproteus Tenax*. Part II: Synthesis of the Trieicosapeptide, Corresponding to the Protein Sequence 79–101

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Summary. For the identification of a protein predicted by DNA sequence analysis of the TTV1 virus from the archaebacterium *Thermoproteus tenax*, the trieicosapeptide H-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Tyr-Asp-Ile-Thr-Tyr-Val-Val-Phe-Asp-Val-Thr-Pro-Ser-Pro-Thr-Pro-OH, corresponding to the protein fragment 79–101, was prepared by conventional methods of peptide synthesis. This sequence portion may possibly represent a suitable protein specific immunepitope.

Keywords. TTV1 virus; Peptide synthesis; Poly-(Thr-Pro)-peptides.

Zur Hypothese eines TTV1 Virus/*Thermoproteus tenax* F154-Proteins. Teil II: Synthese des Proteinfragments 79–101

Zusammenfassung. Für den Nachweis der Expression des Proteins F154 — nach einer Sequenzanalyse des Genoms des TTV1 Virus im Archaebakterium *Thermoproteus tenax* postuliert — wurde das Peptid H-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Tyr-Asp-Ile-Thr-Tyr-Val-Val-Phe-Asp-Val-Thr-Pro-Ser-Pro-Thr-Pro-OH (Proteinfragment 79–101) mit Hilfe konventioneller Peptidsynthese hergestellt. Diese Peptidsequenz sollte ein geeignetes proteinspezifisches Immunepitop darstellen.

Abbreviations

Standard abbreviations as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature are used for amino acids and related derivatives; HO*Su*, N-hydroxysuccinimide; HO*Bt*, 1-hydroxybenzotriazole; *DCC*, dicyclohexylcarbodiimide; *DCHA*, dicychlohexylamine; *DMF*, dimethylformamide; *Me*OH, methanol; *THF*, tetrahydrofuran; *TEA*, triethylamine; tlc, thin layer chromatography; hptlc, high performance thin layer chromatography; hplc, high performance liquid chromatography.

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Introduction

As discussed in the preceding communication [1] the main goal of the present synthetic study is the immunological identification of the hypothetical protein F154 encoded in the DNA of the virus TTV1 [2]. Our rational for selecting appropriate peptides for immunization experiments is based on the characteristic sequence repeats of this protein; conjugation of the corresponding synthetic peptides with appropriate carriers [3] could lead to antisera capable of crossreacting with the native protein if it is expressed in the virus/archaebacterium system. The present communication deals with the synthesis of the second characteristic sequence repeat of the hypothetical protein F-154 (see Scheme 1) corresponding to the sequence portions 85–95 and 116–126, in the latter case with one conservative mutation in position 123 (Ile for Val). Because of its hydrophilicity, this repeat may represent loops exposed on the protein surface and thus suitable immunepitops. For the present study we have selected the sequence portion 85–95; to possibly stabilize a native conformation of this undecapeptide sequence and to mimic its location in the native protein, it was flanked at the N- and C-terminus by two hexapeptide sequences of the poly-(Thr(Ser)-Pro)-repeats. Thus, the protein fragment chosen for our purpose corresponds to the sequence portion 79–101.

Scheme 1. Amino acid sequence of the hypothetical protein F-154 from TTV 1 virus as predicted by nucleotide sequence analysis (cf. Ref. [2]). Poly(Thr-Pro)-repeats (full line) and the undecapeptide repeats (dotted line) are specially indicated

1	Met-Tyr-Leu-Ser-Ile-Asn-Gly-Ser-Thr-Ala-Asn-Val-Lys-Val-Tyr-Lys-Gln-Gly-Ser-Asn-	20
21	Ile-Gly-Thr-Val-Ser-Gly-Asn-Tyr-Ser-Thr-Thr-Pro-Tyr-Gly-Asn-Pro-Ser-Met-Ala-Gly-	40
41	Tyr-Gly-Thr-Val-Asp-Lys-His-Tyr-Ala-Asn-Phe-Ile-Val-Leu-Pro-Tyr-Glu-Pro-Asp-Pro-	60
61	Gln-Val-Thr-Val-Thr-Pro-Ile-Ser-Ser-Pro-Ser-Pro-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Pro-	80
81	Thr-Pro-Thr-Pro-Thr-Tyr-Asp-Ile-Thr-Tyr-Val-Val-Phe-Asp-Val-Thr-Pro-Ser-Pro-Thr-	100
101	Pro-Thr-Pro-Thr-Leu-Thr-Ser-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Tyr-Asp-Ile-Thr-	120
121	Tyr-Val-Ile-Phe-Asp-Val-Thr-Pro-Ser-Pro-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Pro-	140
141	Thr-Pro-Thr-Pro-Thr-Pro-Thr-Ser-Thr-Thr-Ser-Ser-Asn-Ile	

Results and Discussion

For the synthesis of the trieicosapeptide, we have applied the Schwyzer-Wünsch strategy, i.e.

(i) acid labile groups on *tert*-butanol basis for the permanent protection in combination with the benzyloxycarbonyl and the 2-nitrophenylsulfenyl-derivatives for α-amino protection in the intermediate chain elongation steps; whenever possible also the *tert*-butyloxycarbonyl group was applied for temporary protections;

(ii) the fragment condensation procedure based on the preparation of suitably protected segments, in the present case corresponding to the sequences 79–84,

85–88, 89–93, 94–95, and 96–101, respectively, followed by their stepwise assembly from the C-terminal end to the fully protected trieicosapeptide;

(iii) the final acidolytic deprotection followed by purification via chromatographic techniques.

Synthesis of the Fragments

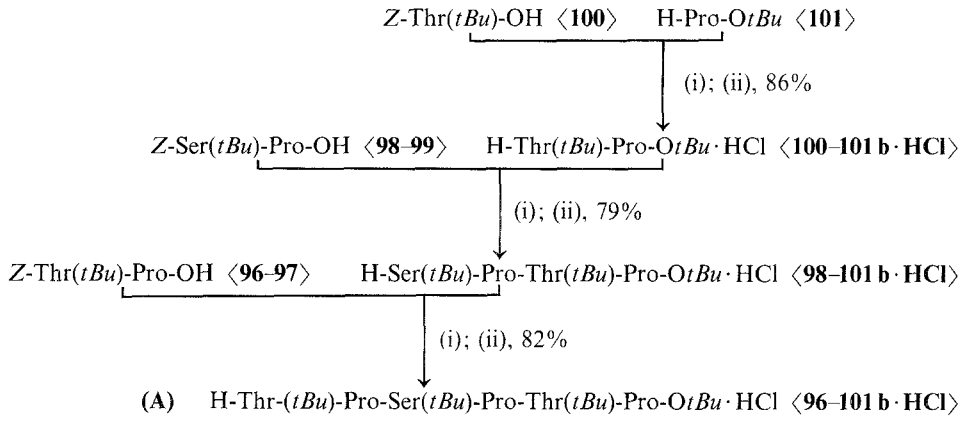
Fragment A (Sequence 96–101)

Following Scheme 2 H-Pro-O*tBu* ⟨101⟩ was coupled with *Z*-Thr(*tBu*)-OH ⟨100⟩ via *DCC*/HO*Bt* to yield the dipeptide derivative ⟨100–101 a⟩; subsequent hydrogenolysis led to the amine-free ⟨100–101 b⟩ which was isolated as hydrochloride; further elongation of the peptide chain with the dipeptide derivatives *Z*-Ser(*tBu*)-Pro-OH ⟨98–99⟩ and *Z*-Thr(*tBu*)-Pro-OH ⟨96–97⟩ respectively, and hydrogenolytic debenzyloxycarbonylation of the intermediate derivatives ⟨98–101 a⟩ and ⟨96–101 a⟩ produced the fragment A, i.e. H-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨96–101 b⟩, in 56% yield over the 6 steps.

