[8] *A. Datyner, P. Rys & H. Zollinger*, Helv. *49*, 755 (1966).
[9] *P. Rys, A. Schmitz & H. Zollinger*, Helv. *54*, 163 (1971).
[10] *E. Siegel*, Chimia *22*, 100 (1968).
[11] *P. Rys & H. Zollinger*, Leitfaden der Farbstoffchemie, Verlag Chemie, Weinheim 1970.
[12] USP. 2741532.
[13] Bg. P. 596945, 585006.
[14] Bg. P. 573466.
[15] DBP. 1108176.
[16] Bg. P. 560033, 565997.
[17] *K. G. Kleb*, Angew. Chem. *76*, 423 (1964); Bg. P. 569439.
[18] Fp. 1212297, 1212298.
[19] *R. Baird & S. Winstein*, J. Amer. chem. Soc. *74*, 756 (1957); *84*, 788 (1962).
[20] *R. Baird & S. Winstein*, J. Amer. chem. Soc. *85*, 567 (1963).
[21] *R. Baird & S. Winstein*, J. Amer. chem. Soc. *79*, 4238 (1957).
[22] *P. Rys, P. Skrabal & H. Zollinger*, Tetrahedron Letters *21*, 1797 (1971).
[23] *G. F. Koser & W. H. Pirkle*, J. org. Chemistry *32*, 1992 (1967).
[24] *A. Mustafa & M. K. Hilmy*, J. chem. Soc. *1952*, 1434.
[25] *R. Adams & C. S. Marvel*, Organic Syntheses, Coll. Vol. *1*, 128 (1956).
[26] *E. Fourneau*, Ber. deutsch. chem. Ges. *43*, 455 (1928).
[27] *R. L. Burwell*, Chem. Rev. *54*, 615 (1954).
[28] *C. Hartmann & L. Gattermann*, Ber. deutsch. chem. Ges. *25*, 3531 (1892).
[29] *H. Hopff & K. Koulen*, Ber. deutsch. chem. Ges. *85*, 897 (1952).
[30] *P. Pfeiffer & E. Haack*, Liebigs Ann. Chem. *460*, 156 (1927).
[31] *V. Prey*, Ber. deutsch. chem. Ges. *74*, 1219 (1941).
[32] *A. Roedig* in: Houben-Weyl; Methoden der org. Chemie, G. Thieme Verlag Stuttgart, 1960, V/4, 418.
[33] *M. H. Karger & Y. Mazur*, J. Amer. chem. Soc. *90*, 3878 (1968).
[34] Organikum; VEB Deutscher Verlag der Wissenschaften, Berlin 1965, S. 180.

288. The Use of Intermediates with Preformed Disulfide Bridge for the Synthesis of Oxytocin and Deamino-oxytocin

by **M. Mühlemann**[1]), **M. I. Titov**[2]), **R. Schwyzer** and **J. Rudinger**

Institute of Molecular Biology and Biophysics, Swiss Federal Institute of Technology, CH-8049 Zürich

(12 IX 72)

Summary. The disulfide-bridged hexapeptides **6a** and **6b** have been prepared from benzyl-protected intermediates. Coupling of **6a** and **6b** with prolyl-leucyl-glycine amide afforded deamino-oxytocin (**7a**) and the protected oxytocin derivative **7b**, respectively; the latter was converted to oxytocin (**7c**) by removal of the Boc protecting group.

All syntheses of the neurohypophysial hormones, and their analogues of similar chain length, which have been reported to date, have involved the preparation of nonapeptide intermediates containing S-protected cysteine residues, the protecting groups being removed and the disulfide bridge formed in the last stage of the synthesis.

---

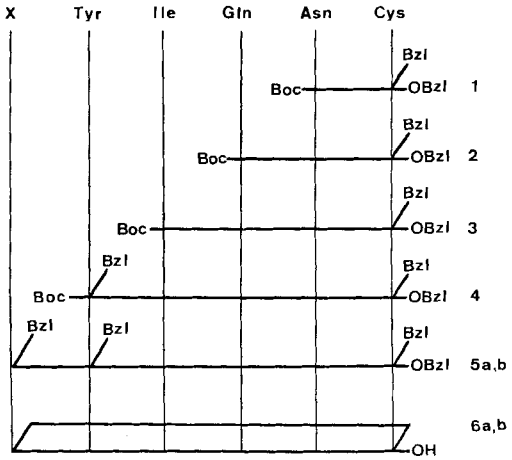
[1]) Taken in part from the Doctoral Dissertation to be submitted by *M. Mühlemann*.

