(11) N. F. Blau, J. W. Johnson, and C. G. Stuckwisch, *J. Am. Chem. Soc.*, **76**, 5106 (1954).
(12) R. M. DeMarinis, J. R. E. Hoover, L. L. Lam, J. V. Uri, J. R. Guarini, L. Phillips, P. Actor, and J. A. Weisbach, *J. Med. Chem.*, **19**, 754 (1976).
(13) H. R. Le Seur and P. Haas, *J. Chem. Soc.*, **97**, 173 (1910).
(14) J. A. Moore and J. E. Kelly, *Org. Prep. Proced. Int.*, **6**, 255 (1974).

Synthesis and Some Pharmacological Properties of [4-Homoserine]oxytocin

Andras Turan, Maurice Manning,*

Department of Biochemistry, Medical College of Ohio, Toledo, Ohio 43699

Jaya Haldar, and Wilbur H. Sawyer

Department of Pharmacology, College of Physicians & Surgeons of Columbia University, New York, New York 10032.
Received March 7, 1977

The incorporation of homoserine [Hse] into synthetic peptides has proved extremely difficult due to the ease with which homoserine derivatives form a γ-lactone. We now wish to report the synthesis of [4-homoserine]oxytocin. We wished to obtain this peptide to test our earlier hypothesis as to why threonine substitution in the 4 position in oxytocin enhanced both oxytocic activity (O) and oxytocic–antidiuretic (O/A) selectivity relative to oxytocin. The key to this synthesis was the preparation of N^{α}-*tert*-butyloxycarbonyl-*O*-benzyl-L-homoserine [Boc-Hse(Bzl)] by a modification of a recently published method used for the benzylation of N^{α}-*tert*-butyloxycarbonylserine (Boc-Ser). Boc-Hse(Bzl) was incorporated into the protected nonapeptide Z-Cys(Bzl)-Tyr(Bzl)-Ile-Hse(Bzl)-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 using the solid-phase method by procedures previously described from these laboratories. Following deblocking, cyclization, and purification in the usual manner the desired peptide, [4-homoserine]oxytocin, was obtained. An examination of its pharmacological properties provided the following potencies (units/mg): rat oxytocic (O) 125 ± 13; rat antidiuretic (A) 0.24 ± 0.03. Its oxytocic–antidiuretic ratio is thus 521. These data indicate that threonine and homoserine when substituted at position 4 in oxytocin exert similar effects on O/A selectivity (cf. [4-threonine]oxytocin has an O/A ratio of 512) but that threonine exerts a unique effect in leading to enhanced oxytocic activity (cf. [4-threonine]oxytocin has an oxytocic potency of 923 ± 96 units/mg). This successful synthesis of Boc-Hse(Bzl) and its incorporation into [4-homoserine]oxytocin now make possible the synthesis of homoserine analogues of other peptides.

The substitution of threonine for glutamine in oxytocin gave a compound [Thr^4]oxytocin which possesses very unusual properties.[1] Its oxytocic potency is almost twice that of oxytocin whereas its antidiuretic potency is less than one-third that of oxytocin. In attempting to explain why threonine had such unusual effects, we proposed that examination of the properties of [Hse^4]oxytocin might shed some light on this intriguing question.[2] Thus in 1970 we undertook the synthesis of this compound. This synthesis proved extremely difficult and has only recently been finally accomplished. The difficulty in incorporating homoserine into synthetic peptides stems from the well-known tendency of γ-hydroxyamino acids and their derivatives to form a γ-lactone.[3-15] Thus to date to our knowledge there is only one reported instance in which homoserine was incorporated into a synthetic peptide.[11] The method employed the coupling of homoserine as the C-terminal lactone of a dipeptide to give an analogue of tetragastrin in a yield of only 20%. We have tried unsuccessfully to adapt this approach to the synthesis of [Hse^4]oxytocin. After many unsuccessful attempts to synthesize [Hse^4]oxytocin, we now report its successful synthesis by an approach which should be widely applicable for the synthesis of homoserine analogues of other peptides.

