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Synthesis and Some Pharmacological Properties of [4-Threonine,7-glycine]oxytocin, [1-(L-2-Hydroxy-3-mercaptopropanoic acid),4-threonine,7-glycine]oxytocin (Hydroxy[Thr⁴,Gly⁷]oxytocin), and [7-Glycine]oxytocin, Peptides with High Oxytocic-Antidiuretic Selectivity

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[4-Threonine,7-glycine]oxytocin and [1-(L-2-hydroxy-3-mercaptopropanoic acid),4-threonine,7-glycine]oxytocin (hydroxy [Thr⁴,Gly⁷]oxytocin) were synthesized by a combination of solid-phase and classical methods of peptide synthesis. A protected octapeptide was synthesized by the solid-phase method and following ammonolysis and purification 1 + 8 couplings in solution were employed to furnish the required key nonapeptide and acyl octapeptide intermediates, respectively. [7-Glycine]oxytocin was prepared from a sample of the protected nonapeptide intermediate used in the original synthesis of this peptide. [7-Glycine]oxytocin has an oxytocic potency (O) of 93 ± 4 units/mg and an antidiuretic potency (A) of 0.0056 ± 0.0003 units/mg. It has an O/A ratio of 16000. [4-Threonine,7-glycine]oxytocin has an oxytocic potency of 166 ± 4 units/mg and an antidiuretic potency of 0.002 ± 0.0004 units/mg. Its O/A ratio is 83000. Threonine substitution has thus brought about a substantial enhancement in oxytocic activity and a fivefold enhancement in O/A selectivity. Hydroxy[Thr⁴,Gly⁷]oxytocin has an oxytocic potency of 218 ± 8 units/mg and antidiuretic potency of 0.0040 ± 0.0005 units/mg. Its O/A ratio is thus 54500. All three 7-glycine-substituted analogues exhibit a marked sensitivity to Mg²⁺ on the rat uterus assay system and in the presence of 0.5 mM Mg²⁺ had oxytocic potencies in the range of 900–1000 units/mg. Should these peptides exhibit enhanced oxytocic selectivity in humans, they might offer a greater margin of safety than oxytocin in those clinical situations in which the latter is currently employed.

In pursuing the goal of designing highly selective synthetic peptides derived from oxytocin and vasopressin, it has proved useful to combine within one molecule those structural changes which individually enhance a particular pharmacological activity in a selective manner.^{1,2a} This approach led to the synthesis of the highly selective antidiuretic peptide [1-deamino,4-valine,8-D-arginine]vasopressin (dVDAVP).³ This compound has high antidiuretic potency (1230 units/mg; cf. arginine-vasopressin, 320 units/mg) and no pressor activity could be detected. The ratio of the antidiuretic/pressor (A/P) potencies thus approaches infinity.

It appeared worthwhile to utilize this approach in attempting to design an analogue of oxytocin with negligible vasopressin-like characteristics, i.e., a peptide exerting substantial oxytocic effects but neither antidiuretic nor pressor effects. Such a compound may have potential clinical use.

The literature shows that oxytocin has been modified in but two positions within the nonapeptide sequence with resulting enhancement of the ratio of oxytocic (O) to antidiuretic (A) potencies. [Gly⁷]oxytocin was reported

to exhibit a greatly enhanced oxytocic/antidiuretic (O/A) ratio relative to oxytocin (O/A 130).^{4,5} The values for the O/A ratio of [Gly⁷]oxytocin ranged from 7000 to 33000, the discrepancies arising from differing estimates of oxytocic activities.^{4,6,7} Although activity on the isolated rat uterus in the absence of Mg²⁺ was substantially less than that of oxytocin, the greater reduction in antidiuretic activity resulted in these high O/A ratios for [Gly⁷]oxytocin.