[2]) Permanent address: Chemical Faculty, *A. A. Zhdanov* State University, Leningrad, USSR.

Riniker, Rittel et al. in their total synthesis of α-thyrocalcitonin [1] and of human calcitonin M [2] made use of N- and O-protected nona- and deca-peptide intermediates [3] in which the disulfide bridge between the cysteine residues in positions 1 and 7 was already preformed, and they showed that the bridge was stable under the conditions of fragment coupling and removal of the *t*-butyl-based protecting groups. A similar approach to the synthesis of the neurohypophysial hormones and their analogues appeared attractive particularly for the synthesis of analogues modified in residues 7–9 (the 'tail' of the molecule). This paper describes the preparation of oxytocin and deamino-oxytocin by such an approach.

The key intermediates were the disulfide-bridged peptides **6a** and **6b**. They were obtained by stepwise synthesis using benzyl protecting groups for the terminal carboxyl, the tyrosine hydroxyl, and the two thiol groups, with *t*-butyloxycarbonyl serving for protection of the terminal amino group (see scheme)[3]. Up to the pentapeptide the couplings were carried out with dicyclohexyl-carbodiimide in the presence of 1-hydroxy-benzotriazole [5]; the terminal *t*-butyloxycarbonyl-S-benzyl-cysteine or 3-benzylthio-propionyl residues were introduced using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydro-quinoline (EEDQ) [6] as the reagent[4]).

The benzyl protecting groups were removed by reduction with sodium in liquid ammonia [8] with the addition of urea, as an acid in the ammonia system, to avoid the formation of sodium amide [9]. The disulfide groups of **6a** and **6b** were formed by treatment with 1,2-diiodo-ethane [10] and the products were isolated by a combina-



a, X = βMp; **b**, X = Boc-Cys

X-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

7a, X = βMp; **7b**, X = Boc-Cys; **7c**, X = H-Cys

[3]) Standard abbreviations [4] are used for amino acids and protecting groups. In addition, βMp stands for the 3-mercaptopropionyl residue, DCCI for dicyclohexyl-carbodiimide, DCHA for dicyclohexylamine, and DMF for dimethylformamide.

[4]) After completion of this work, the preparation of the protected peptides **1** and **2** by a somewhat different procedure was described by *Ferger et al.* [7].

tion of countercurrent distribution and gel chromatography. The deamino derivative **6b** was obtained as a crystalline tetrahydrate.

The disulfide-bridged peptides **6a** and **6b** were coupled with the N-terminal tripeptide amide, prolyl-leucyl-glycine amide [11], again using dicyclohexyl-carbodiimide in the presence of 1-hydroxy-benzotriazole. The products were freed of basic and acidic impurities (including 1-hydroxy-benzotriazole) by filtration through a mixed bed of an acidic and a basic macroreticular ion exchange resin (*Amberlysts* 15 and A-21) in methanol solution.

Deamino-oxytocin (**7a**) was obtained sufficiently pure for crystallisation by a single gel filtration on *Sephadex* G-15 [12]. The N-*t*-butyloxycarbonyl-oxytocin (**7b**) was precipitated from 2-propanol with di-2-propyl ether as an amorphous but chromatographically homogeneous material, which was converted to oxytocin (**7a**) by treatment with 90% trifluoroacetic acid. The analytical and biological properties of the two peptides, **7a** and **7c**, corresponded to those recorded in the literature [13]–[16].

Experimental Part – *General*: Melting-points (uncorrected) were determined with a capillary melting-point apparatus (*Büchi*, Flawil, Switzerland) or, where stated, on a *Kofler* microscope hot-stage (*Mettler AG*, Greifensee, Switzerland). Optical rotations were measured with a *Perkin-Elmer* 141 photoelectric polarimeter in a 1-cm cell and are estimated to be correct to $\pm 1°$.

Materials: Most solvents and reagents as well as some of the protected amino acids were purchased from *Fluka AG*, Buchs, Switzerland. The *Amberlyst* ion-exchange resins are the products of *Rohm & Haas*, Philadelphia, USA, the *Sephadex* dextran gels of *Pharmacia AB*, Uppsala, Sweden.