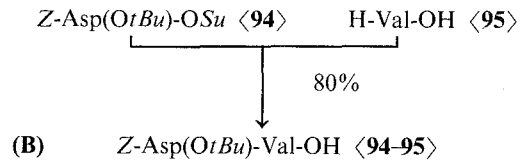
Fragment B (Sequence 94–95)

The dipeptide derivative *Z*-Asp(O*tBu*)-Val-OH was isolated in 80% yield as dicyclohexylamine salt upon acylating H-Val-OH ⟨95⟩ with *Z*-Asp(O*tBu*)-O*Su* ⟨94⟩ (Scheme 3).

Scheme 2. Synthesis of fragment A; reagents used: (i) *DCC*/1-hydroxybenzotriazole; (ii) H_2/Pd, titration with HCl



Scheme 3. Synthesis of fragment B



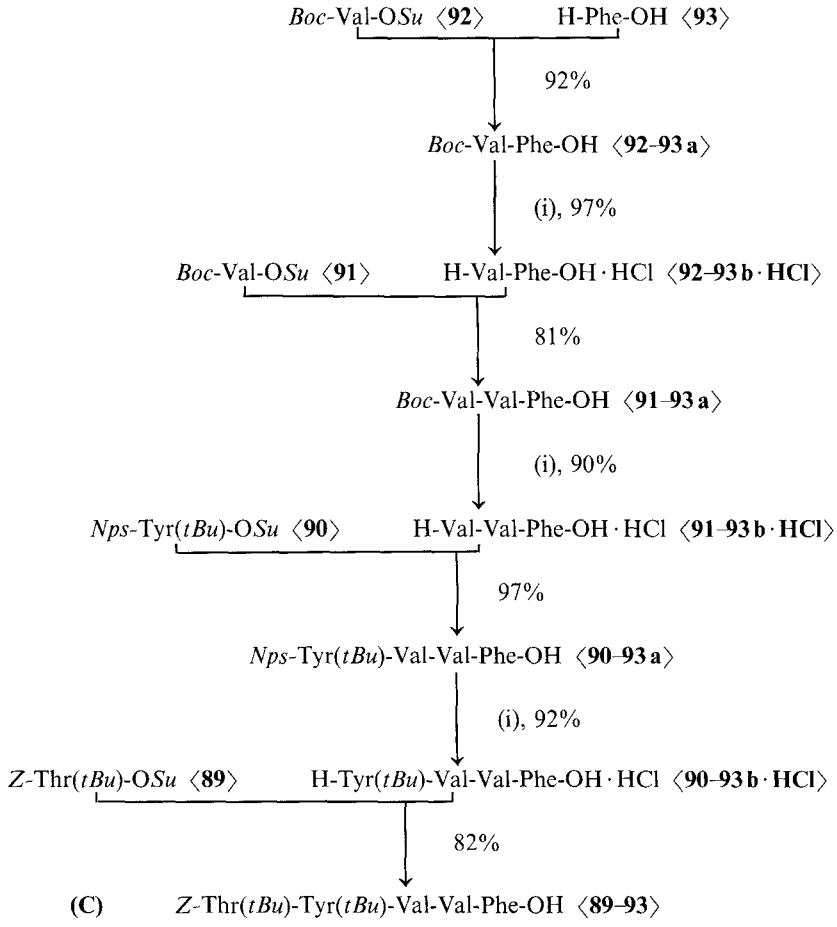
Fragment C (Sequence 89–93)

The pentapeptide derivative ⟨89–93⟩ was synthesized in stepwise manner as outlined in Scheme 4 starting from the C-terminal H-Phe-OH ⟨93⟩ and using *Boc*-Val-O*Su* (positions 92 and 91), *Nps*-Tyr(*tBu*)-O*Su* (position 90) and *Z*-Thr(*tBu*)-O*Su* (position 89) as acylating agents. Intermediate N^{α}-deprotection of both the *tert*-butyloxycarbonyl and 2-nitrophenylsulfenyl derivatives proceeded quantitatively by exposure to hydrogen chloride. The use of the 2-nitrophenylsulfenyl group at the level of the tetrapeptide derivative ⟨90–93 a⟩ was necessary since the corresponding benzyloxycarbonyl-compound was found to be only sparingly soluble, thus seriously impeding hydrogenolytic N^{α}-deprotection. Fragment C, i.e. *Z*-Thr(*tBu*)-Tyr(*tBu*)-Val-Val-Phe-OH ⟨89–93⟩ was obtained in 48% yield over the 7 steps.

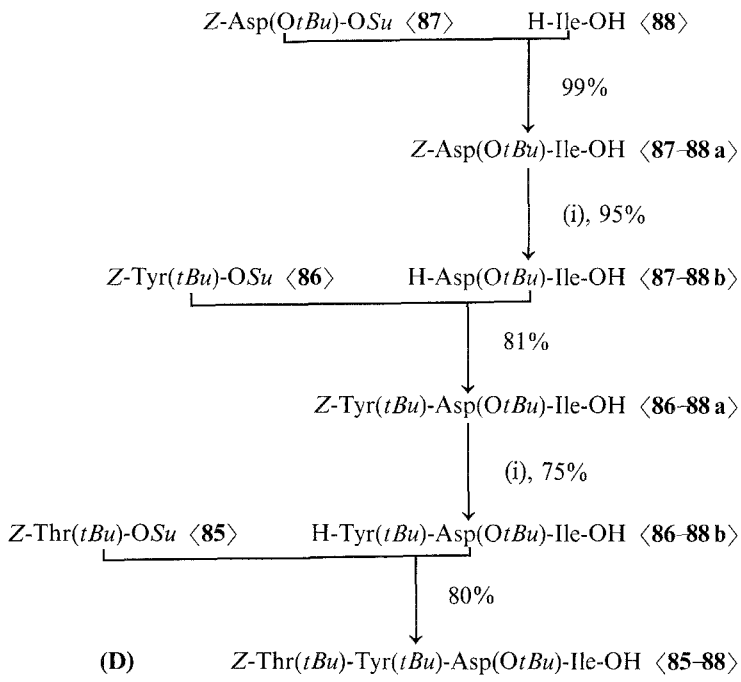
Fragment D (Sequence 85–88)

Starting from the C-terminal H-Ile-OH ⟨88⟩, the tetrapeptide derivative ⟨85–88⟩ was again synthesized in stepwise manner following Scheme 5 and using *Z*-Asp(O*tBu*)-O*Su* ⟨87⟩, *Z*-Tyr(*tBu*)-O*Su* ⟨86⟩, and *Z*-Thr(*tBu*)-O*Su* ⟨85⟩ in the

Scheme 4. Synthesis of fragment C; reagents used: (i) HCl in dioxane



Scheme 5. Synthesis of fragment D; reagents used: (i) H_2/Pd



acylation steps, whereby intermediate catalytic hydrogenation served to remove the N^{α}-benzyloxycarbonyl group. *Z*-Thr(*tBu*)-Tyr(*tBu*)-Asp(O*tBu*)-Ile-OH ⟨85–88⟩ was obtained in 46% yield over the 5 steps.

