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Synthesis of the *N*-Trityl Hexapeptide Hydrazide Corresponding to the Sequence 152—157 of the Coat Protein of Tobacco Mosaic Virus. Comparison of the Homogeneous and the Solid Phase Syntheses

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The pentapeptide derivative benzyloxycarbonyl-threonyl-(benzyl) seryl-glycyl-prolyl-alanine methyl ester was synthesized in solution from alanine methyl ester and on a polymeric support from alanyl-resin (followed by transesterification) by stepwise chain elongation using dicyclohexylcarbodiimide and *tert*-butyloxycarbonyl amino acids. Threonine was introduced as benzyloxycarbonyl-threonine. The over-all yield was 24 % and 28 %, respectively, based on alanine, whereas the efficiency, based on the utilization of amino acids, was 28 % and 9 %, respectively. The products were shown to be identical. Finally, the deprotected pentapeptide methyl ester, obtained by catalytic hydrogenation, was condensed with trityl-tryptophan by the action of dicyclohexylcarbodiimide. The product, trityl-tryptophyl-threonyl-seryl-glycyl-prolyl-alanine methyl ester, was converted to the hydrazide by treatment with hydrazine hydrate in ethanol.

In the present synthesis of a fragment of the C-terminal part of the coat protein of Tobacco Mosaic Virus *(vulgare)* [1] (residues 152 – 157) a pentapeptide derivative, Z-Thr-Ser(Bzl)-Gly-Pro-Ala-OMe,* was used as intermediate. A comparison of its synthesis in solution and on a solid support using the Merrifield method [2] was made. A similar study has been reported for the sequence 81 – 85.[3] Experience with uncontrolled solid-phase synthesis indicates that in general, due to the accumulation of closely related products, only peptides, for which especially favourable isolation procedures are available, can be synthesized in a high state of purity. Cyclic peptides, like antamanid,[4] gramicidin S,[5] and valinomycin,[6] seem especially suited for solid-phase syn-

* Abbreviations used in the text: Z = benzyloxycarbonyl; Boc = *tert*-butyloxycarbonyl; Bzl = benzyl; DMF = dimethylformamide; DCC = dicyclohexylcarbodiimide.

thesis, presumably because cyclization and crystallization is an efficient purification. Unfortunately, very few free peptides can be purified by crystallization from organic solvents. However, in the preparation of protected peptides favourable crystallization properties are occasionally encountered.[7] In the rational synthesis of protected peptides for fragment condensation, the fully automated solid-phase synthesis [8] of such intermediates may be competitive with synthesis in solution. In the present case, an over-all yield of 28 % based on the C-terminal residue was obtained, compared with 24 % in solution. However, when comparing the syntheses on an economical basis, the considerable excess of protected amino acids used in the former method should be taken into account. Thus, by calculating the synthesis efficiency according to Rydon,[9] a figure of 9 % is obtained compared with 28 % in solution. Still, it should be emphasized that neither synthesis has been optimized.