The key to this synthesis was the preparation of N^{α}-*tert*-butyloxycarbonyl-*O*-benzyl-L-homoserine [Boc-Hse(Bzl)] using an adaptation of a recently published procedure for the benzylation of N^{α}-*tert*-butyloxycarbonyl-L-serine (Boc-Ser).[16] Boc-Hse(Bzl) was incorporated into the required protected nonapeptide intermediate by the solid-phase method[17] as described for the synthesis of [Thr^4]oxytocin.[1] Deblocking with sodium in liquid ammonia,[18] cyclization, and purification by gel filtration on Sephadex G-15 by the two-step procedure involving 50% AcOH and 0.2 N AcOH as eluents, respectively, as previously described[19,20] afforded the desired free peptide. The peptide was assayed for oxytocic activity on the isolated rat uterus suspended in solutions containing no Mg^{2+} or 0.5 mM Mg^{2+}.[21] Milk-ejection activity was measured by intravenous injection of the peptide into lactating rats anesthetized with pentobarbital.[22] Antidiuretic activity was estimated by intravenous injection into hydrated rats anesthetized with ethanol.[23] Vasopressor assays were performed on phenoxybenzamine-treated rats under urethane anesthesia.[24] The USP posterior pituitary reference standard was used in all assays.

Results and Discussion

Synthetic Aspects. Attempts to incorporate homoserine into synthetic peptides have to date met with little success. Morley and Smith coupled Z-Trp-DL-Hse lactone and Asp-Phe-NH_2 to obtain Z-Trp-DL-Hse-Asp-Phe-NH_2 in low yield.[11] When these authors attempted to couple Z-Trp-DL-Hse azide (obtained from the above lactone), the end product contained mainly lactone. We attempted unsuccessfully to couple *tert*-butyloxycarbonylhomoserine lactone to Asn-Cys(Bzl)-Pro-Leu-Gly-resin. From these results it was clear that N-protected homoserine lactone or N-protected peptides ending with homoserine lactone are not active enough to give satisfactory yields in the peptide bond forming step unless promoted by other driving forces. Such forces have been very elegantly demonstrated in the synthesis of [52-homoserine] basic pancreatic trypsin inhibitor (BPTI) by cyanagen bromide cleavage of the native molecule at the methionine residue in position 52, followed at neutral pH by a conforma-

Table I. Pharmacological Activities (Units/mg ± Standard Errors) of [4-Homoserine]oxytocin, Oxytocin, and Some Other 4-Substituted Analogues of Oxytocin

Peptide	Substituents at position 4 β	γ	Rat oxytocic, no Mg^{2+} (0)	0.5 mm Mg^{2+}	Rabbit milk ejection[h]	Rat antidiuretic (A)	Rat vasopressor (P)	O/A	O/P
1 [Hse⁴]oxytocin[a]	-CH(H)CH_2OH		125 ± 13	380 ± 28	357 ± 18*	0.24 ± 0.03	0.021 ± 0.001	520	5950
2 Oxytocin[b]	-CH(H)CH_2CONH_2		520 ± 12	486 ± 15	474 ± 16	4.0 ± 0.8	4.3 ± 0.1	130	121
3 [Thr⁴]oxytocin[b]	-CH(CH_3)OH		923 ± 95	719 ± 83	543 ± 23	1.8 ± 0.3	0.43 ± 0.01	510	2150
4 [Ser⁴]oxytocin[c]			195 ± 30		255 ± 45	0.06 ± 0.01	<0.1	3200	>1950
4 [Ser⁴]oxytocin[d]	-CH(H)OH		148 ± 6	452 ± 8		1.04 ± 0.54		140	
4 [Ser⁴]oxytocin[e]			197 ± 5	331 ± 20	423 ± 35	2.4 ± 0.3	0.028 ± 0.002	80	7040
5 [Asn⁴]oxytocin[f]	-CH(H)$CONH_2$		108 ± 29		300 ± 128	0.044 ± 0.005	0.13 ± 0.03	2450	823
5 [Asn⁴]oxytocin[g]			158 ± 6	490 ± 15	382 ± 12*	0.17 ± 0.01	0.12 ± 0.01	929	1320