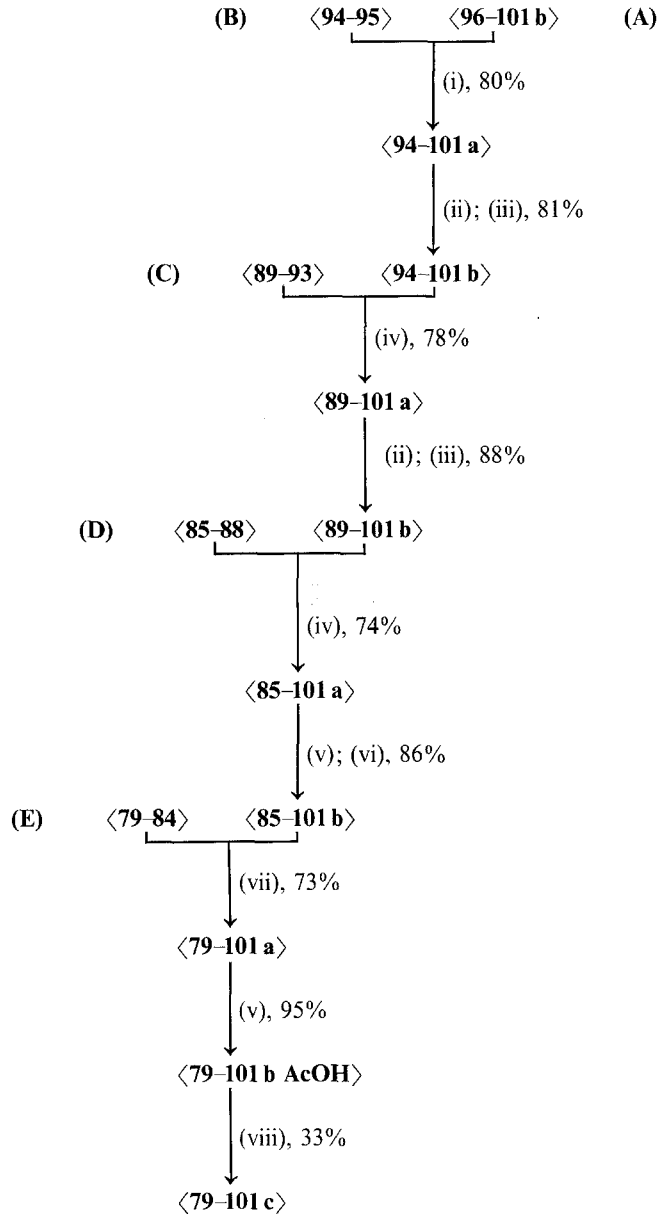
Fragment E (Sequence 79–84)

The hexapeptide derivative *Z*-Thr(*tBu*)-Pro-Thr(*tBu*)-Pro-Thr(*tBu*)-Pro-OH ⟨79–84⟩ was synthesized as described in the preceeding communication [1].

Synthesis of the Trieicosapeptide (Sequence 79–101)

The fragments were assembled in stepwise manner, starting from the C-terminal fragment A and using both the *DCC*/HO*Su* and *DCC*/HO*Bt* condensation procedure as shown in Scheme 6. Only for coupling the fragments A and B the mixed anhydride method proved to be more suitable in terms of yields. Intermediate N^{α}-deprotection proceeded smoothly by catalytic hydrogenolysis, whereby serious solubility problems were not encountered, thus ensuring high average yields in the single acylation and deprotection steps. The fully protected trieicosapeptide ⟨79–101⟩ was deblocked at its N^{α}-amino function by hydrogenolysis in acetic acid and then exposed to treatment with 95% aqueous trifluoroacetic acid containing 0.5% 1,2-ethanedithiol. Upon gel filtration of the resulting crude deprotection product, the desired trieicosapeptide, i.e. H-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Tyr-Asp-Ile-Thr-Tyr-Val-Val-Phe-Asp-Val-Thr-Pro-Ser-Pro-Thr-Pro-OH ⟨79–101 c⟩ was obtained in satisfactory yield as highly pure product as judged by various indicative analytical assays.

Scheme 6. Assembly of fragments; reagents used: (i) isobutylchloroformate (M.A.); (ii) H_2/Pd, titration with HCl; (iii) $NaHCO_3$ (extraction); (iv) *DCC*/N-hydroxysuccinimide; (v) H_2/Pd, acetic acid; (vi) diisopropylethylamine; (vii) *DCC*/1-hydroxybenzotriazole; (viii) 95% trifluoroacetic acid followed by gel chromatography



The detection of 4–5% *D-allo*-isoleucine in the amino acid analysis of the acid hydrolysate and by the gas chromatographic racemization test is due to hydrolysis dependent epimerization as suggested by the following observations: (i) Condensation of fragment D, ⟨85–88⟩ with the C-terminal segment ⟨89–101 b⟩ via *DCC*/HO*Su* and via the mixed anhydride method led to the identical compound in terms of *D-allo*-isoleucine content upon acid hydrolysis, although the mixed

anhydride procedure is known to enhance racemization of C-terminal amino acid residues in fragment condensation steps; (ii) the value of *D-allo*-isoleucine remained constant during the various synthetic steps up to the final product; diasterioisomeric *D-allo*-Ile-trieicosapeptide could not be detected on hptlc and hplc and could not be enriched in single fractions of the peptide peak in the gel filtration; (iii) aminopeptidase M digestion of the trieicosapeptide led to a recovery (79%) identical to the peptide content determined by acid hydrolysis (78%) within the limits of error of the amino acid analyses.

Acknowledgements

The authors thank Mrs. A. Bernert, Mrs. R. Scharf, Mrs. B. Guth, and Mrs. E. Weyher-Stingl for their skillful assistance. Elemental analyses were carried out in the microanalytical laboratory (Dr. E. Jaeger).

Experimental

For materials and methods see Ref. [1]. For tlc the following solvent systems were used: (1) ethyl acetate/1-butanol/acetic acid/water, 5:3:1:1; (2) 1-butanol/acetic acid/water, 3:1:1; (3) ethyl acetate/1-butanol/water/pyridine/acetic acid, 50:27:11:9:3; (4) 1-butanol/water/pyridine/acetic acid, 55:22:18:5; (5) *n*-heptane/*tert*-butanol/acetic acid, 3:2:1; (6) *n*-heptane/*tert*-butanol/acetic acid, 5:1:1; (7) cyclohexane/chloroform/acetic acid, 45:45:10; (8) dichloromethane/ethyl acetate/*Me*OH, 3:2:1; (9) chloroform/trifluoroethanol/propionic acid/water, 46:22:22:10; (10) chloroform/trifluoroethanol/80% propionic acid, 76:16:8; (11) cyclohexane/chloroform/acetic acid/trifluoroethanol, 40:39:20:1; (12) chloroform.