EXPERIMENTAL

Melting points are uncorrected. Ascending thin-layer chromatography was performed on commercial plates (DC-Fertigplatten, Kieselgel F 254, E. Merck AG., Darmstadt). Solvent systems: S1 (chloroform/acetic acid/methanol by volume 90/5/5), S2 (2-butanol/formic acid/water by volume 75/15/10,) S3 (2-butanol/10 % aqueous NH_3 by volume 85/15), S4 (1-butanol/pyridine/acetic acid/water by volume 37/25/8/30), S5 (2-methyl-2-propanol/pyridine/heptane by volume 33/13/54), and S7 (1-butanol/acetone/diethylamine/water by volume 37/37/7/19). Chromatograms were visualized by spraying with *tert*-butyl hypochlorite, followed by *p*-tolidine/potassium iodide, or by spraying with N hydrogen chloride in acetic acid, followed by ninhydrin. Optical rotation was measured on a Perkin-Elmer model 141 photoelectric polarimeter (tube length 1 dm). The resin (Bio-beads S-X2, 200 – 400 mesh) was obtained from Bio-Rad Laboratories, Richmond, California, and was chloromethylated and esterified with the first amino acid, Boc-alanine, according to the general procedure of Merrifield.[10] Methylene chloride (May and Baker, Ltd. Dagenham, Essex) was stored over potassium carbonate and distilled before use. Acetic acid ("Pronalys" glacial acetic acid, May and Baker Ltd.) was used as such. Ethanol was commercial absolute ethanol. Boc-Gly and Boc-Pro were prepared according to Schnabel [11] whose procedure was also used to prepare Z-Thr at 10°C and pH 8.2. Boc-Ser(Bzl) was purchased from the "Reanal" factory of laboratory chemicals, Budapest. Amino acid analysis was carried out on a Beckman model 120C analyzer after hydrolysis in sealed tubes at 110°C in 6 N hydrochloric acid. Unless otherwise stated, the time of hydrolysis was 24 h. Samples were not dried to constant weight prior to amino acid analysis.

Synthesis in solution

Boc-L-Pro-L-Ala-OMe (I). L-Alanine methyl ester hydrochloride (41.7 g, 300 mmol) was dissolved in DMF (56 ml) and neutralized by addition of triethylamine (40 ml). During the addition, the mixture was diluted with methylene chloride (100 ml) to keep it from solidifying. Excess of volatile base was detected over the liquid surface with the aid of moist indicator paper (Neutralit, Merck). The excess was removed by addition of more L-alanine methyl ester hydrochloride (*ca.* 1 g). After further dilution with methylene chloride (100 ml), a solution of Boc-L-Pro (64.5 g, 300 mmol) in methylene chloride (250 ml) was added, and the mixture cooled to 5°C. After addition of a solution of DCC, Merck, (62.0 g, 300 mmol) in methylene chloride (100 ml), the temperature rose to 20 – 25°C for 5 – 10 min. By immersion in a cooling bath, the temperature was quickly readjusted to about 5°C and the mixture was left to stand at this temperature for 16 h. By filtration the formed dicyclohexylurea (75.0 g), m.p. 196 – 203°C, was isolated, and the filtrate was left to stand at room temperature (22 – 25°C) for 4 h. By concentration *in vacuo* to about

half volume, a further quantity of dicyclohexylurea (9.0 g) could be isolated. The total crude yield of this by-product was 84.0 g (theory: 67.2 g); it was stirred with methylene chloride (200 ml), filtered and dried at 0.1 mmHg. Yield: 68.4 g, mp. 205 – 215°C. The combined filtrates were washed with 5 % aqueous citric acid (200 ml), water (3 × 100 ml), 5 % aqueous sodium bicarbonate solution (200 ml), and water (3 × 100 ml), dried over $MgSO_4$, filtered and evaporated to dryness at 0.1 mmHg. The yellow syrup (90.0 g, 100 %) was covered with petroleum ether (b.p. 60 – 80°C.) and left to stand for 18 h at room temperature. The crystalline mass was recrystallized from ethyl acetate (80 ml)/ petroleum ether (600 ml). Yield: 51.5 g of m.p. 79 – 81°C. Upon evaporation of the mother liquor to dryness and crystallization of the resulting syrup from ethyl acetate (20 ml)/ petroleum ether (600 ml) a second crop (15.5 g, m.p. 78 – 80°C.) was obtained. Thus the total yield was 67.0 g (75 %). $[\alpha]_D^{25} = -92{,}3°$; $[\alpha]_{578}^{25} = -96{,}6°$ ($c = 1$ in methanol); R_FS2 = 0.8; R_FS5 = 0.6. (Found: C 56.2; H 8.1; N 9.3. Calc. for $C_{14}H_{24}N_2O_5$ (300.4): C 56.0; H 8.1; N 9.3.) Amino acid content (mol per 300.4 g): Pro 0.98, Ala 0.97.