[a] Present communication. [b] Values reported by Manning et al.[33] [c] Values reported by Guttman et al.[27] [d] Assays done on a solution provided by Dr. Berde. [e] Assays done on a preparation of [Ser⁴]oxytocin synthesized by the solid-phase method in these laboratories but not yet reported. [f] Values reported by Jacquenoud and Boissonnas.[28] [g] Assays done on a preparation of [Asn⁴]oxytocin synthesized by the solid-phase method in these laboratories but not yet reported. [h] An asterisk indicates rat milk ejection.

tionally induced cyclization of the homoserine lactone derivative.[15] The forgoing and other well-documented data[3-15] on the tendency of γ-hydroxyamino acids and their derivatives to lactonize thus pointed to the clear need for protection of the γ-hydroxyl group as a prerequisite for the successful incorporation and retention of homoserine in synthetic peptides. We thus embarked on the synthesis of Boc-Hse(Bzl). *tert*-Butyloxycarbonyl-L-homoserine was obtained in excellent yield free from lactone as an oil by a modification of the Me_2SO method.[25] It bears mentioning, however, that on standing at room temperature spontaneous lactone formation occurred. Conversion to the dicyclohexylammonium (DCHA) salt eliminated this problem and also facilitated the characterization of this material. Boc-DL-Hse was obtained in the same manner and was used in initial exploratory experiments. The method of Hruby et al.[26] employing sodium in liquid ammonia and benzyl bromide for the preparation of Boc-Ser(Bzl) was followed to prepare Boc-DL-Hse(Bzl) in good yield. However, due to differences in the solubilities of Boc-DL-Hse and Boc-L-Hse when this method was employed for the synthesis of Boc-L-Hse(Bzl), the results were unsatisfactory, i.e., elimination of the starting material was more difficult in the latter case. We thus turned to the method of Sugino and Miyoshi[16] for the successful benzylation of Boc-L-homoserine with sodium hydride and benzyl bromide as described in the Experimental Section. Lactonization of the free Boc-Hse was avoided by direct conversion in DMF of the Boc-Hse DCHA salt into the Boc-Hse sodium salt, required for the benzylation step, with aqueous sodium chloride.

Boc-L-Hse(Bzl), obtained as an oil, was characterized as its cyclohexylammonium (CHA) salt. It was incorporated into the required protected nonapeptide intermediate without any difficulty using the Merrifield solid-phase method. The overall yield of the purified homoserine containing protected peptide was 62%. This figure compares very favorably with the yields of other protected peptides obtained by similar methods in these laboratories. Conversion of the protected intermediate to the purified [Hse⁴]oxytocin was effected in 47% yield. Thus the synthesis of Boc-L-Hse(Bzl) has made possible the synthesis of [Hse⁴]oxytocin as reported here. It should also now permit the ready synthesis of homoserine-containing analogues of other peptides.