Fragment A

H-Thr(*tBu*)-Pro-O*tBu*·HCl ⟨100–101 b·HCl⟩

To a chilled mixture of *Z*-Thr(*tBu*)-OH·*DCHA* ⟨100·*DCHA*⟩ (49.0 g, 0.1 mol), H-Pro-O*tBu*·HCl ⟨101·HCl⟩ (20.8 g, 0.1 mol), and HO*Bt* (13.5 g, 0.1 mol) in *DMF* (870 ml) *DCC* (20.6 g, 0.1 mol) in *DMF* (100 ml) was added. After 20 h stirring at room temperature the precipitate was filtered off, the bulk of *DMF* was evaporated and the residue was distributed between 0.1 *M* $KHSO_4$ and ethyl acetate. The organic layer was washed twice with 0.1 *M* $KHSO_4$, water, 0.5 *M* Na_2CO_3, 0.5 *M* $NaHCO_3$, and water and dried over Na_2SO_4. The filtrate was evaporated to dryness and the oily residue, i.e. *Z*-Thr(*tBu*)-Pro-O*tBu* ⟨100–101 a⟩ (homogeneous in tlc: solvent systems 8 and 12) was hydrogenated over Pd/C in *Me*OH-water (4:1 *v/v*, 750 ml) under titrimetric addition of 1 *M* HCl at *pH* 4.0. The catalyst was removed by filtration, the filtrate evaporated to dryness, and the residue recrystallized from ether. Yield: 32.2 g (86%), m.p. 136–139°C; $[\alpha]^{20}_D = -62.60°$ and $[\alpha]^{20}_{546} = -74.60°$ (*c* 1.5, ethanol); tlc: 1, 5; gas chromatographic racemization test: *D-allo*-Thr 0.5%, *D*-Pro 0.8%. Anal. calcd. for $C_{17}H_{32}N_2O_4 \cdot HCl \cdot 0.5\,H_2O$ (373.91): C 54.60, H 9.17, N 7.49, Cl 9.48; found: C 54.03, H 9.33, N 7.31, Cl 10.02.

H-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu*·HCl ⟨98–101 b·HCl⟩

To a solution of ⟨100–101 b·HCl⟩ (30.8 g, 83.4 mmol), *Z*-Ser(*tBu*)-Pro-OH ⟨98–99⟩ [1] (33.4 g, 85.2 mmol) in *DMF* (500 ml) *TEA* (12 ml, 87 mmol) and HO*Bt* (11.5 g, 85.1 mmol) were added followed by *DCC* (17.6 g, 85.4 mmol) in *DMF* (100 ml) at 0°C. After 40 h stirring at room temperature the bulk of

the solvent was evaporated, the residue was taken up in ethyl acetate (400 ml) and insoluble dicyclohexylurea was filtered off. The solution was worked up as described for ⟨100–101 a⟩. The resulting amorphous material, i.e. *Z*-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨98–101 a⟩ (homogeneous according to tlc: 6, 8) was hydrogenated in *Me*OH-water (4 : 1 *v/v*, 1 l) over Pd/C and worked up as described for ⟨100–101 b · HCl⟩. The product was crystallized from ether. Yield: 39.9 g (79%), m.p. 164–165°C; $[\alpha]^{20}_{D} = -76.86°$ and $[\alpha]^{20}_{546} = -91.82°$ (*c* 1, ethanol); tlc: 1, 3, 5; amino acid analysis (24 h, 6 *M* HCl): Thr 1.04 (1), Ser 1.05 (1), Pro 1.91 (2); gas chromatographic racemization test: *D-allo*-Thr 0.6%, *D*-Ser < 0.2%, *D*-Pro 0.3%. Anal. calcd. for $C_{29}H_{52}N_4O_7 \cdot HCl$ (605.20): C 57.55, H 8.83, N 9.26, Cl 5.86; found: C 56.25, H 8.68, N 8.91, C 5.77.

H-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* · HCl ⟨96–101 b · HCl⟩

⟨98–101 b · HCl⟩ (39.0 g, 64.4 mmol), *Z*-Thr(*tBu*)-Pro-OH ⟨96–97⟩ [1] (26.2 g, 64.4 mmol) and *TEA* (8.90 ml, 64.4 mmol) were reacted with HO*Bt* (8.7 g, 64.4 mmol) and *DCC* (13.8 g, 67 mmol) in *DMF* (300 ml). The reaction mixture was worked up as described for ⟨100–101 a⟩ and the resulting product was precipitated from *Me*OH with water. The oily product, i.e. *Z*-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨96–101 a⟩ (homogeneous in tlc: 6, 8) was hydrogenated over Pd/C in *Me*OH-water (4 : 1 *v/v*, 600 ml) as described for ⟨100–101 b⟩. The crude product was reprecipitated from ether-petroleum ether. Yield: 47.0 g (82%), m.p. 138–141°C; $[\alpha]^{20}_{D} = -87.39°$ and $[\alpha]^{20}_{546} = -104.79°$ (*c* 2, ethanol); tlc: 1, 3; amino acid analysis (24 h, 6 *M* HCl): Thr 2.08 (2), Ser 1.08 (1), Pro 2.84 (3); gas chromatographic racemization test: *D-allo*-Thr 0.8%, *D*-Ser < 0.2%, *D*-Pro 0.5%. Anal. calcd. for $C_{42}H_{74}N_6O_{10} \cdot HCl \cdot 1.5\,H_2O$ (886.57): C 56.90, H 8.41, N 9.48, Cl 4.00; found: C 57.06, H 8.59, N 9.15, Cl 4.04.

Fragment B

Z-Asp(O*tBu*)-Val-OH · *DCHA* ⟨94–95 · *DCHA*⟩

H-Val-OH ⟨95⟩ (23.4 g, 0.2 mol) in 1 *M* NaOH (200 ml)/dioxane (200 ml) was reacted in an ice bath with *Z*-Asp(O*tBu*)-O*Su* ⟨94⟩ (42.0 g, 0.1 mol) in dioxane (300 ml). After 24 h the bulk of dioxane was evaporated and the residue was distributed between ethyl acetate and 0.1 *M* $KHSO_4$. The organic layer was washed with water, dried over Na_2SO_4, and concentrated to a small volume. The product was obtained upon addition of *DCHA* (18.1 g, 0.1 mol) and recrystallization from ethanol-water (1 : 1 *v/v*). Yield: 48.1 g (80%); $[\alpha]^{20}_{D} = -9.42°$ and $[\alpha]^{20}_{546} = -11.48°$ (*c* 1, ethanol); tlc: 7; gas chromatographic racemization test: *D*-Val 1.1%, *D*-Asp 1.2%. Anal. calcd. for $C_{33}H_{53}N_3O_7$ (603.78): C 65.64, H 8.85, N 6.96; found: C 65.34, H 8.79, N 6.83.

Fragment C

Boc-Val-Phe-OH ⟨92–93 a⟩

H-Phe-OH ⟨93⟩ (16.5 g, 0.1 mol) in 1 *M* NaOH (100 ml) was combined with a solution of *Boc*-Val-O*Su* ⟨92⟩ (15.7 g, 0.05 mol) in dioxane (100 ml). After 24 h at room temperature the reaction mixture was worked up as described for ⟨94–95⟩ and the residue was recrystallized from ethyl acetate-petroleum ether. Yield: 16.8 g (92%); m.p. 137–139°C; $[\alpha]^{20}_{D} = +13.7°$ and $[\alpha]^{20}_{546} = +16.2°$ (*c* 1, *Me*OH); tlc: 1, 5, 6; amino acid analysis (48 h, 6 *M* HCl): Val 0.99 (1), Phe 1.01 (1); gas chromatographic racemization test: *D*-Val 0.2%, *D*-Phe 0.8%. Anal. calcd. for $C_{19}H_{28}N_2O_5$ (364.43): C 62.62, H 7.74, N 7.69; found: C 62.60, H 7.70, N 7.65.