Boc-Gly-L-Pro-L-Ala-OMe (II). I (60.0 g, 200 mmol) was dissolved in N HCl/glacial acetic acid (500 ml) and left to stand for 90 min at room temperature. Evaporation to dryness at room temperature and 0.2 mmHg left an oil (66 g), which still contained some acetic acid. It was mixed with DMF (10 ml) and kept at 0.2 mmHg and room temperature overnight. Residual acetic acid was removed by adding DMF (15 ml) and precipitating by addition of sodium dried ether (600 ml). The oil was isolated by decantation of the supernatant, and was dried at 0.2 mmHg and room temperature. Yield: 56 g (theory: 47.2 g). It was redissolved in DMF (50 ml) and neutralized by addition of a solution of triethyl amine (27 ml) in methylene chloride (50 ml). The presence of an excess of volatile base was confirmed with the aid of moist indicator paper over the liquid surface. Immediately afterwards Boc-Gly (50.0 g, 286 mmol) was added, dissolved in methylene chloride (200 ml). The mixture was rapidly cooled to −10°C and a solution of DCC (51.5 g, 250 mmol) in methylene chloride (100 ml) was added. After standing for 1 h at −10°C the mixture was kept at +5°C for 4 h, and then at room temperature for 16 h. By filtration, the formed dicyclohexylurea was isolated. It was stirred with methylene chloride (200 ml), filtered and dried at 0.1 mmHg and room temperature. Yield: 51.3 g (92 %), m.p. 215 – 228°C. The combined filtrates were washed with 5 % aqueous citric acid (200 ml), water (3 × 100 ml), 5 % aqueous bicarbonate solution (200 ml), and water (3 × 100 ml), dried over magnesium sulfate, filtered and concentrated to dryness at 1.1 mmHg. The yellow syrup (69 g, 97 %) solidified to a white, compact mass during the evaporation. The product was recrystallized from hot ethyl acetate (270 ml). After standing for 1 h at +5°C, filtration and drying at 0.1 mmHg the yield of white crystalline product was 51.2 g (72 %), m.p. 152 – 153°C. By dilution of the filtrate with petroleum ether (b.p. 60 – 80°C) (270 ml) and leaving the solution for 16 h at −18°C a second crop was obtained (2.5 g, 3 %), m.p. 153 – 154°C. $[\alpha]_D^{25} = -110°$; $[\alpha]_{578}^{25} = -116°$ ($c = 1$ in methanol); R_FS2 = 0.6. An additional spot (R_F 0.8) revealed the presence of a few percent of dicyclohexylurea. (Found: C 54.7; H 7.8; N 12.2. Calc. for $C_{16}H_{27}N_3O_6$ (357.4): C 53.8, H 7.6; N 11.8.) Amino acid content (mol per 357.4 g): Gly 0.99, Pro 0.99, Ala 0.99.

HCl.Gly-L-Pro-L-Ala-OMe (III) II(36 g, 100 mmol) was dissolved in N HCl/glacial acetic acid (250 ml) and left to stand for 1 h at room temperature. Upon evaporation to dryness *in vacuo*, a crystalline residue was obtained. Recrystallization from methanol-abs. ether afforded a white crystalline product (28 g, 95 %) of m.p. 209°C. $[\alpha]_D^{25} = -110°$; $[\alpha]_{578}^{25} = -118°$ ($c = 1$ in methanol). R_FS2 = 0.2 (trace of urea at 0.8). (Found: C 44.7; H 6.9; N 14.2; Cl 12.0. Calc. for $C_{11}H_{20}N_3O_4Cl$ (293.8): C 45.0; H 6.9; N 14.3; Cl 12.1.)

