Synthesis of Corticotropin Peptides. XV. The Synthesis of an Undecapeptide and a Nonapeptide Derivatives Related to the Amino Acid Sequence 11—21 of Corticotropin*

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An undecapeptide derivative, Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-Lys(Mhoc)-Arg-Arg-Pro-Val-Lys-(Mhoc)-$NHNH_2$, corresponding to the amino acid sequence 11—21 of corticotropin (ACTH) is synthesized. In the course of the synthesis a key intermediate H-Arg(NO_2)-Arg(NO_2)-Pro-OH is derived from Z-Arg(NO_2)-Arg-(NO_2)-Pro-OBzl by the treatment with hydrogen bromide in acetic acid. This procedure eliminates the danger of racemization associated with the use of alkali for saponification. The synthesis is also described of a nonapeptide derivative, Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-Lys(Mhoc)-Arg(NO_2)-Arg(NO_2)-Pro-OH (amino acid sequence 11—19 of ACTH), in which a new protecting group, 9-methyl-9-fluorenyloxycarbonyl (Mfoc), is utilized for the temporary protection of an α-amino function.

In the preceding paper[1] we described the syntheses of the amino acid sequence 22—39 of porcine corticotropin (α_p-ACTH) and that of the human hormone (α_h-ACTH). In the present communication we wish to report the synthesis of an undecapeptide derivative, Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-Lys(Mhoc)-Arg-Arg-Pro-Val-Lys(Mhoc)-$NHNH_2$ (VII), and a nonapeptide derivative, Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-Lys(Mhoc)-Arg(NO_2)-Arg(NO_2)-Pro-OH (XI), corresponding to the amino acid sequences 11—21 and 11—19, respectively, of ACTH which has been performed as a step of the total synthesis of α_p-ACTH and α_h-ACTH.[2]

Synthesis of Undecapeptide Derivative VII. The synthetic procedure of undecapeptide VII is outlined in Fig. 1. The ε-amino function of lysines and the guanidino function of arginines were protected by the 1-methylcyclohexyloxycarbonyl (Mhoc) group and by the nitro group, respectively. The Mhoc group is much the same as the *t*-butoxycarbonyl (Boc) group in susceptibility toward acid reagents.[3] The dicyclohexylcarbodiimide (DCC)-mediated coupling of Boc-Arg(NO_2)-OH with proline benzyl ester yielded a dipeptide (I), which was purified on a silica gel column with a chloroform–methanol system as solvent. Compound I was treated with trifluoroacetic acid and the resulting N^{α}-free peptide was allowed to react with the pentachlorophenyl ester[4] of Z-Arg(NO_2)-OH. The product was purified by chromatography to give a tripeptide (II) in a 70% yield. Compound II was treated with hydrogen bromide in acetic acid for the simultaneous removal of benzyloxycarbonyl (Z) and benzyl ester groups to yield III. This was then coupled with Z-Lys(Mhoc)-OSu[5] to give a tetrapeptide (IV) in an 82% yield after chromatographic purification on a silica gel column with a chroloroform–methanol–acetic acid system as solvent.

A dipeptide derivative Z-Val-Lys(Mhoc)-OMe[5] was hydrogenolyzed to remove its Z group and the product was coupled with IV by the DCC method in the presence of 1-hydroxybenzotriazole (HOBt)[6] to produce a hexapeptide (V) in an excellent yield. Catalytic hydrogenolysis of V yielded H-Lys(Mhoc)-Arg-Arg-Pro-Val-Lys(Mhoc)-OMe. This compound, which was found to be homogeneous in TLC and found to have no appreciable absorption owing to the presence of nitro group, was allowed to react with the azide derived from Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-$NHNH_2$[5] in