Pharmacological Aspects. The properties of [Hse⁴]oxytocin are listed in Table I along with those of some other 4-substituted analogues of oxytocin. Whereas its oxytocic–antidiuretic selectivity approximates that of [Thr⁴]oxytocin, its oxytocic activity is only one-seventh that of [Thr⁴]oxytocin. The synthesis of [Hse⁴]oxytocin was originally proposed in an attempt to explain the high oxytocic potency of [Thr⁴]oxytocin. It was selected for this purpose on the basis of the properties of [Ser⁴]oxytocin[27] and [Asn⁴]oxytocin.[28] These molecules differ only in the nature of the substituents attached to the β-carbon atoms at their respective 4 positions (Table I). Thus the former peptide has a hydroxy group and the latter peptide a carboxamide group in this position. Since [Ser⁴]oxytocin is clearly more potent than [Asn⁴]oxytocin in the oxytocic assay system, it was inferred that this was due to the greater effectiveness of the OH group as compared to the $CONH_2$ group. In attempting therefore to explain why the threonine–glutamine interchange in oxytocin brought about a twofold enhancement of oxytocic activity, it was tempting to speculate that by analogy with the serine–asparagine interchange this was due to the greater effectiveness of the OH group in threonine over the $CONH_2$ group in glutamine. Homoserine and glutamine differ only at their γ positions, with the former peptide having an OH group and the latter peptide a $CONH_2$ group in this position. Thus, by comparing the properties of these two peptides, the relative contributions of the OH and $CONH_2$ groups on the γ carbons at position 4 could be ascertained. It is clear from the results reported here that the position of the OH is very critical for the manifestation of optimal oxytocic activity. Thus its presence on the β-carbon of [Thr⁴]oxytocin appears to be the critical factor in giving rise to such a potent oxytocic agent. These findings further point to an interesting position-dependent reversal of the effects of the OH–$CONH_2$ substituents on oxytocic potencies. When on the β-carbon the OH group exerts a greater effect than the $CONH_2$ group in producing more potent oxytocic activity. Thus [Ser⁴]oxytocin is a more potent oxytocic agent than [Asn⁴]oxytocin. When the OH–$CONH_2$ groups are attached to the γ carbon the order is reversed; the $CONH_2$ group produces a greater effect than the OH group. Thus oxytocin is over three times more potent than [Hse⁴]oxytocin as an oxytocic agent. In comparing the properties of [Thr⁴]oxytocin with those of [Ser⁴]oxytocin and [Hse⁴]oxytocin and with other 4-substituted analogues, it is now clear that the combination of the methyl and hydroxyl substituents on the β-carbon exerts a unique effect in producing enhancement of oxytocic potency. Thus of all the 4-substituted analogues of oxytocin synthesized to date, 4-threonine alone exhibits enhanced oxytocic activity relative to oxytocin. The extremely low vasopressor activities of [Hse⁴]oxytocin and [Ser⁴]oxytocin should also be noted (Table I). Thus with respect to oxytocic–pressor (O/P) ratios, these peptides

exhibit the highest selectivities known to date.

Experimental Section

Chloromethylated resin (Bio-Rad Bio-Beads SX-1) was esterified[29] to Boc-Gly to an incorporation of 0.45 mmol g^{-1}. L-Homoserine and *tert*-butyloxycarbonyl azide were purchased from Bachem, Inc. DL-Homoserine was purchased from Calbiochem. The other amino acid derivatives were purchased from Bachem or from Beckman Bioproducts Division. The purity of each one was checked by TLC as described in ref 25 and by a melting point determination. Triethylamine was distilled from ninhydrin; methanol was dried with magnesium methoxide and distilled; dimethylformamide (DMF) was distilled under reduced pressure immediately prior to its use. Other solvents and reagents were analytical grade. Thin-layer chromatography (TLC) was carried out by the ascending technique on silica gel (0.25-mm Brinkman Silplate). Solvent systems were A, butan-1-ol–acetic acid–water (4:1:5 v/v), upper phase; B, ethyl acetate–pyridine–acetic acid–water (240:20:6:11); C, ethyl acetate–pyridine–acetic acid–water (480:20:6:11); D, butan-1-ol–acetic acid–water–pyridine (15:3:3:10); E, chloroform–methanol (70:30).

Loads of 10–50 μg were applied and ninhydrin and chlorine–potassium iodide–tolidine and chloroplatinate were used for detection. For amino acid analysis samples (ca. 0.5 mg) were hydrolyzed with constant boiling hydrochloric acid (400 μL) in evacuated and sealed ampules for 18 h at 110 °C. The analyses were performed by the method of Spackman et al.[30] using a Beckman automatic amino acid analyzer Model 121. Melting points were taken in an open capillary in a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. All optical rotations were measured on a Bellingham Stanley, Ltd., Model A polarimeter.