Boc-L-Ser(Bzl)-Gly-L-Pro-L-Ala-OMe (IV). III(75 g, 255 mmol) was suspended in a mixture of DMF (43 ml) and methylene chloride (170 ml) and treated with triethylamine (36 ml, 259 mmol) under vigorous stirring. After addition of Boc-L-Ser(Bzl) (95 g, 322 mmol) dissolved in methylene chloride (110 ml) the suspension was stirred for 25 min at room temperature and then cooled to −15°C. A solution of DCC (66 g, 320 mmol) in methylene chloride (110 ml), precooled to 0°C, was added, and the mixture was stored at −20°C for 2 h with occasional shaking, and then left overnight at 5°C. After 2 more days at room temperature the mixture was filtered, the filtrate concentrated to dryness, and the resulting oil redissolved in ethyl acetate (800 ml). Undissolved dicyclohexylurea was removed by filtration, and the filtrate was washed with 5 % aqueous citric acid (3 × 100 ml), water (3 × 100 ml), 5 % aqueous sodium bicarbonate solution (2 × 100 ml), and water (3 × 100 ml), and dried over magnesium sulfate. Upon concentrating the

filtrate to dryness, a yellowish oil (140 g, 100 %) was obtained. Attempts at crystallization from various solvent mixtures (ether/petroleum ether, ethyl acetate/petroleum ether, and methanol/water) were unsuccessful. Being fairly homogeneous (one major spot in TLC, R_FS2 = 0.7; R_FS5 = 0.4) apart from contamination by some dicyclohexylurea, the product was used in the following step without further purification.

*HCl.*L-*Ser(Bzl)-Gly*-L-*Pro*-L-*Ala-OMe (V).* IV (160 g) was dissolved in 1.7 N HCl/glacial acetic acid (500 ml). After 30 min at room temperature, carbon dioxide evolution had ceased, and the solution was concentrated to dryness *in vacuo*. The resulting syrup was triturated three times with hot ethyl acetate. By this process, two impurities, detected by TLC (S5), were removed, leaving a yellowish, crystalline product (120 g, 85 %), m.p. 80 – 82°C d. (Found: C 53.8; H 7.0; N 11.5; Cl 8.2. Calc. for $C_{21}H_{31}N_4O_6Cl$ (471.0): C 53.6; H 6.6; N 11.9; Cl 7.5.) $[\alpha]_D^{25} = +10.7$; $[\alpha]_{578}^{25} = +11.0$ ($c = 1$ in methanol). R_FS2 = 0.3 (impurity at 0.4); R_FS5 = 0.0.

Z-L-*Thr*-L-*Ser(Bzl)-Gly*-L-*Pro*-L-*Ala-OMe (VI).* V (94.2 g, 200 mmol) was suspended in a mixture of methylene chloride (100 ml) and ethyl acetate (300 ml) and cooled to 0°C before the addition of triethylamine (30 ml, 216 mmol) under vigorous stirring. When the mixture had reached room temperature it was filtered (25.9 g triethylammonium chloride, 95 %), and to the filtrate was added Z-L-Thr (75.9 g, 300 mmol). To the resulting solution, after cooling to 0°C, was added solid DCC (61.8 g, 300 mmol), and the mixture was left overnight at 5°C. After dilution with ethyl acetate (100 ml), the mixture was left at room temperature for further 24 h. Upon filtration was obtained a white product (160 g), contaminated with dicyclohexylurea. Treatment with boiling methanol (200 ml) afforded dicyclohexylurea (57 g, 85 %) of m.p. 229 – 223°C and a filtrate, which upon standing at room temperature overnight deposited a crystalline product (84 g, 63 %) of m.p. 153 – 156°C (with sintering at 85°C). Recrystallization of this product (100 g) twice from hot methanol (220 ml) gave pure VI (85 g) of m.p. 88 – 90°C, re-solidification and final m.p. 160 – 161°C. The identity of the substance was proved by a mixed melting point determination with the purified product from the solid-phase synthesis (X).