H-Pro-OBzl
↓ Boc-Arg(NO_2)-OH, DCC
Boc-Arg(NO_2)-Pro-OBzl (I)
↓ CF_3COOH
H-Arg(NO_2)-Pro-OBzl
↓ Z-Arg(NO_2)-OPcp[4]
Z-Arg(NO_2)-Arg(NO_2)-Pro-OBzl (II)
↓ HBr/AcOH
H-Arg(NO_2)-Arg(NO_2)-Pro-OH (III)
↓ Z-Lys(Mhoc)-OSu[5]
Z-Lys(Mhoc)-Arg(NO_2)-Arg(NO_2)-Pro-OH (IV)
↓ H-Val-Lys(Mhoc)-OMe, DCC (+HOBt)
Z-Lys(Mhoc)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Mhoc)-OMe (V)
↓ H_2/Pd
H-Lys(Mhoc)-Arg-Arg-Pro-Val-Lys(Mhoc)-OMe
↓ Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-N_3[5]
Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-Lys(Mhoc)-Arg-Arg-Pro-Val-Lys(Mhoc)-OMe (VI)
↓ NH_2NH_2
Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-Lys(Mhoc)-Arg-Arg-Pro-Val-Lys(Mhoc)-$NHNH_2$ (VII)

Fig. 1. Synthesis of amino acid sequence 11—21 of ACTH.
Mhoc: 1-methylcyclohexyloxycarbonyl; HOPcp: pentachlorophenol.

* All the amino acid residues mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by the IUPAC-IUB Commission of Biochemical Nomenclature [*Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972)], and include Mhoc: 1-methylcyclohexyloxycarbonyl, Mfoc: 9-methyl-9-fluorenyloxycarbonyl, and HOPcp: pentachlorophenol.

the usual manner. The product was purified on a silica gel column with chloroform–methanol–acetic acid systems as solvent to give an undecapeptide ester (VI) in a pure form. The hydrazinolysis of ester VI yielded the desired undecapeptide hydrazide (VII) whose sufficient purity was confirmed by TLC and elemental analysis. The acid hydrolysates of VI and VII were also found to contain the constituent amino acids in the correct ratios expected by theory.

In the above synthesis of VII the three peptide subunits, which were respectively synthesized in the step-by-step manner from their C-terminal, were connected by the azide procedure and the DCC–HOBt method[6] to form a Lys–Lys bond and a Pro–Val bond, respectively. In addition, there was not employed any alkaline treatment throughout the synthesis. Thus, the cause of racemization associated with chemical processes was fully eliminated. Compound VII has successfully been employed as an intermediate in the total synthesis of α_p-ACTH and α_h-ACTH.[2]

Synthesis of Nonapeptide Derivative XI. The synthetic route to XI was illustrated in Fig. 2. In introducing a lysine residue to position 16, its N^{α}-protecting group was required to be the one selectively removable in the presence of the N^{ε}-Mhoc and the N^{G}-nitro groups. A purpose of the present synthesis is to examine if a new protecting group 9-methyl-9-fluorenyloxycarbonyl (Mfoc), which has been introduced by us recently,[3] meets this requirement.

Z–Arg(NO_2)–Arg(NO_2)–Pro–OMe[5]
↓ HBr/AcOH
H–Arg(NO_2)–Arg(NO_2)–Pro–OMe
↓ Mfoc–Lys(Mhoc)–ONp (VIII)
Mfoc–Lys(Mhoc)–Arg(NO_2)–Arg(NO_2)–Pro–OMe (IX)
↓ 20% HCOOH/AcOH
H–Lys(Mhoc)–Arg(NO_2)–Arg(NO_2)–Pro–OMe
↓ Z–Lys(Mhoc)–Pro–Val–Gly–Lys(Mhoc)–N_3[5]
Z–Lys(Mhoc)–Pro–Val–Gly–Lys(Mhoc)–Lys(Mhoc)–Arg(NO_2)–Arg(NO_2)–Pro–OMe (X)
↓ OH^-
Z–Lys(Mhoc)–Pro–Val–Gly–Lys(Mhoc)–Lys(Mhoc)–Arg(NO_2)–Arg(NO_2)–Pro–OH (XI)

Fig. 2. Synthesis of amino acid sequence 11—19 of ACTH.
Mhoc: 1-methylcyclohexyloxycarbonyl; Mfoc: 9-methyl-9-fluorenyloxycarbonyl.