Thin-layer chromatography (tlc.) and *electrophoresis*: Chromatograms were run on silica gel (thickness 0.25 mm; DC-Fertigplatten, *Merck*, Darmstadt, Germany) in the solvent systems (composition by volume): A, *n*-BuOH/H_2O/AcOH 100:35:15; B, *n*-BuOH/H_2O/AcOH 4:1:1; C, $CHCl_3$/MeOH/AcOH 95:5:3; D, dioxan/MeOH 1:1. The Rf values were often rather variable and should be regarded as merely a general indication of chromatographic behaviour. Electrophoresis was carried out in a moist-chamber apparatus (*Camag*, Muttenz, Switzerland) on cellulose-coated (0.1 mm thickness) plates (*Macherey-Nagel*, Düren, Switzerland) at a potential gradient of about 23 V/cm during 45–60 min, using the electrolytes (composition by volume): E, 88% HCOOH/AcOH/H_2O 15:10:75, pH 1.9; F, pyridine/AcOH/H_2O 1:10:90, pH 3.6. Mobilities are expressed as m_{Arg}, the ratio of distances from the origin for the sample and arginine reference. Detection was carried out with ninhydrin [17] and by a modification [18] of the procedure of *Reindel & Hoppe* [19]. In testing for purity, samples were applied for chromatography in 5 μg and 0.1 μg amounts and detection was carried out by the *Reindel-Hoppe* procedure.

Analytical: Samples for elemental analysis were dried at room temperature and 0.01 Torr for 12–24 h unless otherwise stated. For amino-acid analysis, samples were hydrolysed with 6M HCl at 110° for 24 h and run on a *Beckman* 120B automatic amino-acid analyser.

Standard preparative procedures: Peptide ester hydrochlorides were prepared from the Boc derivatives by treatment with a saturated solution of HCl in ethyl acetate at room temperature during 30 min and isolated by dilution with an excess of dry ether, washing with ether, and drying *in vacuo* over KOH. To remove any excess HCl the hydrochlorides were dissolved in DMF, stirred with dry $NaHCO_3$ (about 100 mg) at room temperature for 15 min, and filtered; the filtrates were directly used for the coupling reaction.

Couplings with DCCI were carried out by adding the reagent (1.1 mol) to a solution of the amino-ester hydrochloride (1 mol), the protected amino-acid (1 mol), N-methylmorpholine (1 mol), and 1-hydroxy-benzotriazole (1.5–2 mol) in DMF at 0°, stirring at 0° for 1 h and at room temperature for 3–16 h, and working up as described. The crude protected peptides obtained by precipitation were washed by trituration with water, 5% $NaHCO_3$, water, 0.05M H_2SO_4, water, and finally on the filter with a suitable organic solvent.

All evaporations were carried out in a rotary evaporator (*Büchi*, Flawil, Switzerland) under reduced pressure at a temperature not exceeding 40°.

Bioassay: Uterotonic activity was assayed [20] on organs taken from virgin rats in natural oestrus or oestrus to metoestrus, using *van Dyke-Hastings* medium without magnesium [21] and isotonic recording, against the 'Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances'.

Boc-Asn-Cys(Bzl)-OBzl (**1**). Boc-Asn-OH (5.80 g) and TosOH, Cys(Bzl)-OBzl [22] (11.8 g) in DMF (70 ml) were treated with N-methylmorpholine (2.52 g), 1-hydroxy-benzotriazole (6.75 g) and DCCI (5.66 g) under standard conditions. The reaction mixture was filtered, evaporated to half the volume, set aside at 0° overnight, once more filtered, and diluted with water (25 ml). The product was collected, dissolved in ethyl acetate, and the solution was washed with 5% $NaHCO_3$, water, 0.05 M H_2SO_4, and water, dried ($MgSO_4$), and evaporated to dryness. The residue was recrystallised from ethyl acetate (60 ml). Yield 9.06 g (70%), m.p. 146–149°, $[\alpha]_D^{25} = -37.2°$ ($c = 1$, DMF), Rf 0.72 (A), 0.59 (B), 0.60 (C).

$C_{26}H_{33}N_3O_6S$	Calc.	C 60.56	H 6.45	N 8.15	S 6.22%
(515.6)	Found	,, 60.39	,, 6.57	,, 8.20	,, 6.14%

The literature [7] records m.p. 145–147°, $[\alpha]_D^{23} = -37.7°$, for a product obtained in 66% yield by the nitrophenyl ester procedure.