A number of 4-substituted analogues of oxytocin have enhanced O/A selectivity.^{8–11} In most this stems from reduced antidiuretic activity rather than enhanced oxytocic activity. An exception is [Thr⁴]oxytocin which shows both enhanced oxytocic activity and depressed antidiuretic activity, relative to those of oxytocin. We thus decided to incorporate threonine in the 4 position of [Gly⁷]oxytocin in the hope of further enhancing its oxytocic activity and selectivity. We report here the synthesis and some pharmacological properties of the peptide [Thr⁴,Gly⁷]oxytocin designed according to this rationale.^{2b}

The discrepancy in the values reported from different laboratories for the oxytocic potency of [Gly⁷]oxytocin^{4,6,7}

Table I. Biological Activities (Units/mg \pm Standard Errors) of [Gly⁷]oxytocin, [Thr⁴,Gly⁷]oxytocin, Hydroxy[Thr⁴,Gly⁷]oxytocin and Related Peptides

Peptide	Rat oxytocic		Rat mammary strip	Rat milk ejection	Rat antidiuretic (A)	Rat vasopressor (P)	O/A
	No Mg ²⁺ (O)	0.5 mM Mg ²⁺					
1. [Gly ⁷]oxytocin ^a	93 \pm 4	965 ^f	478 \pm 14	392 \pm 13	0.0056 \pm 0.0003 (\sim 0.01, ^d <0.01 ^e)	<0.01	16 600
2. [Thr ⁴ ,Gly ⁷]oxytocin ^a	204, ^b 330, ^d 65 ^e	857 \pm 26	490 \pm 25	802 \pm 23	0.002 \pm 0.0004	<0.01	83 000
3. Hydroxy[Thr ⁴ ,Gly ⁷]oxytocin ^a	218 \pm 8	1002 \pm 29	623 \pm 13	950 \pm 32	0.0040 \pm 0.0005	<0.01	54 500
4. Oxytocin ^g	520 \pm 12	486 \pm 15	533 \pm 62 ^h	474 \pm 16 ⁱ	4.0 \pm 0.8	4.3 \pm 0.12	130
5. [Thr ⁴]oxytocin ^g	923 \pm 95	719 \pm 83		543 \pm 23 ^h	1.8 \pm 0.3	0.43 \pm 0.01	510
6. Hydroxy[Thr ⁴]oxytocin ^j	4179 \pm 222	937 \pm 55	378 \pm 10	808 \pm 19	5.3 \pm 0.5	4.92 \pm 0.09	790

^a Present communication. ^b Value reported by Bepalova et al.⁶ ^c Value reported by Hechter et al.⁷ ^d Values reported by Bodanszky and Bath.⁴ ^e Value reported by Walter et al.²⁰ ^f Estimates of activity varied among different assays (range 316–2112 units/mg). Figure shown is the geometric mean of results from six separate assays. ^g Values reported by Manning et al.^{10,11,30} ^h Value reported by Polacek et al.¹⁹ ⁱ Rabbit. ^j Values reported by Manning et al.¹²

made it essential that we ascertain the correct oxytocic potency of this peptide in order to evaluate properly the effect of Thr⁴ substitution on oxytocic activity and selectivity. The pharmacological properties of [Gly⁷]oxytocin, synthesized from a sample of protected nonapeptide intermediate⁴ kindly furnished for this purpose by Professor M. Bodanszky, are also reported here. We recently reported the synthesis of [1-(L-2-hydroxy-3-mercaptopropanoic acid,4-threonine)]oxytocin (hydroxy-[Thr⁴]oxytocin).¹² This peptide is eight times more potent than oxytocin in the in vitro rat uterus assay system and has an O/A ratio of \sim 790. The possibility that glycine substitution in the 7 position of this peptide might give a peptide with a considerably enhanced O/A ratio while still retaining substantial oxytocic activity prompted the design of hydroxy[Thr⁴,Gly⁷]oxytocin. Its synthesis and some of its pharmacological properties are also reported here.