N^{α}-*tert*-Butyloxycarbonyl-L-homoserine (I).[34] A suspension of 5.95 g (50 mmol) of homoserine, 100 mL of DMF, 5 mL of H_2O, 8.58 mL (60 mmol) of *tert*-butyloxycarbonyl azide, and 15.4 mL (110 mmol) of triethylamine was stirred at room temperature for 36 h. Complete solution was achieved after 12 h. Following evaporation of the solvent the residual oil was dissolved in 26 mL of 2 N NaOH and 26 mL of H_2O. The combined extracts were washed with chloroform saturated with water (3 × 20 mL). The combined chloroform washings were washed with H_2O (10 mL). The aqueous phases were combined, acidified with 25 mL of cooled 2 N HCl, and extracted with *n*-BuOH saturated with water (30 mL × 6). The combined *n*-BuOH extracts were washed with water saturated with *n*-BuOH (10 mL × 3) and the butanol was evaporated to give the required *tert*-butyloxycarbonyl-L-homoserine (I) as an oil: R_f (A) 0.64, R_f (B) 0.35 (cf. *tert*-butyloxycarbonyl-DL-homoserine lactone, R_f (A) 0.7, R_f (B) 0.88, R_f (C) 0.85). This material was immediately converted to the dicyclohexylammonium salt (II) for characterization and to avoid spontaneous lactone formation. It was thus stored as the DCHA salt.

N^{α}-*tert*-Butyloxycarbonyl-L-homoserine Dicyclohexylammonium Salt (II). *tert*-Butyloxycarbonylhomoserine (I) was dissolved without delay in 250 mL of ether and the cloudy solution dried over $MgSO_4$. The $MgSO_4$ was collected on a filter and washed with ether (50 mL × 3). The combined ethereal filtrate and washings, now clear, were treated with 9.8 mL (50 mmol) of dicyclohexylamine and the desired salt precipitated out of solution. The suspension was stored in the ice box for 12 h and the precipitate was collected, washed with ether, and dried in vacuo over P_2O_5 to give 16.05 g (80.16% based on homoserine) of white crystalline product: mp 148–149 °C; $[\alpha]^{23}_D$ −2.6° (*c* 2, DMF). This material was used in the subsequent benzylation procedure. For analytical purposes a sample (1 g) was recrystallized from 16 mL of ethyl acetate and 18 mL of ether to give 0.89 g (89%): mp 149–150 °C; $[\alpha]^{23}_D$ −3.5° (*c* 2, DMF); R_f (A) 0.64, R_f (B) 0.35, R_f (C) 0.29. Anal. ($C_{21}H_{40}O_5N_2$) C, H, N.

N^{α}-*tert*-Butyloxycarbonyl-*O*-benzyl-L-homoserine (IIIA and IIIB). II (4.0056 g, 10 mmol) was dissolved in 70 mL of DMF with gentle warming. NaCl (0.5844 g, 10 mmol) in 6 mL of H_2O was added and the resultant cloudy solution cooled in an ice box for 2 h. The precipitated dicyclohexylammonium hydrochloride was removed by filtration and washed on a filter with cold DMF (3 × 5 mL). The combined filtrate and washings were evaporated. The residual oil was redissolved in DMF (10 mL) and the undissolved material removed by filtration and washed with DMF (3 × 2 mL). The filtrate and washings were reevaporated. The residual oil was redissolved in 30 mL of DMF and cooled to 0 °C. To this solution was added sodium hydride (50% oily suspension) (0.528 g, 11 mmol). The suspension was stirred in an ice bath for 1.5 h to give a viscous solution. Benzyl bromide (1.32 mL, 10 mmol) was added with stirring. The reaction mixture was stirred for 2 h at 0 °C and at room temperature overnight and evaporated. The residue was taken up with water (10 mL) and ether (20 mL). The aqueous phase was further extracted with ether (3 × 20 mL). The combined ethereal washings were washed with 5% $NaHCO_3$ (20 mL). The combined aqueous layers were acidified with solid citric acid and extracted with ether (3 × 80 mL). The ethereal solution was washed with H_2O until neutral. The combined ethereal solutions were extracted with $NaHCO_3$ (3 × 40 mL). The aqueous extract was reacidified with solid citric acid and extracted with chloroform (3 × 40 mL). The combined chloroform layers were washed (5 × 30 mL) with water and dried over $MgSO_4$. Following removal of the drying agent the chloroform was removed on a rotary evaporator to give Boc-Hse(Bzl) (IIIA) as an oil (1.55 g, 49.5%). This was shown to be homogeneous by TLC examination: R_f (A) 0.80, R_f (B) 0.74. For characterization purposes IIIA was converted to its cyclohexylammonium salt IV. A repeat preparation gave IIIB also as an oil: 1.5 g (48.4%); R_f (A) 0.80, R_f (B) 0.74. This material was shown to be identical with IIIA and with material liberated from IV and was used directly, i.e., without conversion to IV in the synthesis of the protected nonapeptide on the resin as described below.