Boc-Gln-Asn-Cys(Bzl)-OBzl (**2**). The protected dipeptide **1** (5.16 g) was treated with HCl in dry ethyl acetate (50 ml) and the dipeptide ester hydrochloride was isolated by the standard procedure: Yield 4.35 g (96%) of hygroscopic product, Rf 0.37 (A), 0.52 (D); m_{Arg} 0.44 (E), 0.52 (F). This product was coupled under the standard conditions with Boc-Gln-OH (2.38 g) in the presence of N-methylmorpholine (0.98 g) and 1-hydroxy-benzotriazole (2.01 g) using DCCI (2.19 g). The reaction mixture was filtered, diluted with water (10 ml), and the product was collected. It was washed by the standard procedure and finally with acetone, dried, and recrystallised from DMF/ethyl acetate. Yield 4.90 g (78%), m.p. 197–199°, $[\alpha]_D^{25} = -25.2°$ ($c = 1$, DMF), Rf 0.67 (A), 0.61 (B), 0.19 (C), 0.71 (D).

$C_{31}H_{41}N_5O_8S$	Calc.	C 57.84	H 6.42	N 10.88	S 4.98%
(643.8)	Found	,, 57.95	,, 6.63	,, 10.72	,, 4.89%

The literature [7] records m.p. 199–201°, $[\alpha]_D^{23} = -25.0°$, for a product obtained by the nitrophenyl ester procedure in 71% yield.

Boc-Ile-Gln-Cys(Bzl)-OBzl (**3**). The protected tripeptide **2** was treated with HCl by the standard procedure to afford the ester hydrochloride in 96% yield; Rf 0.24 (A), 0.29 (D); m_{Arg} 0.39 (E), 0.46 (F). The hydrochloride (1.13 g) in DMF (10 ml) was coupled with Boc-Ile-OH (468 mg of the crystalline hemihydrate) in the presence of N-methylmorpholine (197 mg) and 1-hydroxy-benzotriazole (405 mg) using DCCI (434 mg) in DMF (2 ml). The reaction mixture was filtered, evaporated to half the volume, set aside at −25° overnight, once more filtered, and diluted with 2 volumes of ethyl acetate. After standing at 3° for 2 days the product was collected, washed by the standard procedure and finally with acetone/ether (4:1) and with ether. Yield 1.04 g (69%) of the monohydrate, m.p. 238–240° (dec.), $[\alpha]_D^{25} = -23.9°$ ($c = 1$, DMF), Rf 0.78 (A), 0.71 (B).

$C_{37}H_{52}N_6O_9S \cdot H_2O$	Calc.	C 57.55	H 7.02	N 10.84	S 4.14%
(774.9)	Found	,, 57.81	,, 6.85	,, 10.83	,, 4.12%

Boc-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-OBzl (**4**). The protected tetrapeptide **3** was treated with HCl by the standard procedure to afford the ester hydrochloride in 97% yield; Rf 0.26 (A), 0.35 (B); m_{Arg} 0.33 (E), 0.29 (F). The hydrochloride (1.39 g) in DMF (10 ml) was coupled with Boc-Tyr(Bzl)-OH (added as the DCHA salt; 1.11 g), using DCCI (453 mg) in the presence of 1-hydroxy-benzotriazole (540 mg), and the product was isolated as in the preparation of **3**. The crude product was taken up in the minimal amount of DMF, the solution was kept at 3° overnight, filtered, and diluted with ethyl acetate to afford 1.44 g (70%) of **4**, m.p. 245–248°, $[\alpha]_D^{25} = -22.6°$ ($c = 1$, DMF), Rf 0.65 (A), 0.37 (C).

$C_{53}H_{67}N_7O_{11}S$	Calc.	C 63.01	H 6.68	N 9.71	S 3.17%
(1010.2)	Found	,, 62.84	,, 6.78	,, 9.60	,, 3.20%

βMp(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-OBzl (**5a**). The protected pentapeptide **4** was treated with HCl by the standard procedure to afford the ester hydrochloride in 88% yield; Rf 0.58 (A), 0,66 (D); m_{Arg} 0.08 (E). The hydrochloride (950 mg; 1 mmole) in DMF (20 ml) and tetrahydrofuran (8 ml) was treated with N-methylmorpholine (101 mg; 1 mmole) and coupled

with 3-benzylthio-propionic acid [23] (216 mg; 1.1 mmole) using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydro-quinoline (EEDQ) (272 mg; 1.1 mole). The mixture was stirred at room temperature for 15 h, the disappearance of the amino ester being followed chromatographically. The solvent was evaporated and the residue was washed by the standard procedure, finally with acetone/ether and with ether. Recrystallisation from DMF/ethyl acetate afforded 880 mg (81%) of **5a**, m.p. above 262° (dec.; *Kofler* block), $[\alpha]_D^{25} = -22.8°$ (c = 1, DMF), Rf 0.69 (A), 0.23 (C).