Another synthesis of VI was carried out, starting from II, without isolation of III, IV, and V. The condensation of *Z*-L-Thr and oily V, was carried out in methylene chloride, and after filtration of dicyclohexyl urea the filtrate was washed with 5 % aqueous citric acid, water, 5 % aqueous sodium bicarbonate solution and water, dried over magnesium sulfate and concentrated to dryness. The resulting yellowish oil was crystallized from methanol/ethyl acetate/petroleum ether (200/150/600 ml) to yield a white product (42 % based on II) of m.p. 81 – 83°C. $[\alpha]_D^{25} = -68.0°$; $[\alpha]_{578}^{25} = -71.6°$ ($c = 1$ in methanol). (Found: C 58.7; H 6.8; N 10.7; O 24.1; OCH_3 4.4. Calc. for $C_{33}H_{43}N_5O_{10}$ (669.7): C 59.2; H 6.5; N 10.5; O 23,9; OCH_3 4.6.) Amino acid content (mol per 669.7 g): Thr 0.89, Ser 0.75, Gly 0.92, Pro 0.90, Ala 0.92.

On recrystallization from hot methanol, crystals of m.p. 85 – 89°C, re-solidification above 90°C and final m.p. 161 – 162°C were obtained. In TLC, complete agreement was also found between the two products and the purified product from the solid-phase synthesis (X).

*HCl.*L-*Thr*-L-*Ser-Gly*-L-*Pro*-L-*Ala-OMe (VII).* VI (6.7 g, 10 mmol) was dissolved in methanol (70 ml), and 2.8 N methanolic HCl (6.0 ml) was added. Palladium black, obtained by reducing $PdCl_2$ (2.0 g) with formic acid, was added, and hydrogen was passed through the mixture with vigorous stirring at room temperature and atmospheric pressure. After 4 h the evolution of CO_2 had virtually ceased. After further 2 h, the mixture was filtered, and the filtrate concentrated to dryness. The resulting colourless oil was dissolved in water (50 ml) and lyophilized. Yield of white, amorphous material: 4.7 g (92 %). M.p. 103 – 106°C d. $[\alpha]_D^{25} = -92.5$; $[\alpha]_{578}^{25} = -96.6$ ($c = 1$ in methanol); homogeneous in TLC R_FS2 = 0.1; R_FS4 = 0.4; R_FS7 = 0.5. (Found: C 42,3; H 6.5; N 13.4; O 30.3; Cl 9.4; OCH_3 7.1. Calc. for $C_{18}H_{32}N_5O_8$. 1.3HCl,H_2O (510.9): C 42.3; H 6.8; N 13.7; O 28.2; Cl 9.0; OCH_3 6.1.) Amino acid content (mol per 510.9 g): Thr 0.99, Ser 0.94, Pro 1.00, Gly 1.00, Ala 1.01.

Trt-L-*Trp*-L-*Thr*-L-*Ser-Gly*-L-*Pro*-L-*Ala-OMe (VIII).* VII [4.2 g, 8.2 mmol (10.7 mmol HCl)] was dissolved in DMF/H_2O (10 ml/1ml) by warming. A solution of *N*-trityl-L-tryptophan diethyl ammonium salt (5.7 g, 11.0 mmol) in methylene chloride (35 ml) was added, and the resulting solution cooled to – 18°C. DCC (2.3 g, 11.2 mmol) was added, and the reaction mixture was left to stand, with occasional shaking, at – 18°C for 20 h, at + 5°C for 48 h, and at room temperature for 4 days. After filtration of the

wine-red mixture, and washing on the filter with methylene chloride, dicyclohexylurea (1.5 g, 60 %) of m.p. 231 – 232°C was isolated.

The combined filtrate and washings were diluted to 100 ml with methylene chloride and washed with water (8 × 25 ml) in a separatory funnel. The organic phase was then concentrated to dryness, and the residue precipitated from methylene chloride by addition of ethyl acetate and petroleum ether, b.p. 60 – 80°C. By filtration and drying to 0.01 mmHg the product was obtained as a yellow solid, (4.0 g, 56 %), m.p. 145 – 155°C. In TLC one large UV-absorbing spot at R_FS1 = 0.2 and R_FS5 = 0.1 was present. On treatment with chlorine/tolidine, an additional spot was found at R_FS1 = 0.8 and R_FS5 = 0.6 corresponding to dicyclohexyl urea.