H-Val-Phe-OH · HCl ⟨92–93 b · HCl⟩

⟨92–93 a⟩ (16.7 g, 0.046 mol) was treated with 1 *M* HCl in dioxane (200 ml) for 3 h at room temperature. The reaction mixture was concentrated and the precipitate formed upon addition of ethyl acetate was

recrystallized from 2-propanol-diisopropylether. Yield: 16.0 g (97%); m.p. 221–223°C; $[\alpha]^{20}_{D} = +33.9°$ and $[\alpha]^{20}_{546} = +41.1°$ (*c* 1, *Me*OH); tlc: 1, 3; Anal. calcd. for $C_{14}H_{20}N_2O_3 \cdot HCl \cdot C_3H_7OH$ (360.86): C 56.58, H 8.10, N 7.76, Cl 9.82; found: C 56.30, H 8.03, N 7.78, Cl 9.81.

Boc-Val-Val-Phe-OH ⟨91–93 a⟩

To a chilled solution of ⟨92–93 b · HCl⟩ (12.2 g, 0.034 mol) in N-methylpyrrolidone (250 ml), *TEA* (9.4 ml, 0.068 mol) and *Boc*-Val-O*Su* ⟨91⟩ (15.9 g, 0.051 mol) in N-methylpyrrolidone (50 ml) were added. After 48 h at room temperature the solvent was evaporated and the residue recrystallized from ethanol-ethyl acetate. Yield: 13.0 g (81%); $[\alpha]^{20}_{D} = -32.3°$ and $[\alpha]^{20}_{546} = -38.5°$ (*c* 1, *Me*OH); tlc: 5, 6; amino acid analysis (48 h, 6 *M* HCl): Val 1.12 (2), Phe 1.00 (1); gas chromatographic racemization test: *D*-Val 2.0%, *D*-Phe 0.9%. Anal. calcd. for $C_{24}H_{37}N_3O_6$ (463.57): C 62.18, H 8.05, N 9.07; found: C 61.47, H 7.94, N 8.96.

H-Val-Val-Phe-OH · HCl ⟨91–93 b · HCl⟩

Upon reaction of ⟨91–93 a⟩ (16.6 g, 0.035 mol) with 2 *M* HCl in dioxane (160 ml) for 12 h at room temperature the solution was concentrated and the product precipitated with ether and recrystallized from 2-propanol-ether. Yield: 14.6 g (90%); m.p. 252°C (dec.); $[\alpha]^{20}_{D} = +8.3°$ and $[\alpha]^{20}_{546} = +10.5°$ (*c* 1, 80% acetic acid); tlc: 1, 3; Anal. calcd. for $C_{19}H_{29}N_3O_4 \cdot HCl \cdot C_3H_7OH$ (460.00): C 57.44, H 8.33, N 9.14, Cl 7.71; found: C 56.53, H 8.09, N 9.02, Cl 8.17.

Nps-Tyr(*tBu*)-Val-Val-Phe-OH ⟨90–93 a⟩

To a cold stirred solution of ⟨91–93 b · HCl⟩ (10.0 g, 0.022 mol) in N-methylpyrrolidone (300 ml) *TEA* (6.0 ml, 0.043 mol) and *Nps*-Tyr(*tBu*)-O*Su* ⟨90⟩ (12.5 g, 0.026 mol) in N-methylpyrrolidone (50 ml) were added. After 24 h at room temperature the reaction mixture was worked up as described for ⟨94–95⟩; then the product was recrystallized from ethyl acetate. Yield: 15.6 g (97%); m.p. 117–118°C; $[\alpha]^{20}_{D} = +16.3°$ and $[\alpha]^{20}_{546} = +36.2°$ (*c* 1, *Me*OH); tlc: 3, 6; amino acid analysis (48 h, 6 *M* HCl): Val 1.60 (2), Tyr 0.99 (1), Phe 1.0 (1); gas chromatographic racemization test: *D*-Val 0.8%, *D*-Phe 0.6%, *D*-Tyr < 0.5%. Anal. calcd. for $C_{38}H_{49}N_5O_8S$ (735.89): C 62.02, H 6.71, N 9.52, S 4.36; found: C 61.47, H 6.80, N 9.30, S 4.27.

H-Tyr(*tBu*)-Val-Val-Phe-OH · HCl ⟨90–93 b · HCl⟩

A chilled solution of ⟨90–93 a⟩ (21.7 g, 0.029 mol) in *DMF* (220 ml) was treated with HCl (0.065 mol) in dioxane (240 ml). After 2 h at room temperature the reaction mixture was concentrated to small volume and on addition of ether the product was filtered off and reprecipitated from *DMF*-ether. Yield: 17.0 g (92%); $[\alpha]^{20}_{D} = -10.0°$ and $[\alpha]^{20}_{546} = -11.9°$ (*c* 1, trifluoroethanol); Anal. calcd. for $C_{32}H_{46}N_4O_6 \cdot HCl \cdot H_2O$ (637.22): C 60.31, H 7.75, N 8.79, Cl 5.56; found: C 60.33, H 7.55, N 8.54, Cl 5.35.

Z-Thr(*tBu*)-Tyr(*tBu*)-Val-Val-Phe-OH ⟨89–93⟩

Reaction of ⟨90–93 b · HCl⟩ (16.7 g, 0.026 mol) with *TEA* (7.3 ml, 0.052 mol) and *Z*-Thr(*tBu*)-O*Su* ⟨89⟩ (12.8 g, 0.032 mol) in N-methylpyrrolidinone (420 ml) was allowed to proceed for 24 h at room temperature. The solvent was evaporated and the residue was recrystallized from 2-propanol and then reprecipitated from *DMF* with water. Yield: 18.7 g (82%); m.p. 212–214°C; $[\alpha]^{20}_{D} = -16.7°$ and $[\alpha]^{20}_{546} = -19.9°$ (*c* 1, trifluoroethanol); tlc: 6, 10; amino acid analysis (3 h, propionic acid/HCl 1 : 1 *v/v*, 130°C): Thr 1.03 (1), Val 1.59 (2), Tyr 0.96 (1), Phe 1.01 (1) uncomplete hydrolysis of Val-Val; gas chromatographic racemization test: *D*-Val 1.1%, *D-allo*-Thr 0.4%, *D*-Phe 0.7%, *D*-Tyr 0.3%. Anal. calcd. for $C_{48}H_{67}N_5O_{10}$ (874.07): C 65.95, H 7.73, N 8.01; found: C 65.14, H 7.60, N 7.93.