First, H–Lys(Mhoc)–OH[3] was acylated with 9-methyl-9-fluorenyl azidoformate[3] in the presence of Triton B (benzyltrimethylammonium hydroxide) as base and under anhydrous conditions[7] to give Mfoc–Lys(Mhoc)–OH, from which the *p*-nitrophenyl ester (VIII) was derived by the DCC method in the usual manner. A tripeptide derivative Z–Arg(NO_2)–Arg(NO_2)–Pro–OMe[5] was treated with hydrogen bromide in acetic acid and the resulting N^{α}-free compound isolated in the form of acetic acid salt was allowed to react with VIII. The product was purified on a silica gel column with a chloroform–methanol system as solvent to give a tetrapeptide (IX) in pure form. The subsequent treatment of IX with 20% formic acid in acetic acid achieved the selective removal of the N^{α}-Mfoc group. The removal was complete within 7 h, in which any appreciable cleavage of the Mhoc group was not observed. The N^{α}-free tetrapeptide thus obtained was then coupled with the pentapeptide azide, derived from Z–Lys(Mhoc)–Pro–Val–Gly–Lys(Mhoc)–$NHNH_2$[5] by the treatment with nitrous acid in the usual manner, to give a protected nonapeptide ester (X) in an excellent yield. In the final step ester X was saponified with alkali under the same conditions as those employed for the saponification of Z–Lys(Mhoc)–Arg(NO_2)–Arg(NO_2)–Pro–OMe, in which no appreciable racemization seemed to occur,[5] and the product was purified on a silica gel column with a chloroform–methanol system as solvent to give the desired compound (XI). The purity of XI was confirmed by TLC and elemental analysis. Nonapeptide XI has been utilized as an intermediate in our first synthesis of α_h-ACTH.[2]

Experimental

Thin-layer chromatography (TLC) was performed on silica gel plates (Kieselgel GF_{254} or precoated Kieselgel $60F_{254}$, Merck) with the following solvent systems: A, chloroform–methanol (85: 15); B, chloroform–methanol (3: 1); C, chloroform–methanol–acetic acid (90: 10: 3); D, chloroform–methanol–acetic acid (80: 20: 3); E, ethyl acetate–acetic acid–water (4: 1: 1); F, 1-butanol–acetic acid–water (4: 1: 2).

Boc–Arg(NO_2)–Pro–OBzl (I). An aqueous solution of H–Pro–OBzl·HCl (2.42 g, 10 mmol) was treated with 50% potassium carbonate (5 ml) at 0 °C in the presence of dichloromethane. The organic phase separated was dried over magnesium sulfate and evaporated *in vacuo* at a bath temperature of 20 °C. The resulting proline ester free base and Boc–Arg(NO_2)–OH (3.19 g, 10 mmol) were coupled with dicyclohexylcarbodiimide (DCC; 2.06 g, 10 mmol) in *N,N*-dimethylformamide (DMF)–ethyl acetate in the presence of 1-hydroxybenzotriazole (HOBt; 1.35 g, 10 mmol) at 4 °C; for 20 h. The crude product was purified on a column of silica gel (100 g, Kieselgel H, Merck) with chloroform–methanol (93: 7) as solvent. The fractions (10 g/tube) were examined by TLC in system A and those containing the desired compound as a single component (tubes 62—70) were combined and evaporated *in vacuo* to afford I as a sirupy residue.

Z–Arg(NO_2)–Arg(NO_2)–Pro–OBzl (II). Compound I obtained above was treated with trifluoroacetic acid (10 ml) at room temperature for 60 min. The precipitates which separated upon addition of ether were dissolved in DMF (30 ml) together with HOBt (1.3 g, 10 mmol) and triethylamine (1.4 ml, 10 mmol) and to this was added Z–Arg(NO_2)–OPcp (6.0 g, 10 mmol)[4] at 0 °C. The mixture was stirred at 4 °C overnight followed by evaporation *in vacuo* at a bath temperature of 45 °C. The oily residue was dissolved in aqueous ethyl acetate and the solution was washed with 1M acetic acid and evaporated *in vacuo*. The crude product thus obtained was submitted to a silica gel column (140 g, Kieselgel H, Merck) with chloroform–methanol (90: 10) as solvent. The fractions (12 g/tube) were examined by TLC in system A and those containing the desired product as a single component (tubes 41—60) were combined and evaporated *in vacuo*. The residue was precipitated from ethyl acetate–ether; yield 5.35 g (70%), $[\alpha]_D^{28}$ $-48.4\pm0.9°$

(*c* 1.0, methanol).