Conditions for preparative silica gel column separation were evaluated using mixtures of chloroform and methanol. By application of the crude product (2.5 g) on a 20 × 4 cm column filled with Kieselgel HF 254 (Merck) (80 g), and elution first with chloroform and then with 5 % methanol in chloroform, a fraction (1.8 g) was obtained, which was almost chromatographically pure (TLC in S1). A previously eluted fraction (0.3 g) contained the impurities with high TLC R_F values. A second portion (1.1 g), applicated on the same column, gave 0.9 g of almost pure product. The entire purified quantity (2.7 g) was applicated on a fresh column, and eluted in the same way. Yield: 2.5 g (35 % overall yield) of a glass, m.p. 100 – 110°C d. Chromatographically homogeneous, R_FS1 = 0.2; R_FS5 = 0.1; $[\alpha]_D^{25} = -80.4$; $[\alpha]_{578}^{25} = -84.1$ ($c = 1$ in methanol). On microanalysis, the sum of C, H, N, and O was 89.3 % due to contamination with inorganic material from the silica gel. The values were corrected to give 100 %. (Found C 65.5; H 6.3; N 11.6; O 16.6; OCH_3 3.4. Calc. for $C_{48}H_{55}N_7O_9$ (874.0): C 66.0; H 6.3; N 11.2; O 16.5; OCH_3 3.6.) Amino acid content (mol per 874 g): Trp 0.74, Thr 0.83, Ser 0.80, Gly 0.86, Pro 0.86, Ala 0.86. (8 h hydrolysis).

Trt-L-*Trp*-L-*Thr*-L-*Ser*-*Gly*-L-*Pro*-L-*Ala*-N_2H_3 *(IX).* VIII (1.7 g, 1.9 mmol) was dissolved in methanol (15 ml). After addition of hydrazine hydrate (0.6 ml, 12 mmol), the solution was left to stand for 24 h at room temperature. It was then concentrated to half volume on a water-bath at 65 – 70°C, and left for further 24 h. In TLC VIII (R_FS2 = 0.6) had disappeared, and only one other spot, visible with UV, acid/ninhydrin, and hypochlorite/tolidine was present at R_FS2 = 0.3. By precipitation with water, and reprecipitation from ethanol/water, a suspension (400 ml) was obtained, which could only be filtered with difficulty. On centrifugation for 20 min at 2000 rpm the product was isolated as a yellow glass (1.0 g) of m.p. 160 – 170°C. By concentration of the supernatant to dryness *in vacuo* and precipitating the residue from ethanol/water a further quantity (0.4 g) was obtained. Chromatographically homogeneous; R_FS2 = 0.3; R_FS3 = 0.2; R_FS7 = 0.7. The combined fractions were precipitated from ethanol (5 ml) by addition of ethyl acetate (40 ml) and ether (200 ml). Yield of almost white product: 1.3 g (79 %) and m.p. 160 – 165°C, decomposition above 200°C (evolution of gas, and red colour). $[\alpha]_D^{25} = -76.2$; $[\alpha]_{578}^{25} = -78.2$ ($c = 1$ in methanol). (Found: C 64.5; H 6.0; N 13.9; O 15.5. Calc. for $C_{47}H_{55}N_9O_8 \cdot \frac{1}{2}\ C_2H_5OH$ (897.1): C 64.3; H 6.5; N 14.1; O 15.2.)