Fragment D

Z-Asp(O*tBu*)-Ile-OH · *DCHA* ⟨87–88 a · *DCHA*⟩

H-Ile-OH ⟨88⟩ (26.2 g, 0.2 mol) in 1 *M* NaOH (200 ml)/dioxane (100 ml) was reacted with *Z*-Asp(O*tBu*)-O*Su* ⟨87⟩ (42.0 g, 0.1 mol) and worked up as described for ⟨94–95 · *DCHA*⟩ using 1 equivalent of *DCHA*. Yield 61.0 g (99%), m.p. 160–161°C; $[\alpha]^{20}_{D} = -7.14°$ and $[\alpha]^{20}_{546} = -8.43°$ (*c* 1, *Me*OH); tlc: 3, 7; gas chromatographic racemization test: *D-allo*-Ile 0.2%, *D*-Asp 2%. Anal. calcd. for $C_{34}H_{55}N_3O_7$ (617.81): C 66.10, H 8.97, N 6.80; found: C 66.23, H 8.83, N 6.79.

H-Asp(O*tBu*)-Ile-OH ⟨87–88 b⟩

Z-Asp(O*tBu*)-Ile-OH · *DCHA* ⟨87–88 a · *DCHA*⟩ (35.2 g, 57 mmol) was distributed between ethyl acetate (400 ml) and 1 *M* sulfuric acid, the organic layer was washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue was hydrogenated over Pd/C in *Me*OH-water (4 : 1 *v/v*). The filtrate was evaporated and the product precipitated with ethyl acetate. Yield: 16.4 g (95%); m.p. 235°C; $[\alpha]^{20}_{D} = +15.88°$ and $[\alpha]^{20}_{546} = +19.30°$ (*c* 1, *Me*OH); tlc: 1, 2, 4. Anal. calcd. for $C_{14}H_{26}N_2O_5$ (302.37): C 55.61, H 8.67, N 9.27; found: C 55.30, H 8.85, N 9.27.

Z-Tyr(*tBu*)-Asp(O*tBu*)-Ile-OH ⟨86–88 a⟩

To a chilled solution of ⟨87–88 b⟩ (15.1 g, 50 mmol) in 1 *M* NaOH (50 ml) and dioxane (50 ml) *Z*-Tyr(*tBu*)-O*Su* ⟨86⟩ (23.4 g, 50 mmol) in dioxane (70 ml) was added followed by $NaHCO_3$ (4.2 g, 50 mmol). The reaction mixture was stirred for 4 h and worked up as described for ⟨94–95⟩; the product was precipitated from ether with hexane. Yield: 26.5 g (81%), m.p. 107–110°C; $[\alpha]^{20}_{D} = -4.75°$ and $[\alpha]^{20}_{546} = -5.85°$ (*c* 1, dioxane); tlc: 6, 8; amino acid analysis (24 h, 6 *M* HCl): Asp 0.99 (1), Ile 0.98 (1), Tyr 1.02 (1); gas chromatographic racemization test: *D-allo*-Ile 0.3%, *D*-Asp 2.3%, *D*-Tyr 0.4%. Anal. calcd. for $C_{35}H_{49}N_3O_9$ (655.77): C 64.10, H 7.53, N 6.41; found: C 63.14, H 7.24, N 6.15.

H-Tyr(*tBu*)-Asp(O*tBu*)-Ile-OH ⟨86–88 b⟩

⟨86–88 a⟩ (26 g, 39.6 mmol) was hydrogenated over Pd/C in *Me*OH/water (4 : 1 *v/v*, 1.2 l). The catalyst was removed by filtration upon addition of warm *Me*OH (400 ml) and the filtrate was evaporated to dryness. The product was precipitated from *Me*OH with ether and recrystallized from *Me*OH. Yield: 16.1 g (75%), m.p. 197–198°C (dec.); $[\alpha]^{20}_{D} = +35.9°$ and $[\alpha]^{20}_{546} = +43.0°$ (*c* 1, acetic acid); tlc: 1, 5. Anal. calcd. for $C_{27}H_{43}N_3O_7 \cdot H_2O$ (539.66): C 60.08, H 8.41, N 7.79; found: C 60.22, H 8.43, N 7.75.

Z-Thr(*tBu*)-Tyr(*tBu*)-Asp(O*tBu*)-Ile-OH ⟨85–88 a⟩

⟨86–88 b⟩ (15.65 g, 29 mmol) in 0.5 *M* NaOH (58 ml) and dioxane (200 ml) was reacted with *Z*-Thr(*tBu*)-O*Su* ⟨85⟩ (12.2 g, 30 mmol) in dioxane (100 ml) upon addition of $NaHCO_3$ (2.5 g, 30 mmol). After 48 h the bulk of dioxane was evaporated and the aqueous solution was acidified with 0.2 *M* $KHSO_4$. The product was extracted with ethyl acetate and the combined organic layers were washed twice with 0.2 *M* Na_2CO_3, 0.2 *M* $KHSO_4$ and water and were dried over Na_2SO_4. After evaporation of the solvent the residue was precipitated twice from ether with petroleum ether. Yield: 19.0 g (80%); m.p. 88–92°C; tlc: 6, 7; amino acid analysis (24 h, 6 *M* HCl): Asp 1.00 (1), Thr 1.02 (1), Ile 0.98 (1), Tyr 0.94 (1); gas chromatographic racemization test: *D-allo*-Thr 0.5%, *D-allo*-Ile 0.6%, *D*-Asp 1.7%, *D*-Tyr 0.6%. Anal. calcd. for $C_{43}H_{64}N_4O_{11}$ (812.98): C 63.52, H 7.94, N 6.89; found: C 63.06, H 8.07, N 6.62.

Assembly of the Fragments

Z-Asp(O*tBu*)-Val-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨94–101 a⟩

⟨96–101 b · HCl⟩ (17.2 g, 19.4 mmol) was desalted by distribution between ethyl acetate and 0.5 *M* $NaHCO_3$. The organic layer was washed with water and evaporated to dryness. The residue was dissolved in chloroform (50 ml) and was cooled to − 20°C (solution A). *Z*-Asp(O*tBu*)-Val-OH ⟨94–95⟩ (8.5 g, 20.1 mmol)—obtained in the usual manner from the corresponding *DCHA* salt—and N-methylmorpholine (2.2 ml, 20 mmol) in tetrahydrofuran (200 ml) were reacted with isobutylchloroformate (2.63 ml, 20 mmol) for 1 min at − 20°C; then solution A was added. The reaction mixture was allowed to reach room temperature within 4 h; then the solvents were evaporated and the residue was distributed between water and ethyl acetate. The organic layer was washed in the usual manner, dried over Na_2SO_4 and evaporated to dryness. The residue was reprecipitated from ether with petroleum ether. Yield: 19.0 g (80%); m.p. 116–119°C; $[\alpha]^{20}_D = -80.66°$ and $[\alpha]^{20}_{546} = -96.91°$ (*c* 1.4, ethanol); tlc: 6; amino acid analysis (24 h, 6 *M* HCl): Asp 1.01 (1), Thr 1.96 (2), Ser 1.02 (1), Pro 3.01 (3), Val 0.99 (1); gas chromatographic racemization test: *D*-Val 1.4%, *D-allo*-Thr < 1%, *D*-Pro 0.4%, *D*-Ser < 0.2%, *D*-Asp 0.9%. Anal. calcd. for $C_{63}H_{102}N_8O_{16}$ (1 227.53): C 61.64, H 8.38, N 9.13; found: C 60.65, H 8.33, N 9.05.