Found: C, 51.79; H, 6.04; N, 20.17%. Calcd for $C_{33}H_{43}N_{11}O_{11}$: C, 51.49; H, 5.63; N, 20.02%.

Z-Lys(Mhoc)-Arg(NO_2)-Arg(NO_2)-Pro-OH (IV). Compound II (1.49 g, 1.93 mmol) was treated with 25% hydrogen bromide in acetic acid (20 ml) at room temperature for 3 h. The precipitates which separated upon addition of ether were dissolved in DMF (10 ml) together with triethylamine (1.33 ml, 9.7 mmol) and to the resulting solution was added Z-Lys(Mhoc)-OSu[5] prepared from the dicyclohexylamine salt of Z-Lys(Mhoc)-OH (1.16 g, 1.93 mmol).[3] The mixture was kept at 4 °C for 2.5 days followed by evaporation *in vacuo*. To the residue were added 1-butanol–ethyl acetate (1: 1, 20 ml) and 1M acetic acid (20 ml), and the mixture was shaken vigorously. The organic phase separated was evaporated *in vacuo* (1.6 g). This was purified on a column of silica gel (70 g, Kieselgel H, Merck) with chloroform–methanol–acetic acid (80: 20: 3) as solvent to give IV in pure form; yield 1.50 g (82%), mp 138—140 °C, $[\alpha]_D^{25}$ −37.5 ±0.8° (*c* 1.0, methanol).

Z-Lys (Mhoc)-Arg (NO_2)-Arg (NO_2)-Pro-Val-Lys (Mhoc)-OMe (V). A solution of Z-Val-Lys(Mhoc)-OMe (0.54 g, 1 mmol)[5] in 10% acetic acid in methanol was submitted to hydrogenolysis over palladium for 2 h followed by evaporation *in vacuo*. The residue was dissolved in dichloromethane and the solution was shaken with 50% potassium carbonate at 0 °C. The organic phase was dried over magnesium sulfate at 0 °C and evaporated *in vacuo* at a bath temperature of 20 °C. The resulting dipeptide ester free base and IV (0.94 g, 1 mmol) were coupled with DCC (0.41 g, 2 mmol) in DMF in the presence of HOBt (0.27 g, 2 mmol). The reaction was allowed to proceed at 4 °C for 2.5 days. The crude product was repeatedly precipitated from methanol–ether to give a pure preparation of V; yield 1.26 g (95%), mp 125—130 °C, $[\alpha]_D^{26}$ −55.6±0.9° (*c* 1.0, methanol). TLC: homogeneous (sulfuric acid) in system D.

Found: C, 53.63; H, 7.48; N, 17.13%. Calcd for $C_{59}H_{96}N_{16}O_{17}\cdot H_2O$: C, 53.70; H, 7.49; N, 16.99%.

Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-Lys(Mhoc)-Arg-Arg-Pro-Val-Lys(Mhoc)-OMe (VI). Compound V (0.90 g, 0.69 mmol) was hydrogenolyzed over palladium in acetic acid to give the partially deblocked hexapeptide ester (acetate); no appreciable absorption at 275 nm. TLC: homogeneous (ninhydrin and Sakaguchi reagents) in system F.

An ethyl acetate solution of the azide, derived from Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-$NHNH_2$ (0.73 g, 0.76 mmol) in the same manner as described previously,[5] was combined with a solution of the hexapeptide ester obtained above and triethylamine (0.5 ml, 3.5 mmol) in DMF (3 ml). The mixture was, after removal of ethyl acetate by evaporation at a bath temperature of 5 °C, stirred at 4 °C for 2.5 days. After addition of another quantity of the pentapeptide azide, freshly prepared from the hydrazide (0.33 g, 0.35 mmol), the mixture was stirred for one more day followed by evaporation *in vacuo*. The residue was dissolved in aqueous ethyl acetate and the solution was washed with 1M acetic acid and evaporated. The crude product thus obtained (1.50 g) was then purified on a silica gel column (50 g, Kieselgel 60, Merck) with chloroform–methanol–acetic acid system (90: 10: 3, 100 ml; 80: 20: 3, 100 ml; 60: 40: 3, 200 ml) as solvent to give a pure preparation of VI; yield 1.04 g (76%), mp 129—130 °C, $[\alpha]_D^{24}$ −58.7±1.0° (*c* 1.0, methanol). TLC: a single component (ninhydrin, after preheating at 150 °C) in systems D, E, and F. Amino acid ratios in acid hydrolysate (theoretical values are given in parentheses): Lys 4.00 (4), Arg 1.92 (2), Pro 1.84 (2), Gly 1.00 (1), Val 2.01 (2).