$C_{58}H_{69}N_7O_{10}S$	Calc.	C 64.01	H 6.39	N 9.01	S 5.89%
(1088.4)	Found	,, 63.87	,, 6.56	,, 9.00	,, 5.72%

Boc-Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-OBzl (**5b**). The pentapeptide ester hydrochloride prepared as above (350 mg; 0.37 mmol) and Boc-Cys(Bzl)-OH, DCHA (197 mg; 0.4 mmole) in DMF (12 ml) were treated with EEDQ (99 mg; 0.4 mmole) in tetrahydrofuran (4 ml) and the product was isolated as described for **5a** above. The product was finally washed with 2-propanol and with ether (yield 330 mg; chromatographically homogeneous) and crystallised from DMF/2-propanol to afford 300 mg (67%) of the monohydrate, m.p. 251–254°, $[\alpha]_D^{25} = -32.0°$ (c = 1, DMF), Rf 0.62 (A), 0.26 (C).

$C_{63}H_{78}N_8O_{12}S_2 \cdot H_2O$	Calc.	C 61.94	H 6.60	N 9.17	S 5.25	H_2O 1.48%
(1221.5)	Found	,, 61.90	,, 6.52	,, 9.10	,, 5.17	,, 0.94%

βMp-Tyr-Ile-Gln-Asn-Cys-OH (**6a**). The protected derivative **5a** (540 mg: 0.5 mmole) and urea (120 mg; 4 mmoles), dissolved in liquid ammonia (250 ml) freshly distilled from sodium, were treated with sodium, added in small portions, by the extraction procedure [24]; the end-point (blue coloration persisting for 20 s) was reached after about 90 mg (4 mg-at.) Na had been added. After addition of NH_4Cl (214 mg; 4 mmoles) the ammonia was removed by lyophilisation and the residue was dissolved in 20% aq. MeOH (250 ml). This solution was added dropwise, and simultaneously with a solution of freshly recrystallised 1,2-diiodo-ethane (155 mg; 0.55 mmole) in MeOH (150 ml), during 3 h to a stirred mixture of MeOH (50 ml) and water (150 ml), the whole addition being carried out under nitrogen and the pH kept at 7.5 by the addition of 5% $NaHCO_3$ [10]. The mixture was stirred under nitrogen until the reaction for free thiol groups [25] was negative (3.5 h), evaporated to about 100 ml, filtered, acidified to pH 2.5–3 with acetic acid, and lyophilised. The residue was taken up in 20 ml each of the upper and lower phase of the solvent system *n*-BuOH/2.5% aq. AcOH (1:1), filtered, and subjected to countercurrent distribution in a 'Steady State Distribution Apparatus' (*Quickfit & Quartz*, Stone, England). After 142 transfers of upper and 112 of lower phase the peptide was located by tlc. and colorimetrically by the *Lowry* reaction [26]. The material in tubes 70–105 (K = 4.1) was recovered by evaporation followed by lyophilisation, and the product (140 mg) was precipitated from solution in MeOH with 2-propanol and ether to give 120 mg (33%) of **6a**, m.p. 174–176° (*Kofler* block), Rf 0.43 (A), 0.47 (B). In both systems a trace impurity (<1–2%) was seen. Amino-acid ratios: NH_3 2.1 (2), Asp. 1.03 (1), Glu 1.05 (1), $\frac{1}{2}Cys_2$ 0.48 (1), Ile 0.96 (1), Tyr 1.0 (1). Elemental analysis indicated the presence of 0.5 mol H_2O.

$C_{30}H_{43}O_{10}N_7S_2 \cdot \frac{1}{2}H_2O$	Calc.	C 49.03	H 6.03	N 13.34	H_2O 1.22%
(734.8)	Found	,, 49.11	,, 6.33	,, 13.11	,, 1.40%

This material was used in the next synthetic step. A sample (20 mg) was dissolved in 0.1 M AcOH (0.4 ml) at 80° and allowed to crystallise during 1 day at room temperature and 4 days at 4°. Yield 14.2 mg (71%) of material, m.p. 155–158° with sintering from 147° (*Kofler* block), $[\alpha]_D^{25} = -67.6°$ (c = 0.5, 1 M AcOH), which by analysis was the tetrahydrate.