N^{α}-*tert*-Butyloxycarbonyl-*O*-benzyl-L-homoserine Cyclohexylammonium Salt (IV). IIIA (1.55 g) was dissolved in 100 mL of diisopropyl ether–heptane (1:1). Cyclohexylamine (0.59 mL, 5 mmol) was added. A precipitate formed. The suspension was stored in the refrigerator overnight. The product was collected, washed with cold diisopropyl ether–heptane (1:1), and dried in vacuo over P_2O_5: wt 2.02 g (99%); mp 145–147 °C; $[\alpha]^{23}_D$ +3° (*c* 3, methanol); R_f (A) 0.80, R_f (B) 0.74, R_f (C) 0.63. Anal. ($C_{22}H_{36}N_2O_5$) C, H, N, O.

Z-Cys(Bzl)-Tyr(Bzl)-Ile-HSe(Bzl)-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (V). Boc-Gly-resin (1.11 g, 0.5 mmol of glycine) was treated as described for the synthesis of [4-threonine]oxytocin[1] except that Boc-Hse(Bzl) (IIIB) was used in place of Boc-Thr(Bzl) in the fifth coupling step. Also the chloroform washes were omitted and a pyridine hydrochloride treatment[31] followed by neutralization was included after the final coupling step. Eight cycles of deprotection, neutralization, and coupling were carried out on successive days.

At the conclusion of the synthesis the protected peptide resin was washed out of the reaction vessel with methylene chloride, methanol, and ether, collected on a filter, and dried in vacuo: wt 1.71 g. The weight gain of 0.6 g (0.46 mmol) at this stage indicated at 92.1% incorporation of protected peptide based on the initial glycine (0.5 mmol) on the resin.

Ammonolytic cleavage of the protected peptide resin (1.71 g) was carried out as described earlier[1,32] except that the ammonia-saturated methanolic suspension was stirred at room temperature for 3 days. Following removal of the methanolic ammonia the cleaved protected peptide was extracted with warm DMF (10 mL). The resin was further washed with warm DMF (3 × 5 mL), DMF–MeOH (1:1, 5 mL), and MeOH (3 × 5 mL). Addition of H_2O (55 mL) to the combined washings caused the protected peptide to precipitate as a gelatinous solid. The suspension was cooled in the ice box for 2 h; the precipitate was collected, washed with MeOH–H_2O (1:1), MeOH, and ether, and dried in vacuo over P_2O_5: wt 0.569 g (83.6%). TLC examination in solvent A revealed faint traces of impurities. This material was redissolved in warm AcOH (10.5 mL) and EtOH (47 mL) added. On standing in the ice box for 2 h the precipitate was collected, washed with MeOH and ether, and dried in vacuo: wt 0.47 g (68.62%). TLC examination still revealed the presence of traces of impurities. This material was dissolved in warm DMF (8 mL), reprecipitated by the addition of 80% MeOH (45 mL), and cooled in an ice box for 2 h. The precipitate was collected, washed with cooled 50% MeOH, MeOH, and ether, and dried in vacuo over P_2O_5 to give the required protected nonapeptide amide as a white amorphous