Z-Lys (Mhoc)-Pro-Val-Gly-Lys (Mhoc)-Lys (Mhoc)-Arg-Arg-Pro-Val-Lys(Mhoc)-$NHNH_2$ (VII). Compound VI (0.98 g, 0.49 mmol) was treated with hydrazine hydrate (0.25 ml) in DMF (5 ml) at 37 °C for 40 h. The solvent was evaporated *in vacuo*. To the resulting residue were added 1M acetic acid (15 ml) and 1-butanol–ethyl acetate (1: 1, 15 ml) and the mixture was shaken vigorously. The organic phase was separated and evaporated *in vacuo* (0.95 g). A 0.85 g portion of this product was dissolved in aqueous ethyl acetate and the solution was repeatedly extracted with 1 M acetic acid. The aqueous solutions combined were lyophilized to afford VII; yield 0.79 g (90%), $[\alpha]_D^{24.5}$ −55.4 ±1.1° (*c* 0.9, methanol). TLC: almost homogeneous (ninhydrin, after preheating at 150 °C) in system F. Amino acid ratios in acid hydrolysate: Lys 4.07 (4), Arg 2.01 (2), Pro 1.95 (2), Gly 1.00 (1), Val 2.10 (2).

Found: C, 55.49; H, 8.41; N, 14.78%. Calcd for $C_{97}H_{165}N_{23}O_{21}\cdot 2CH_3COOH\cdot 4H_2O$: C, 55.60; H, 8.36; N, 14.77%.

Mfoc-Lys(Mhoc)-ONp (VIII). N^{ε}-Mhoc-lysine (1.43 g, 5 mmol)[3] and 40% benzyltrimethylammonium hydroxide in methanol (Triton B, 2.4 ml) were dissolved in DMF (4 ml) and the solvent was evaporated *in vacuo* at a bath temperature of 45 °C. The residue was redissolved in DMF (10 ml) and 9-methyl-9-fluorenyl azido-formate (1.85 g, 7 mmol)[3] was added. The reaction mixture was stirred at 4 °C for 24 h followed by evaporation *in vacuo*. The residue was dissolved in ethyl acetate and the solution was washed with 1M citric acid and water, dried over magnesium sulfate and evaporated *in vacuo*. The residue was purified on a column of silica gel (50 g, Kieselgel H, Merck) with chloroform–methanol (95: 5) as solvent to give Mfoc-Lys(Mhoc)-OH as an oil; yield 1.65 g (59%).

The diacyllysine thus obtained was dissolved in ethyl acetate along with *p*-nitrophenol (0.42 g, 3 mmol) and to this was added DCC (0.62 g, 3 mmol). The mixture was stirred at 4 °C for 20 h followed by evaporation *in vacuo*. The resulting oily residue was precipitated from methanol–petroleum ether to give VIII; yield 1.95 g (97%). This was used for the subsequent coupling reaction without further purification.

Mfoc-Lys(Mhoc)-Arg(NO_2)-Arg(NO_2)-Pro-OMe (IX). Z-Arg(NO_2)-Arg(NO_2)-Pro-OMe (2.05 g, 3 mmol)[5] was treated with 28% hydrogen bromide in acetic acid (10 ml) at room temperature for 60 min. The precipitates which formed upon addition of ether (1.40 g) were dissolved in water (10 ml) and Amberlite CG-400 (acetate form, wet vol. 10 ml) was added. The mixture was shaken for 30 min and then filtered off. The filtrate was evaporated to give a residue which was dried over sodium hydroxide pellets and phosphorus pentoxide *in vacuo* (1.30 g).