H-Asp(O*tBu*)-Val-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨94–101 b⟩

⟨94–101 a⟩ (18.15 g, 14.8 mmol) was hydrogenated in *Me*OH-water (4 : 1 *v/v*, 1.2 l) and worked up as described for ⟨100–101 b · HCl⟩. The product was obtained as hydrochloride by precipitation from ether/2-propanol with petroleum ether. Yield: 15.8 g (92%), m.p. 160°C (dec.); $[\alpha]^{20}_D = -64.72°$ and $[\alpha]^{20}_{546} = -77.69°$ (*c* 1.5, ethanol); tlc: 1, 3, 10. Anal. calcd. for $C_{55}H_{96}N_8O_{14} \cdot HCl \cdot 2\,H_2O$ (1 165.93): C 56.65, H 8.64, N 9.61, Cl 3.04; found: C 55.59, H 8.43, N 9.40, Cl 3.11.

The compound ⟨94–101 b · HCl⟩ (12.0 g, 10.3 mmol) was desalted as described for ⟨96–101 b⟩ and isolated as crystalline compound from ether-petroleum ether. Yield: 9.9 g (88%), m.p. 125–129°C; $[\alpha]^{20}_D$ − 83.52° and $[\alpha]^{20}_{546} = -100.22°$ (*c* 2, ethanol); tlc: 1, 10, 11. Anal. calcd. for $C_{55}H_{96}N_8O_{14}$ (1 093.40): C 60.41, H 8.85, N 10.25; found: C 60.57, H 8.90, N 9.70.

Z-Thr(*tBu*)-Tyr(*tBu*)-Val-Val-Phe-Asp(O*tBu*)-Val-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨89–101 a⟩

⟨94–101 b⟩ (5.47 g, 5 mmol), ⟨89–93⟩ (4.20 g, 4.8 mmol) and HO*Su* (2.3 g, 20 mmol) were dissolved in *DMF* (75 ml) and reacted in an ice bath with *DCC* (1.05 g, 5.1 mmol) in *DMF* (25 ml). The reaction mixture was stirred for 1 h at 0°C and for additional 48 h at room temperature. Then water (5 ml) was added to the clear solution obtained after warming to 60°C and the precipitate formed upon cooling was collected and recrystallized from *Me*OH/water. Yield: 7.3 g (78%), m.p. 245°C (dec.); $[\alpha]^{20}_D = -50.68°$ and $[\alpha]^{20}_{546} = -60.79°$ (*c* 0.7, acetic acid); tlc: 6, 10, 11; amino acid analysis (48 h, 6 *M* HCl): Asp 1.04 (1), Thr 2.94 (3), Ser 1.02 (1), Pro 3.01 (3), Val 2.53 (3), Tyr 0.94 (1), Phe 1.04 (1); uncomplete hydrolysis of the Val-Val sequence; gas chromatographic racemization test: *D*-Val 1.7%, *D-allo*-Thr 1.1%, *D*-Pro 0.5%, *D*-Ser 0.2%, *D*-Asp 1.6%, *D*-Phe 1.5%, *D*-Tyr 0.5%. Anal. calcd. for $C_{103}H_{161}N_{13}O_{23}$ (1 949.50): C 63.46, H 8.32, N 9.34; found: C 62.24, H 8.23, N 9.38.

H-Thr(*tBu*)-Tyr(*tBu*)-Val-Val-Phe-Asp(O*tBu*)-Val-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨89–101 b⟩

The tridecapeptide derivative ⟨89–101 a⟩ (7.15 g, 3.7 mmol) was suspended in *Me*OH-water (4 : 1 *v/v*, 1.7 l) and hydrogenated over Pd/C under titrimetric addition of 0.5 *M* HCl. After usual work up the residue was washed with ether and filtered off. Yield of ⟨89–101 b · HCl⟩: 6.45 g (95%), m.p. 230°C

(dec.); $[\alpha]^{20}_D = -94.01°$ and $[\alpha]^{20}_{546} = -112.79°$ (*c* 1.7, ethanol); tlc: 10. Anal. calcd. for $C_{95}H_{155}N_{13}O_{21} \cdot HCl$ (1851.79): C 61.61, H 8.49, N 9.84, Cl 1.91; found: C 59.34, H 8.25, N 9.24, Cl 1.97. ⟨89–101 b · HCl⟩ (4.0 g, 2.16 mmol) was desalted by distribution between 1-butanol and 0.5 *M* $NaHCO_3$ and the crystalline product was obtained from 1-butanol upon addition of a large excess of ether-hexane (1 : 4 *v/v*). Yield: 3.8 g (93%); m.p. 220°C (dec.); $[\alpha]^{20}_D = -100.19°$ and $[\alpha]^{20}_{546} = -120.05°$ (*c* 1.5, ethanol). Anal. calcd. for $C_{95}H_{155}N_{13}O_{21} \cdot C_4H_{10}O$ (1889.44): C 62.93, H 8.80, N 9.63; found: C 62.82, H 8.70, N 9.53.

Z-Thr(*tBu*)-Tyr(*tBu*)-Asp(O*tBu*)-Ile-Thr(*tBu*)-Tyr(*tBu*)-Val-Val-Phe-Asp(O*tBu*)-Val-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨85–101 a⟩

⟨89–101 b⟩ (5.45 g, 3.0 mmol), ⟨85–88⟩ (2.68 g, 3.3 mmol), and HO*Su* (0.76 g, 6.6 mmol) were dissolved in *DMF* (45 ml) and reacted with *DCC* (0.68 g, 3.3 mmol) in *DMF* (10 ml) at −10°C. The reaction mixture was stirred in an ice bath for 6 h and at room temperature for additional 60 h; then *DMF* (50 ml) was added. The product was precipitated with water, collected by filtration and suspended in warm methanol/ethanol/water (7 : 7 : 1 *v/v*, 150 ml). The precipitate formed upon cooling was collected by centrifugation. Yield: 5.8 g (74%); $[\alpha]^{20}_D = -37.20°$ and $[\alpha]^{20}_{546} = -44.59°$ (*c* 0.8, acetic acid); tlc: 7, 11; amino acid analysis (48 h, 6 *M* HCl): Asp 1.97 (2), Thr 3.99 (4), Ser 1.07 (1), Pro 3.03 (3), Val 2.53 (3), Ile 0.91 (1), *D-allo*-Ile 0.04, Tyr 2.00 (2), Phe 1.12 (1); gas chromatographic racemization test: *D*-Val 1.2%, *D-allo*-Thr 0.7%, *D-allo*-Ile 4.0%, *D*-Pro < 1%, *D*-Ser 0.5%, *D*-Asp 0.9%, *D*-Phe 0.3%, *D*-Tyr 0.2%. Anal. calcd. for $C_{138}H_{217}N_{17}O_{31}$ (2610.3): C 63.49, H 8.38, N 9.12; found: C 62.53, H 8.29, N 8.97.