$C_{30}H_{43}N_7O_{10}S_2 \cdot 4H_2O$ (797.9) Calc. C 45.16 H 6.44 N 12.29% Found C 45.11 H 6.18 N 12.20%

Boc-Cys-Tyr-Ile-Gln-Asn-Cys-OH (**6b**). Following the procedures described for the preparation of **6a** above, the protected pentapeptide **5b** (200 mg; 0.16 mmole) and urea (40 mg; 0.66 mmole) in liquid ammonia (150 ml) were treated with sodium (30 mg; 1.3 mg-at.) followed by NH_4Cl (70 mg; 1.3 mmoles) and, after removal of the ammonia by lyophilisation, the residue, dissolved in 20% aq. MeOH (100 ml), was added, together with 1,2-diiodo-ethane (45 mg; 0.16 mmole) in MeOH (40 ml), to 25% aq. MeOH under nitrogen, the pH being kept at 7.6. After 3 h the solution was evaporated to about 50 ml, adjusted to pH 2.5–3, filtered, and lyophilised. The residue was extracted with EtOH (3×15 ml), the combined extracts were filtered, evaporated

to 10–15 ml and diluted with ethyl acetate (10 ml). The precipitate was collected, washed with ethyl acetate, dried (125 mg), and dissolved in 20 ml each of the upper and lower phase of the solvent system *n*-BuOH/2.5% aq. AcOH (1:1). After 140 distribution steps (80 transfers of upper and 60 of lower phase) the material was recovered from tubes 90–110 ($K \sim 18$) by evaporation and lyophilisation. The residue (72 mg) in EtOH (2 ml) was chromatographed on a column (1.5 × 90 cm) of *Sephadex* LH-20 and eluted with the same solvent at 13–15 ml/h. The fractions containing the desired product (absorption at 280 nm, tlc.) were pooled, evaporated to 2 ml, and diluted with ether. The precipitated peptide **6b** (41.8 mg; 30%), m.p. 195–198°, $[\alpha]_D^{25} = -67.3°$ ($c = 0.5$, EtOH) appeared homogeneous by tlc., Rf 0.25 (A), 0.31 (B). Amino-acid ratios: Asp 1.05 (1), Glu 1.09 (1), Ile 1.0 (1), Tyr 0.85 (1), $\frac{1}{2}Cys_2$ 1.38 (2), NH_3 2.88 (2). Elemental analysis indicated the presence of about 3 mol H_2O; the water content was determined from the loss on drying at 100°/0.01 Torr.

$C_{35}H_{52}N_8O_{12}S_2 \cdot 3H_2O$	Calc.	C 46.97	H 6.53	N 12.52	H_2O 6.04%
(895.0)	Found	,, 46.78	,, 6.26	,, 12.27	,, 5.8%

Deamino-oxytocin (**7a**). A solution of **6a** (36 mg; 0.05 mmole), Pro-Leu-Gly-NH_2 (20 mg hemihydrate; 0.07 mmole), and 1-hydroxy-benzotriazole (13.5 mg; 0.1 mmole) in DMF (0.5 ml) was treated at 0° with DCCI (12 mg; 0.06 mmole) in DMF (0.2 ml), stirred at 0° for 1.5 h and at room temperature for 24 h, filtered, and evaporated to dryness. The residue was taken up in methanol (2.5 ml), the solution was filtered and passed through a mixed-bed column (0.6 × 10 cm) of *Amberlyst* 15 (H^+ form) and *Amberlyst* A-21 (OH^- form). The methanolic eluates showing absorption at 280 nm were pooled (50 ml), evaporated to about 10 ml, diluted with 0.2 M AcOH (15 ml), the rest of the MeOH was evaporated, the solution was filtered and lyophilised. The residue (51 mg) in 0.2 M AcOH (1.8 ml) was chromatographed on a column (1.5 × 118 cm) of *Sephadex* G-15 and eluted with the same solvent (16 ml/h, fractions 2.4 ml). The product, appearing as a symmetrical peak centered at Rf 0.41, was eluted and recovered from fractions 48–58 by evaporation followed by lyophilisation; yield 25 mg. Of this material, 20 mg were recrystallised from water (0.6 ml) to give 13.9 mg (overall yield 33%) of **7a** as the trihydrate, m.p. 174–176°, $[\alpha]_D^{25} = -92.4°$ ($c = 0.5$ in 1 M AcOH), Rf 0.27 (A), 0.34 (B). The uterotonic activity was 698 ± 58 IU/mg. Amino-acid ratios: NH_3 3.0 (3), Asp 0.99 (1), Glu 1.03 (1), Pro 1.05 (1), Gly 1.0 (1), $\frac{1}{2}Cys_2$ 0.49 (1), Ile 0.95 (1), Leu 0.99 (1), Tyr 0.95 (1)

$C_{43}H_{65}N_{11}O_{12}S_2 \cdot 3H_2O$	Calc.	C 49.37	H 6.84	N 14.73	H_2O 5.17%
(1046.2)	Found	,, 49.51	,, 6.65	,, 14.60	,, 6.20%

The literature records m.p. 179°, $[\alpha]_D^{20} = -90.4°$ ($c = 0.5$, 1 M AcOH) for crystalline deamino-oxytocin [13] and a uterotonic activity of 551 ± 17 IU/mg (oestrus) or 899 ± 24 IU/mg (met-oestrus) [27].