The tripeptide methyl ester obtained above was allowed to react with VIII (1.95 g, 2.88 mmol) in DMF (5 ml) in the present of triethylamine (0.6 ml, 4.4 mmol) at 4 °C for 24 h. The crude product was chromatographed on a column of silica gel (70 g, Kieselgel H, Merck) with chloroform–methanol (95: 5) as solvent to give IX in pure form; yield 2.10 g (92%) mp 135—140 °C decomp., $[\alpha]_D^{23}$ −60.9±1.0° (*c* 1.0, methanol). TLC: homogeneous (sulfuric acid) in system A.

Found: C, 54.55; H, 6.66; N, 17.15%. Calcd for $C_{47}H_{67}N_{13}O_{13}\cdot H_2O$: C, 54.27; H, 6.69; N, 17.51%.

Z-Lys(Mhoc)-Pro-Val-Gly-Lys(Mhoc)-Lys(Mhoc)-Arg-(NO_2)-Arg(NO_2)-Pro-OMe (X). Compound IX (0.52 g, 0.5 mmol) was dissolved in 20% formic acid in acetic acid (6 ml) and the solution was allowed to stand at room temperature for 7 h. The solvent was evaporated and the residue was triturated with ether. The resulting precipitates were filtered off and dried over potassium hydroxide pellets *in vacuo*. To this were added ethyl acetate (15 ml) and 1M

acetic acid (15 ml) and the mixture was shaken vigorously. The organic phase was extracted again with 1M acetic acid. The combined aqueous solution was washed with ethyl acetate, concentrated *in vacuo* and lyophilized.

The N^{α}-free tetrapeptide obtained above was dissolved in DMF and triethylamine (0.07 ml, 0.5 mmol) was added. To this was added an ethyl acetate solution of Z–Lys(Mhoc)–Pro–Val–Gly–Lys(Mhoc)–N_3, derived from the corresponding hydrazide (0.72 g, 0.75 mmol),[5] and the mixture was concentrated *in vacuo* at a bath temperature of 0—5 °C to remove ethyl acetate. The resulting solution was stirred at 4 °C for 20 h followed by evaporation *in vacuo*. The precipitates which formed upon addition of ether were reprecipitated from ethyl acetate to yield X in pure form; yield 0.83 g (96%), mp 130—135 °C, $[\alpha]_D^{23}$ $-52.7\pm0.9°$ (*c* 1.0, methanol). TLC: homogeneous (ninhydrin, after pretreatment with hydrobromic acid) in system A.

Found: C, 55.71; H, 7.68; N, 15.37%. Calcd for $C_{80}H_{130}N_{20}O_{22}$: C, 55.73; H, 7.60; N, 16.25%.

Z–Lys(Mhoc)–Pro–Val–Gly–Lys(Mhoc)–Lys(Mhoc)–Arg(NO₂)–Arg(NO₂)–Pro–OH (XI). Compound X (0.83 g, 0.48 mmol) in methanol (2 ml) was treated with 2 M sodium hydroxide (0.48 ml) at room temperature for 2 h. The mixture was neutralized by the addition of ice-cold 1M hydrochloric acid (0.96 ml) and then diluted with water. This was repeatedly extracted with 1-butanol–ethyl acetate (1: 1). The organic solutions were combined, washed three times with water and evaporated *in vacuo*. The residue was chromatographed on a column of silica gel (30 g, Kieselgel 60, Merck) with chloroform–methanol (95: 5) as solvent for the first 20 fractions (10 ml/tube) and with chloroform–methanol (80: 20) for the rest. The fractions containing the desired product as a single component (tubes 37—55), as examined by TLC in system B, were combined and evaporated *in vacuo*. The residue was solidified by tritutation with ether to afford XI; yield 0.43 g (50%), mp 160—170 °C, $[\alpha]_D^{24.5}$ $-35.5\pm0.8°$ (*c* 1.0, methanol). TLC: homogeneous (ninhydrin, after pretreatment with hydrobromic acid) in system B.

Found: C, 53.12; H, 7.53; N, 15.40%. Calcd for $C_{79}H_{128}N_{20}O_{22}\cdot 4H_2O$: C, 53.24; H, 7.69; N, 15.72%.

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