H-Thr(*tBu*)-Tyr(*tBu*)-Asp(O*tBu*)-Ile-Thr(*tBu*)-Tyr(*tBu*)-Val-Val-Phe-Asp(O*tBu*)-Val-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨85–101 b⟩

Compound ⟨85–101 a⟩ (2.8 g, 1.07 mmol) was hydrogenated over Pd/C in 80% aqueous acetic acid (250 ml). The reaction mixture was worked up in the usual manner; the resulting residue was suspended in *DMF*-water (17 : 1 *v/v*, 180 ml) and reacted with diisopropylethylamine (0.9 ml, 5.3 mmol). After 12 h the mixture was warmed up to 60° and water (200 ml) was added; the precipitate formed upon cooling was collected by filtration and washed with ether-hexane. Yield: 2.3 g (86%); $[\alpha]^{20}_D = -37.54°$ and $[\alpha]^{20}_{546}$ −44.74° (*c* 0.6, acetic acid); tlc: 10, 11. Anal. calcd. for $C_{130}H_{211}N_{17}O_{29} \cdot 2H_2O$ (2512.19): C 62.15, H 8.63, N 9.48; found: C 61.90, H 8.61, N 9.34.

Z-Thr(*tBu*)-Pro-Thr(*tBu*)-Pro-Thr(*tBu*)-Pro-Thr(*tBu*)-Tyr(*tBu*)-Asp(O*tBu*)-Ile-Thr(*tBu*)-Tyr(*tBu*)-Val-Val-Phe-Asp(O*tBu*)-Val-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* ⟨79–101 a⟩

To a suspension of the heptadecapeptide derivative ⟨85–101 b⟩ (1.9 g, 0.76 mmol) in *DMF*-dichloromethane (5 : 1 *v/v*, 300 ml) *Z*-Thr(*tBu*)-Pro-Thr(*tBu*)-Pro-Thr(*tBu*)-Pro-OH ⟨79–84⟩ (1.06 g, 1.15 mmol) [1], HO*Bt* (155 mg, 1.15 mmol), and *DCC* (237 mg, 1.15 mmol) were added. After stirring for 2 d at room temperature and additional 3 d under occasional heating to 50°C the product was precipitated by addition of water (50 ml) and filtered off. Upon reprecipitation from *DMF*/ethanol, followed by extensive washings with ether-petroleum ether the homogeneous product was obtained. Yield: 1.87 g (73%); $[\alpha]^{20}_D = -57.62°$ and $[\alpha]^{20}_{546} = -68.17°$ (*c* 0.6, acetic acid); tlc: 10, 11; amino acid analysis (48 h, 6 *M* HCl): Asp 2.04 (2), Thr 6.27 (7), Ser 1.07 (1), Pro 5.61 (6), Val 2.40 (3), Ile 0.99 (1), *D-allo*-Ile 0.04, Tyr 2.00 (2), Phe 1.05 (1); uncomplete hydrolysis of the Val-Val sequence; gas chromatographic racemization test: *D*-Val 1.0%, *D-allo*-Thr 0.6%, *D-allo*-Ile 5%, *D*-Pro 0.5%, *D*-Ser < 0.4%, *D*-Asp 0.5%, *D*-Phe < 0.5%, *D*-Tyr < 0.5%. Anal. calcd. for $C_{177}H_{283}N_{23}O_{40}$ (3373.4): C 63.02, H 8.46, N 9.55; found: C 62.37, H 8.38, N 9.29.

H-Thr(*tBu*)-Pro-Thr(*tBu*)-Pro-Thr(*tBu*)-Pro-Thr(*tBu*)-Tyr(*tBu*)-Asp(O*tBu*)-Ile-Thr(*tBu*)-Tyr(*tBu*)-Val-Val-Phe-Asp(O*tBu*)-Val-Thr(*tBu*)-Pro-Ser(*tBu*)-Pro-Thr(*tBu*)-Pro-O*tBu* · *Ac*OH
〈79–101 b · *Ac*OH〉

The trieicosapeptide derivative 〈79–101 a〉 (1.4 g, 0.41 mmol) was hydrogenated over Pd/C in 80% acetic acid (100 ml). The reaction mixture was worked up in usual manner, and the residue was precipitated from *DMF* with water. Yield: 1.3 g (95%); $[\alpha]^{20}_{D} = -58.51°$ and $[\alpha]^{20}_{546} = -67.28°$ (*c* 0.8, acetic acid); tlc: 10, 11. Anal. calcd. for $C_{169}H_{277}N_{23}O_{38} \cdot CH_3COOH$ (3299.19): C 62.24, H 8.59, N 9.77; found: C 62.66, H 8.49, N 9.44.

H-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Tyr-Asp-Ile-Thr-Tyr-Val-Val-Phe-Asp-Val-Thr-Pro-Ser-Pro-Thr-Pro-OH 〈79–101 c〉

〈79–101 b · *Ac*OH〉 (1.1 g, 0.33 mmol) was dissolved in trifluoroacetic acid (11 ml) containing water (0.22 ml), and 1,2-ethanedithiol (22 µl). After 2 h stirring at room temperature ether-ethyl acetate (10 : 1 *v/v*, 55 ml) was added, the precipitate was filtered off, washed with ether, and dried over KOH pellets.

The crude product was purified batchwise by gel filtration on Fractogel TSK HW-40S (Merck) (140 × 2.3 cm), eluting with 0.05 *M* ammonium acetate buffer (*pH* 6.05)/methanol (4 : 1 *v/v*) at a flow rate of 23 ml/h. Yield: 0.35 g (33%, based on the peptide content of 78%).

The product behaved homogeneously on hptlc (solvent systems: 4,9) and hplc [µ-Bondapak C18 (30 × 0.4 cm); eluent: acetonitrile/0.1 *M* sodium phosphate (*pH* 5.4); linear gradient elution from 12 to 35% acetonitrile in 40 min at a flow rate of 1 ml/min; detection: uv at 214 nm]; amino acid analysis (24 h, aminopeptidase M); Asp 1.99 (2), Thr 6.70 (7), Ser 1.03 (1), Pro 5.55 (6), Val 2.85 (3), Ile 1.00 (1), Tyr 2.14 (2), Phe 0.97 (1) (recovery: 79% calcd. for $M = 2509.8$); amino acid analysis (48 h, 6 *M* HCl): Asp 2.00 (2), Thr 6.56 (7), Ser 0.97 (1), Pro 5.69 (6), Val 2.48 (3), Ile 0.96 (1), *D-allo*-Ile 0.04, Tyr 1.98 (2), Phe 1.04 (1); uncomplete hydrolysis of the Val-Val dipeptide sequence (peptide content: 78%, calcd. for $M_r = 2509.8$); gas chromatographic racemization test: *D*-Val 1.2%, *D-allo*-Thr 1.2%, *D-allo*-Ile 3.7%, *D*-Pro 1%, *D*-Ser 1.1%, *D*-Asp 1.1%, *D*-Phe 0.6%, *D*-Tyr 0.3%.

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Received April 21, 1989. Accepted May 17, 1989

Verleger: Springer-Verlag KG, Mölkerbastei 5, A-1010 Wien. — Herausgeber: Österreichische Akademie der Wissenschaften, Dr.-Ignaz-Seipel-Platz 2, A-1010 Wien, und Gesellschaft Österreichischer Chemiker, Eschenbachgasse 9, A-1010 Wien. — Redaktion: Währinger Straße 38, A-1090 Wien. — Hersteller: Adolf Holzhausens Nachfolger, Kandlgasse 19-21, A-1070 Wien. — Verlagsort: Wien. — Herstellungsort: Wien. — Printed in Austria.