Peptide Synthesis. Solid-phase peptide synthesis employed the Merrifield technique¹³ as described in the synthesis of oxytocin¹⁴ and [Thr⁴]oxytocin,¹⁰ the fully protected sequence being cleaved from the resin by ammonolysis.^{10,14} The fully protected nonapeptide amide precursor of [Thr⁴,Gly⁷]oxytocin posed difficulties in extraction from the resin-peptide mixture and in subsequent purification by virtue of its extreme insolubility in solvents such as dimethylformamide and hexamethylphosphoramide, generally useful in these stages. Ammonolysis of the Boc octapeptide C-terminal sequence of [Thr⁴,Gly⁷]oxytocin from the resin gave a less intractable product, however, and purification was achieved with no difficulty. Removal of the N-protecting group from this product and further acylation with the appropriate N-terminal derivative by coupling in DMF solution as previously described¹² yielded the required protected intermediates of [Thr⁴,Gly⁷]oxytocin and of hydroxy-[Thr⁴,Gly⁷]oxytocin, respectively. Both protected intermediates and that of [Gly⁷]oxytocin⁴ were converted to the disulfide-bridged cyclic peptides and the products purified by methods described previously.^{10,14,15} The pharmacological properties of [Gly⁷]oxytocin, [Thr⁴,Gly⁷]oxytocin, and hydroxy[Thr⁴,Gly⁷]oxytocin were evaluated by methods previously described^{16–18} and by assays on isolated strips of rat mammary gland.¹⁹

Results and Discussion

The pharmacological data on [Thr⁴,Gly⁷]oxytocin reveal an 80% enhancement in oxytocic potency and a threefold reduction in antidiuretic potency relative to the values

obtained for the newly synthesized preparation of [Gly⁷]oxytocin (Table I). Thus [Thr⁴,Gly⁷]oxytocin has an O/A ratio of 83 000, representing a fivefold enhancement in O/A selectivity relative to [Gly⁷]oxytocin, and is the highest value reported to date. The oxytocic potency obtained for [Gly⁷]oxytocin is consistent with the lowest of the three previously reported values (Table I) and with a very recently reported value²⁰ also obtained from a sample of protected nonapeptide⁴ furnished by Professor Bodanszky. Curiously, the substitution of Gly for proline in the highly potent hydroxy[Thr⁴]oxytocin brought about drastic reductions in both oxytocic and antidiuretic activities. However, although the oxytocic potency of this peptide is greatly reduced compared to that of its parent, it still is appreciable. This coupled with its very low antidiuretic potency gives hydroxy[Thr⁴,Gly⁷]oxytocin an O/A ratio of 54 000. It is interesting to note that when viewed as the 1-hydroxy analogue of [Thr⁴,Gly⁷]oxytocin, these data indicate that the effect of hydroxy substitution has been to increase both oxytocic and antidiuretic activities disproportionately with the latter increase being more pronounced. Thus hydroxy[Thr⁴,Gly⁷]oxytocin possesses reduced O/A selectivity relative to [Thr⁴,Gly⁷]oxytocin. These effects of hydroxy substitution are consistent with previous observations.^{12,28}

The marked enhancement of O/A selectivity obtained by combining threonine and glycine substitutions in positions 4 and 7 of oxytocin, respectively, is in striking contrast to the diminishment of O/A selectivity obtained by combining deamination of position 1 and glycine substitution at position 7 in oxytocin. [1-Deamino,7-glycine]oxytocin was recently reported to have oxytocic and antidiuretic activities of 355 and 0.062 units/mg, respectively, and O/A ratio of 5200.²⁰ Thus in comparing these values with those for [7-glycine]oxytocin presented here, it is clear that deamination while increasing the oxytocic activity about fourfold has also brought about a tenfold enhancement of antidiuretic activity and consequently its overall effect is a threefold reduction in O/A selectivity. This reduction of O/A selectivity effected by deamination is consistent with previous findings.^{1,8,29} Thus the validity of the approach to designing peptides with enhanced selectivity by combining in the same molecule only those modifications which individually lead to enhanced selectivity has been further substantiated by the data on [1-deamino,7-glycine]oxytocin²⁰ and by those for hydroxy[Thr⁴,Gly⁷]oxytocin and [Thr⁴,Gly⁷]oxytocin reported here. Because the latter compound incorporates changes which individually give rise to enhanced O/A

selectivity, i.e., Thr and Gly substitutions, it alone exhibits the desired further enhanced O/A selectivity whereas the former two peptides which incorporate changes at position 1, i.e., deamination and hydroxy substitution, which individually lead to reduced O/A selectivity,^{12,29} exhibit reduced O/A selectivity relative to their respective parents, i.e., [Gly⁷]oxytocin and [Thr⁴,Gly⁷]oxytocin.