Boc-Oxytocin (**7b**). The hexapeptide derivative **6b** (20 mg; 24 μmoles), Pro-Leu-Gly-NH_2 (10.2 mg hemihydrate; 35 μmoles) and 1-hydroxy-benzotriazole (4.8 mg; 36 μmoles) in DMF (0.4 ml) were treated at 0° with DCCI (6.2 mg; 30 μmoles) in DMF (0.4 ml). After standing at 0° for 1 h and at room temperature for 16 h, the reaction mixture was evaporated to dryness, the residue was taken up in MeOH (1.5 ml), filtered, and passed through a mixed-bed column (0.6 × 11 cm) of *Amberlyst* 15 and *Amberlyst* A-21 as described for the preparation of **7a**. The eluates containing the product were pooled, evaporated to 4–5 ml and diluted with 0.2 M AcOH (5 ml). The solution was kept at 4° for 24 h, filtered from dicyclohexylurea, evaporated down and finally lyophilised. The residue appeared homogeneous by tlc., Rf 0.44 (A), 0.35 (B). It was dissolved in 2-propanol (0.4 ml) and precipitated by dilution with di-2-propyl ether to give 11.3 mg (39%) of **7b**, m.p. 164–166° (sinters from 152°), $[\alpha]_D^{25} = -72.2°$ ($c = 0.4$, EtOH). Amino-acid ratios: NH_3 3.5 (3), Asp 1.0 (1), Glu 1.07, Pro 1.07 (1), Gly 0.97 (1), $\frac{1}{2}Cys_2$ 1.43 (2), Ile 1.01 (1), Leu 0.93 (1), Tyr 0.85 (1). Elemental analysis indicated the presence of about 6 mol H_2O.

$C_{48}H_{74}N_{12}O_{14}S_2 \cdot 6H_2O$ (1215.4) Calc. C 47.43 H 7.13 N 13.54% Found C 47.74 H 6.67 N 13.83%

Oxytocin (**7c**). The Boc derivative **7b** (7 mg; 6 μmoles) was treated with 90% trifluoroacetic acid (1 ml) at room temperature for 30 min, the solution was diluted with water to 40 ml, evaporated to about 5 ml, once more diluted with water to 40 ml, evaporated down, and lyophilised. The residue, which contained traces of the dimer (tlc.), was purified by chromatography [12] on a column (1.5 × 108 cm) of *Sephadex* G-15 in 0.2 M AcOH. The fractions centered around Rf 0.55 were pooled, evaporated down and lyophilised to afford 4.2 mg (70%) of oxytocin, Rf 0.47 (A),

0.39 (B); m_{Arg} 0.29 (F), homogeneous and identical with a sample of natural oxytocin in all three systems; $[\alpha]_D^{25} = -25.5°$ (c = 0.4, 1 M AcOH). The uterotonic activity was 511 ± 24 IU/mg. Amino-acid ratios: NH_3 3.22 (3), Asp 0.96 (1), Glu 1.11 (1), Pro 1.04 (1), Gly 1.0 (1), $\frac{1}{2}Cys_2$ 1.84 (2), Ile 0.97 (1), Leu 1.03 (1), Tyr 0.92 (1). The literature gives $[\alpha]_D = -26.2°$ for natural [14] and $-26.1 \pm 1°$ for synthetic [15] oxytocin (c = 0.53, H_2O), and $-23.1°$ (c = 0.51, 1 M AcOH) for the synthetic peptide [16], and a maximal uterotonic activity of about 540 IU/mg [27].

We are obliged to Mrs *H. Marzoll* for measurement of the optical rotations and to Miss *H. Schneider* for the amino acid analyses. The elemental analyses were generously carried out by the Analytical Laboratory of *F. Hoffmann-La Roche & Co.*, Basel, Switzerland.

This work was supported by *Swiss National Science Foundation* grants No. 4883, 3.372.70, and 3.424.70. One of us (*M.I.T.*) is obliged to the Swiss Federal Government for an Exchange Studentship.