powder: wt 457 mg (0.31 mmol); mp 225–227 °C; $[\alpha]_D$ −47.5° (c 1, DMF); R_f (A) 0.83, R_f (E) 0.84. Anal. ($C_{78}H_{97}N_{11}O_{14}S_2$) C, H, N, O, S. Amino acid analysis gave Asp, 1.01; Pro, 0.96; Gly, 1.00; Hse lactone, 0.97; Ile, 0.89; Leu, 0.97; Cys(Bzl), 1.99; NH_3 1.94; Tyr, 0.55. In the presence of 1% phenol; Tyr 0.93 and Ile, 1.04 relative to Gly, 1.0. The yield of the purified protected peptide amide from the ammonolysis step was 67.3%. The overall yield based on the amount of glycine incorporated on the resin was 62%.

[4-Homoserine]oxytocin (VI). The protected nonapeptide V (100 mg, 0.068 mmol) in sodium-dried and redistilled ammonia (300 mL) was treated at the boiling point and with stirring with sodium[18] from a stick of the metal contained in a small bore glass tube until a light blue color persisted in the solution for 20 s.[1,14] The color was discharged by the addition of a few drops of dry acetic acid and the clear solution was evaporated. The residue was taken up in aqueous acetic acid (0.2%, 600 mL) and aqueous ammonia (2 M) added gradually to give a solution of pH 6.8. An excess of potassium ferricyanide solution (0.01 M, 13 mL) was added gradually and the yellow solution stirred for 10 min. Anion-exchange resin [Bio-Rad AG3, 100–200 mesh, chloride form (10 g damp weight)] was added and stirring continued for 10 min. The suspension was filtered through a bed of the resin (70 g damp weight) and the bed washed twice with aqueous acetic acid (0.2%, aliquot 100 mL). The combined filtrate and washings were lyophilized and the residue was desalted on a Sephadex G-15 column (2.8 × 102 cm) with 50% AcOH as the eluting solvent.[20,33] A total of 253 fractions of 2.0 mL were collected at a flow rate of 2.65 mL/h. Fractions 124–150 contained the desired monomer as monitored by TLC [R_f (A) 0.25], clearly separated from dimer (tubes 105–120) and salt (tubes 185–236). Fractions 124–150 were combined and lyophilized. The lyophilysate (46 mg) was dissolved in 0.5 mL of 50% AcOH and applied to the second column of Sephadex G-15 (1.6 × 80 cm) for elution with 0.2 × AcOH.[20] A total of 89 fractions of 1.6 mL were collected at a flow rate of 1.66 mL/h. The required peptide emerged as a single peak as monitored by UV absorbance at 280 nm, with a maximum at tube 60. The contents of the peak (tubes 58–63) were lyophilized to give [4-homoserine]oxytocin as a white fluffy powder: wt 33 mg (46.9% yield from protected nonapeptide; 29% from Boc-Gly-resin); $[\alpha]^{23}_D$ −12.0° (c 0.5, 1 N AcOH). This material was shown to be homogeneous by TLC examination, R_f (A) 0.25, R_f (D) 0.72, R_f (E) 0.11. Amino acid analysis gave Asp, 1.03; Pro, 0.98; Gly, 1.00; Hse, 0.91, Hse lactone, 0.08; Ile, 1.02; Leu, 1.02; Cys, 1.80; NH_3, 2.32.

Acknowledgment. This work was supported in part by research grants from the National Institute of Child Health and Human Development (No. HD 06351), the National Institute of Arthritis, Metabolism and Digestive Diseases (No. AM 01940), and National Heart and Lung Institute (No. HL 12738). The authors wish to thank Dr. John Lowbridge for helpful discussion and with Ms. Jacqueline Moehring for the amino acid analyses, Dr. Krystoff Bankowski for TLC examinations, and Ms. Cindy Licata for invaluable assistance in the preparation of the manuscript.