It should be noted that all three Gly⁷-substituted peptides exhibited a marked sensitivity to Mg²⁺ ion in the rat uterus assay, the presence of Mg²⁺ greatly potentiating rat uterotonic potency (Table I). The exceptional magnesium potentiation of the activities of these analogues on the isolated rat uterus suggests that the ionic composition of the bathing medium is critical. This may explain in part the variance among previously reported oxytocic activities of [Gly⁷]oxytocin.^{4,6,7}

Should [Thr⁴,Gly⁷]oxytocin exhibit a similar spectrum of pharmacological activities in humans, it might offer advantages as a potent and selective oxytocic agent in those clinical situations in which oxytocin is currently used.

The marked selective diminution in vasopressin-like behavior caused by the replacement of proline with glycine reported here is consistent with findings for the effect of this substitution in oxytocin,^{4,5} deamino-oxytocin,²⁰ deamino-"dicarba" oxytocin,^{5,21} and in lysine-vasopressin.²² Plainly, the greater conformational constraint imposed upon the "tail" portion of these peptides by the proline residue has a greater significance in the manifestation of the renal and vascular responses than in interactions with uterine smooth muscle receptors. A further interesting aspect of the Gly⁷-substituted peptides reported here should be mentioned. Estimation of their antidiuretic activities by intravenous injection into ethanol-anesthetized rats is complicated by the fact that they can produce diuresis similar to that often seen after oxytocin injection.²³ This diuretic property is presently under investigation and will be reported elsewhere.

Experimental Section

The procedure of solid-phase peptide synthesis conformed to that published^{10,13,14} with the exception that the chloroform washes were omitted. Chloromethylated resin (Bio-Rad Bio-Beads SX-1) was esterified²⁴ to Boc-Gly to an incorporation of 0.25 mmol g⁻¹. The amino acid derivatives were supplied by Bachem, Inc., or by Beckman Bioproducts Division. Triethylamine and *N*-methylmorpholine (NMM) were distilled from ninhydrin; trifluoroacetic acid (TFA) was distilled from P₂O₅; 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, chloroform-methanol (1:1 v/v); B, butan-1-ol-acetic acid-water (4:1:5 v/v, upper phase); C, butan-1-ol-acetic acid-water-ethyl acetate (1:1:1:1 v/v); D, butan-1-ol-water (3.5% in acetic acid, 1.5% in pyridine) (1:1 v/v, upper phase); E, butan-1-ol-acetic acid-water-pyridine (15:3:3:10 v/v). Loads of 10–50 µg were applied and chloroplatinate reagent, ninhydrin, iodine vapor, and chlorine-potassium iodide-tolidine 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.²⁵ using a Beckman Automatic Amino Acid Analyzer Model 121.

Boc-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Gly-Leu-Gly-NH₂ (I). Boc-Gly resin (6.0 g, 1.5 mmol of Gly) was subjected to seven cycles of deprotection, neutralization, and coupling to yield the protected octapeptide resin (7.3 g, weight gain of 1.3 g, 81% of theory). A portion (2.4 g) was ammonolyzed¹⁴ in methanol-DMF (1:1 v/v, 50 ml). The methanol was evaporated, the DMF suspension filtered, and the resin washed with warm (60 °C) DMF. The combined filtrate and washings were diluted

Table II. Properties of Peptide Intermediates, X-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Gly-Leu-Gly-NH₂

	X	Mp, °C ^a	[α] _D , deg ^b	Formula ^c
I	Boc-	244–247	–18.5	C ₆₂ H ₈₄ N ₁₀ O ₁₃ S
II	Z-Cys(Bzl)-	269–272	–16.3	C ₇₅ H ₉₃ N ₁₁ O ₁₄ S ₂
III	HO·CH ₂ (CH ₂ ·S·Bzl)·CO-	255–258	–19.5	C ₆₇ H ₈₆ N ₁₀ O ₁₃ S ₂

^a Melting points were taken in open capillaries with a Thomas-Hoover apparatus and are uncorrected. All compounds decomposed upon melting. ^b Optical rotations measured with a Bellingham Stanley Ltd. Model A polarimeter; c 1 (DMF), T 24, 24, and 22°, respectively. ^c Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. The compounds were analyzed for C, H, and N; results were within ±0.4% with the exception of the analysis for C of compound III [Anal. (C₆₇H₈₆N₁₀O₁₃S₂) C: calcd, 61.70; found 61.25].