Note Added in Proof: The acid **6a** has now also been described by *V. J. Hruby, C. W. Smith, D. K. Linn, M. F. Ferger & V. du Vigneaud* [J. Amer. chem. Soc. *94*, 5478 (1972)] as the amorphous monohydrate, $[\alpha]_D^{22} = -57.0°$ (c = 0.5, 1 M AcOH). More recent preparations in the same laboratory (*W. C. Jones, J. Nestor & V. du Vigneaud*, unpublished; personal communication from Professor *V. du Vigneaud*) have shown $[\alpha]_D^{24} = -60.2°$ and $[\alpha]_D^{23} = -62.5°$.

BIBLIOGRAPHY

[1] *W. Rittel, M. Brugger, B. Kamber, B. Riniker & P. Sieber*, Helv. *51*, 924 (1968); *B. Riniker, M. Brugger, B. Kamber, P. Sieber & W. Rittel, ibid 52*, 1058 (1969).
[2] *P. Sieber, M. Brugger, B. Kamber, B. Riniker & W. Rittel*, Helv. *51*, 2057 (1968); *P. Sieber, B. Riniker, M. Brugger, B. Kamber & W. Rittel, ibid 53*, 2135 (1970).
[3] *B. Kamber & W. Rittel*, Helv. *52*, 1074 (1969); *B. Kamber, H. Brückner, B. Riniker, P. Sieber & W. Rittel, ibid 53*, 556 (1970).
[4] *IUPAC-IUB Commission on Biochemical Nomenclature*, European J. Biochem. *1*, 375 (1967).
[5] *W. König & R. Geiger*, Chem. Ber. *103*, 788 (1970).
[6] *B. Belleau & G. Malek*, J. Amer. chem. Soc. *90*, 1651 (1968).
[7] *M. F. Ferger, W. C. Jones Jr., D. F. Dyckes & V. du Vigneaud*, J. Amer. chem. Soc. *94*, 982 (1972).
[8] *R. H. Sifferd & V. du Vigneaud*, J. biol. Chemistry *108*, 753 (1935).
[9] *H. Zimmermannová-Maassen van den Brink & J. Rudinger*, manuscript in preparation; *H. Zimmermannová*, Thesis, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague 1966.
[10] *F. Weygand & G. Zumach*, Z. Naturforsch. *17b*, 807 (1962).
[11] *M. Zaoral & J. Rudinger*, Coll. Czechoslov. chem. Commun. *20*, 1183 (1955); *R. A. Boissonnas, S. Guttmann, P.-A. Jaquenoud & J.-P. Waller*, Helv. *38*, 1491 (1955).
[12] *M. Manning, T. C. Wuu & J. W. M. Baxter*, J. Chromatogr. *38*, 396 (1968).
[13] *B. M. Ferrier, D. Jarvis & V. du Vigneaud*, J. biol. Chemistry *240*, 4264 (1965).
[14] *S. Gordon & V. du Vigneaud*, Proc. Soc. exp. biol. Med. *84*, 723 (1953).
[15] *V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts & P. G. Katsoyannis*, J. Amer. chem. Soc. *76*, 3115 (1954).
[16] *C. Ressler & V. du Vigneaud*, J. Amer. chem. Soc. *79*, 4511 (1957).
[17] *A. J. Woiwod*, J. Chromatogr. *3*, 278 (1960).
[18] *C. G. Greig & D. H. Leaback*, Nature *188*, 310 (1960); *E. von Arx & R. Neher*, J. Chromatogr. *12*, 329 (1963).
[19] *F. Reindel & W. Hoppe*, Chem. Ber. *87*, 1103 (1954).
[20] *P. Holton*, Brit. J. Pharmacol. *3*, 328 (1948).
[21] *R. A. Munsick*, Endocrinology *66*, 451 (1960).
[22] *K. C. Hooper, H. N. Rydon, J. A. Schofield & G. S. Heaton*, J. chem. Soc. *1956*, 3148.
[23] *D. B. Hope, V. V. S. Murti & V. du Vigneaud*, J. biol. Chemistry *237*, 1563 (1962).
[24] *H. Nesvadba & H. Roth*, Mh. Chem. *98*, 1432 (1967).
[25] *G. E. Ellman*, Arch. Biochem. Biophysics *82*, 70 (1959).
[26] *O. H. Lowry, N. J. Rosebrough, A. Z. Farr & R. J. Randall*, J. biol. Chemistry *193*, 265 (1951).
[27] *W. Y. Chan, M. O'Connell & S. R. Pomeroy*, Endocrinology *72*, 279 (1963).