References and Notes

(1) M. Manning, E. Coy, and W. H. Sawyer, *Biochemistry*, **9**, 3925 (1970).
(2) M. Manning and W. H. Sawyer in "Progress in Peptide Research" (Proceedings of the Second American Peptide Symposium), S. Lande, Ed., Gordon and Breach, New York, N.Y., 1972, p 79.
(3) M. D. Armstrong, *J. Am. Chem. Soc.*, **71**, 3399 (1949).
(4) Y. Watanabe, S. Konishi, and K. Shimura, *J. Biochem.*, **42**, 837 (1955).
(5) J. P. Greenstein and M. Winitz in "Chemistry of the Amino Acids", Vol. 3, Wiley, New York, N.Y., 1961, p 2589.
(6) B. Witkop, *Adv. Protein Chem.*, **16**, 221 (1961).
(7) M. Flavin and C. Slaughter, *J. Biol. Chem.*, **235**, 1103 (1960).
(8) E. A. Bell and A. S. L. Tirimanna, *Biochem. J.*, **91**, 356 (1964).
(9) Y. Fujita, J. Kollomitsch, and B. Witkop, *J. Am. Chem. Soc.*, **87**, 2030 (1965).
(10) T. Wieland, *Fortschr. Chem. Org. Naturst.*, **25**, 214 (1967).
(11) J. S. Morley and J. M. Smith, *J. Chem. Soc.*, 726 (1968).
(12) F. Faulstich and H. Trischmann, *Justus Liebigs Ann. Chem.*, **74**, 55 (1970).
(13) C. S. Pande, J. Rudick, and R. Walter, *J. Org. Chem.*, **35**, 1440 (1970).
(14) T. F. Fickel and C. Gilvarg, *J. Org. Chem.*, **38**, 1421 (1973).
(15) D. F. Dyckes, T. Creighton, and R. C. Sheppard, *Nature (London)*, **247**, 202 (1974).
(16) H. Sugano and M. Miyoshi, *J. Org. Chem.*, 41, 2352 (1976).
(17) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
(18) V. du Vigneaud, C. Ressler, J. M. Swan, P. Katsoyannis, and C. W. Roberts, *J. Am. Chem. Soc.*, **76**, 3115 (1954).
(19) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, **237**, 1563 (1962).
(20) M. Manning, T. C. Wuu, and J. W. M. Baxter, *J. Chromatogr.*, **38**, 396 (1968).
(21) R. A. Munsick, *Endocrinology*, **66**, 451 (1960).
(22) G. W. Bisset, B. J. Clark, J. Haldar, M. C. Harris, G. P. Lewis, and M. Roche e Silva, Jr., *Br. J. Pharmacol.*, 31, 537 (1967).
(23) W. H. Sawyer, *Endocrinology*, **63**, 694 (1958).
(24) J. Dekanski, *Br. J. Pharmacol.*, 7, 567 (1952).
(25) J. M. Stewart and J. D. Young in "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969, p 29.
(26) V. J. Hruby and K. W. Ehler, *J. Org. Chem.*, **35**, 1690 (1970).
(27) St. Guttman, B. Berde, and E. Stumer, *Helv. Chim. Acta*, **46**, 1626 (1963).
(28) P. A. Jaquenoud and R. A. Boissonnas, *Helv. Chim. Acta*, **45**, 1601 (1962).
(29) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).
(30) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(31) L. C. Dorman, *Tetrahedron Lett.*, **28**, 2319 (1969).
(32) M. Manning, *J. Am. Chem. Soc.*, **90**, 1348 (1968).
(33) M. Manning, E. J. Coy, and W. H. Sawyer, *Experientia*, **27**, 1372 (1971).
(34) The preparation of *tert*-butyloxycarbonyl-DL-homoserine by the Me_2SO method[25] has been reported.[14] The product was obtained as an oil and was not further characterized. The preparation of *tert*-butyloxycarbonyl-L-homoserine was mentioned in this same report but experimental details were not given. We used the Me_2SO method in early attempts to synthesize *tert*-butyloxycarbonyl-DL-homoserine. Due to lactone formation and the more ready removal of DMF, it was decided to use DMF as the solvent rather than Me_2SO. Using this approach as described here *tert*-butyloxycarbonyl-L-homoserine was obtained in excellent yield free from contamination by the lactone.