with water and the precipitated material was filtered, dried, and reprecipitated by methanol-ether (ca. 1:3 v/v) from a filtered solution in a minimum quantity of warm DMF to yield the protected octapeptide amide (305 mg, 61% based upon weight gain in peptidyl resin, Table II) *R*_{f(A)} 0.54, *R*_{f(B)} 0.87. Amino acid analysis gave Tyr, 0.93; Ile, 0.88; Thr, 0.65; Asp, 0.95; Cys(Bzl), 0.81; Gly, 1.97; Leu, 1.00; NH₃, 2.00.

Z-Cys(Bzl)-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Gly-Leu-Gly-NH₂ (II). The foregoing Boc octapeptide amide (100 mg, 0.083 mmol) was dissolved in freshly distilled TFA (2 ml). The solution was left to stand for 1 h with occasional vortex mixing. The cleavage product was precipitated by the addition of ice-cold ether (20 ml) and washed well with ether by repeated centrifugation and decantation and dried in vacuo over soda lime to yield a white powder (*R*_{f(A)} 0.66, *R*_{f(B)} 0.62, chloroplatinate and ninhydrin-positive spot). This material was dissolved in DMF, and NMM (9 µl) was added gradually to give a solution of pH ~7 to moist pH paper. A solution of Z-Cys(Bzl) (31.5 mg, 0.091 mmol) and *N*-hydroxybenzotriazole monohydrate (HOBt·H₂O)²⁶ (14 mg, 0.091 mmol) in DMF (0.2 ml) was added and the mixture, chilled in ice, treated with a solution of dicyclohexylcarbodiimide (DCCI)²⁷ (19 mg, 0.091 mmol) in DMF (0.2 ml) with vortex mixing. Ice cooling was maintained for 1 h and then the reaction mixture was set aside overnight at room temperature. The consumption of the ninhydrin-positive material with the formation of a product (*R*_{f(A)} 0.75) giving no color with the reagent was demonstrated by TLC (both spots detected by chloroplatinate reagent). The crude product, precipitated by the addition of ethanol (85%, 10 ml) and washed with ethanol by repeated centrifugation and decantation, was reprecipitated by ethanol from solution in a minimum quantity of DMF and washed well with ethanol, and ether, to give the protected nonapeptide amide II (100 mg, 75%, Table II). Amino acid analysis gave Tyr, 0.96; Ile, 0.88; Thr, 0.64; Asp, 0.99; Cys(Bzl), 1.88; Gly, 2.02; Leu, 1.00; NH₃, 2.20.

S-Benzyl-L-2-hydroxy-3-mercaptopropanoyl-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Gly-Leu-Gly-NH₂ (III). The product of the treatment of the Boc octapeptide amide I (150 mg, 0.125 mmol) with TFA (2 ml), isolated as described above, was dissolved in DMF (1 ml), and NMM (25 µl) was added gradually to give a solution of pH ~7 to moist pH paper. A solution of S-benzyl-L-2-hydroxy-3-mercaptopropanoic acid²⁸ (29.5 mg, 0.138 mmol) and HOBt·H₂O (29 mg, 0.188 mmol) in DMF (0.2 ml) was added; the mixture was cooled in ice and treated with a solution of DCCI (29 mg, 0.138 mmol) in DMF (0.2 ml) with vortex mixing. Further NMM (5 µl) was added such that moist pH paper registered a slightly alkaline reaction to the atmosphere within the reaction vessel. This test remained positive after the mixture was set aside overnight at room temperature. A trace of unreacted amino component remaining (TLC, product *R*_{f(B)} 0.73), a second aliquot of the acylation reactants was added to the chilled reaction mixture and vortex mixing continued for 30 min, when ethanol (10 ml) containing acetic acid (2 drops) was added, forming a clear solution which gradually deposited a white precipitate upon cooling. Further ethanol (10 ml) was added and the precipitate centrifuged and washed with ethanol (10 ml), ethanol-ether (1:1 v/v, 10 ml), and ether (10 ml) by successive centrifugation and