Synthesis on a solid support

Z-L-*Thr*-L-*Ser(Bzl)*-*Gly*-L-*Pro*-L-*Ala*-*OMe (X).* Boc-L-alanyl-resin (45 g, 0.47 mmol Ala/g) was placed in a 1 litre reaction vessel and subjected to four cycles of deblocking, neutralization and coupling. The sequence and quantities of the reagents and solvents employed were the same as previously described.[12] Chloride determination by Volhard titration of the neutralization filtrates gave the following values: 22.3, 23.5, 20.6, and 20.8 mmol Cl^-. The quantities of amino acid derivatives and coupling reagent employed were: Boc-L-Pro (21.5 g, 100 mmol) and DCC (20.6 g, 100 mmol), Boc-Gly (17.5 g, 100 mmol) and DCC (20.6 g 100 mmol). To reduce waste of the Ser derivative, a smaller excess was used. To ensure a quantitative reaction, two consecutive couplings were carried out, without an intermediate deblocking step. Accordingly: Boc-L-Ser(Bzl) (14.7 g, 50 mmol) and DCC (10.3 g, 50 mmol), having reacted for 90 min were followed, after filtration, by Boc-L-Ser(Bzl) (7.4 g, 25 mmol) and DCC (5.1 g, 25 mmol), which were left to react overnight. In order to reduce the extent of O-acylation Z-L-Thr was also coupled twice, but before each addition of DCC, the derivative solution was filtered

off, and the resin washed three times with methylene chloride. Thus, Z-L-Thr (10.0 g, 40 mmol) and DCC (8.2 g, 40 mmol) was followed by Z-L-Thr (5.0 g, 20 mmol) and DCC (4.1 g, 20 mmol) without an intermediate deblocking step. The combined filtrate and washings following the first salt-formation period were concentrated to dryness at 0.1 mmHg. The recovery of Z-L-Thr was only 1.3 g, m.p. 99 – 101°C. Like Ser, Thr was allowed to react first for 90 min, and then overnight.

After the last coupling the resin was washed once with methylene chloride, six times with ethanol, and once with methanol. Then the entire quantity of peptide resin was transferred to a 3-litre beaker where it was transesterified [13] for 12 h at room temperature in 1.5 litres of 1 N methanolic triethylamine. Concentration of the filtrate to dryness at 0.1 mmHg yielded a colourless oil (13.5 g, 90 % based on the first Volhard titration value). Crystallization from methanol/ethyl acetate/petroleum ether (60 – 80°C) (40 ml/40 ml/800 ml) gave a nearly white product (11.2 g, 75 %) of m.p. 150 – 154°C. $[\alpha]_D^{25} = -66.6$; $[\alpha]_{578}^{25} = -70.0°$ ($c = 1$ in methanol). R_FS2 = 0.6; R_FS4 = 0.6. Inhomogeneous in S5 with $R_F = 0.4$ and 0.7 and in S1 with $R_F = 0.4$ and 0.7, respectively. (Found: C 58.6; H 6.8; N 10.7; O 24.1; OCH_3 5.2. Calc. for $C_{33}H_{43}N_5O_{10}$ (669.7): C 59.2; H 6.5; N 10.5; O 23.9; OCH_3 4.6.) Amino acid content (mol per 669.7 g): Thr 0.87, Ser 0.77, Gly 0.89, Pro 0.95, Ala 0.96.

A second treatment of the resin with 1.5 l of 1 N methanolic triethylamine resulted in the isolation of only 0.5 g of a brown oil. The resin was washed with methanol and dried at 0.1 mmHg. Yield: 40.8 g.

The protected peptide (10.1 g) was dissolved in methanol (25 ml) by warming, and the solution was left to stand at room temperature, in an open flask, for 2 – 3 days. The crystalline product (1.5 g) was collected by filtration, and the yellow filtrate and washings were left to stand for a further period of time. By repeating this procedure for some weeks, a white, crystalline product (4.0 g) of m.p. 161 – 163°C (with sintering at 80 – 90°C) was isolated. A second fraction (1.6 g), m.p. 156 – 160°C, without sintering, was isolated in the same way, leaving a yellow, oily residue.

The combined fractions (5.6 g) could now be recrystallized from hot methanol (20 ml). Yield of white crystals: 4.2 g (28 % over-all). M.p. 86 – 89°C, re-solidification above 90° and final m.p. 162 – 163°C. A mixed melting point with VI was unchanged. In TLC (S1) X was unchanged after being heated to 150°C for 2 min.

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