Table III. Properties of [Gly⁷]oxytocin, [Thr⁴,Gly⁷]oxytocin, and Hydroxy[Thr⁴,Gly⁷]oxytocin

Peptide	[α] _D , deg ^a	R _f in solvent system					M _{Arg} ^b
		B	C	D	E		
[Gly ⁷]oxytocin	-11.9	0.30	0.74	0.17	0.75	0.36	
[Thr ⁴ ,Gly ⁷]oxytocin	+6.3	0.37	0.85	0.27	0.83	0.37	
Hydroxy[Thr ⁴ ,Gly ⁷]oxytocin	-23.2	0.56	0.89	0.52	0.88	0.05	

^a c 0.50 (T 24°), 0.35 (T 27°), 0.42 (T 27°), respectively (1 M acetic acid). ^b Electrophoretic mobility (M) relative to Arg in direction of the cathode (30% acetic acid, 450 V, 5 h). Single bands detected in each case (chloroplatinate, chlorine-potassium iodide-tolidine).

decantation, the precipitate being suspended in each wash by vortex mixing. This crude product was reprecipitated (ethanol-ether, 10:1 v/v) from solution in a minimum quantity of DMF to yield the acyloctapeptide amide (110 mg, 68%, Table II), isolated by filtration, and washed with ethanol-ether (10:1 v/v) and ether. Amino acid analysis gave Tyr, 0.86; Ile, 0.88; Thr, 0.97; Asp, 1.07; Cys(Bzl), 1.03; Gly, 1.98; Leu, 1.00; NH₃, 1.90.

[Thr⁴,Gly⁷]oxytocin (IV). A solution of the peptide intermediate II (80 mg, 0.056 mmol) in sodium-dried and redistilled ammonia (~300 ml) was treated at the boiling point, and with stirring, with sodium¹⁵ from a stick of the metal contained in a small bore glass tube^{10,29} until a light blue color persisted in the solution for 20 s. 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 7. An excess of potassium ferricyanide solution (0.01 M, 11.5 ml) was added gradually and the yellow solution stirred for 5 min. Anion exchange resin (Bio-Rad AG3-X4A, chloride form, ~10 g damp weight) was added and stirring continued for 5 min. The mixture was filtered through a bed of the resin (~80 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 Sephadex G-15 (column 110 × 2.7 cm)^{30,31} eluting with aqueous acetic acid (50%) with flow rate ~3.5 ml h⁻¹. The eluate was monitored for absorbance at 280 nm and fractionated. Those fractions comprising the major peak were pooled and lyophilized, and the residue (45 mg) was further subjected to gel filtration with Sephadex G-15³¹ (column 100 × 1.5 cm) eluting with aqueous acetic acid (0.2 M, flow rate ~4.5 ml h⁻¹). The oxytocin analogue (34 mg, ~60%, Table III) was isolated from the fractions comprising the single symmetrical peak by lyophilization and dried in vacuo over P₂O₅. Amino acid analysis gave Tyr, 0.88; Ile, 0.98; Thr, 0.93; Asp, 1.03; 1/2 Cys, 1.93; Gly, 1.96; Leu, 1.00; NH₃, 1.93.

[1-(L-2-Hydroxy-3-mercaptopropionic acid),Thr⁴,Gly⁷]oxytocin (V). Analogue V (33 mg, ~40%, Table III) was prepared from the precursor III (98 mg, 0.075 mmol) as detailed above for compound IV. Amino analysis gave Tyr, 0.93; Ile, 0.97; Thr, 0.95; Asp, 1.03; 1/2 Cys, 0.43; Gly, 1.99; Leu, 1.00; NH₃, 1.60.

[7-Gly]oxytocin (VI). The protected nonapeptide Z-Cys(Bzl)-Try-Ile-Gln-Asn-Cys(Bzl)-Gly-Leu-Gly-NH₂⁴ (reprecipitated from DME-methanol, 100 mg, 0.075 mmol) was deblocked, re-oxidized, and purified as described for compound IV to give the required free peptide VI as a fluffy white powder (35 mg, ~50%, Table III). Amino acid analysis gave Tyr, 0.94; Ile, 0.89; Glu, 1.05; Asp, 0.97; 1/2 Cys, 1.99; Gly, 1.91; Leu, 1.00; NH₃, 2.65.

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