mL of methanol. After 16 h, at 25 °C the clear solution was concentrated to about 30 mL and diluted with 800 mL of ether to precipitate the product. The solid was collected by filtration, washed with ether, and dried under high vacuum to give colorless material (7.10 g, 109% containing some ether and methanol as indicated by NMR): IR (Nujol) 5.84, 6.08–6.29 μm; NMR (D_2O) δ 5.82 (br s, C_5H), 5.35 (s, $C_{1'}H$), 2.15 (s, C_6CH_3), 0.85, 0.82 [each d, J = 6 Hz, $-CH(CH_3)_2$].

4-Acetoxy-6-methyl-3-(1'-acetoxy-2'-oxo-4'-methylpentyl)-2-pyridinone (4a). Acetic anhydride (50 mL, 0.529 mol) was added to a stirred sample of **3** (6.76 g, 95.2% of the above sodium salt); the latter dissolved with a slight exotherm, followed by the appearance of crystalline sodium acetate. After 16 h, the reaction mixture was evaporated at 25 °C and flushed with toluene three times to remove acetic anhydride. The residue was dissolved in methylene chloride, extracted with 10% aqueous potassium bicarbonate, dried (Na_2SO_4), and evaporated to give 7.840 g of a mixture (80:20) of **4a** and the corresponding triacetate (see text) as shown by NMR. Recrystallization from ether provided crystalline diacetate (5.175 g; mp 129–130 °C). The triacetate present in the mother liquor underwent partial hydrolysis to **4a** when allowed to stand in ether at 25 °C for several weeks. An additional 1.873 g of **4a** was thus obtained: total weight 7.048 g (92%); IR ($CHCl_3$) 5.63, 5.73, 5.79, 6.08 μm; NMR ($CDCl_3$) δ 6.43 (s, $C_{1'}H$), 6.05 (br s, C_5H), 2.37 (s, C_6CH_3), 2.28 and 2.12 (each s, 2 OAc), 0.93 and 0.90 [each d, J = 6 Hz, $-CH(CH_3)_2$]. Anal. ($C_{16}H_{21}O_6N$) C, H, N.

(±)-Flavipucine and (±)-*epi*-Flavipucine (5a). To a stirred solution of **4a** (3.233 g, 0.01 mol) in *t*-BuOOH (40 mL, Pennwalt product dried over $MgSO_4$) cooled in a dry ice–acetone bath was added 1 M KO-*t*-Bu–*t*-BuOH (20 mL, 0.02 mol) in 7 min at 0–5 °C. After an additional 5 min at 0–5 °C, the bright yellow reaction mixture was diluted with methylene chloride, washed with 10% $KHCO_3$, dried (Na_2SO_4), and evaporated to a thick paste first on a water aspirator then high vacuum. The last traces of *t*-BuOOH were removed by flushing with toluene a number of times. The semicrystalline product (1.963 g) was slurried in cold ether to provide 1.746 g of **5a** (nat and epi in about equal amounts). The ether layer was evaporated and the residue further purified via preparative TLC (silica gel; $CHCl_3$–acetone, 65:35) to provide additional **5a** (0.076 g, total weight 1.822 g, 77%). Fractional crystallization from benzene provided (±)-flavipucine (mp 154–155 °C, identical with natural (−)-flavipucine in TLC, UV, IR, NMR, and mass spectrum) and (±)-*epi*-flavipucine: mp 136–138 °C; IR ($CHCl_3$) 2.92, 3.06, 3.14, 5.82, 6.00, 6.12 μm; NMR ($CDCl_3$) δ 5.65 (br s, C_5H), 3.92 (s, $C_{1'}H$), 2.20 (s, C_6CH_3), 0.98 [d, J = 6.5 Hz, $-CH(CH_3)_2$]. Anal. ($C_{12}H_{15}O_4N$) C, H, N.

Reaction of Flavipucine and *epi*-Flavipucine (5a) with Thiophenol. To a stirred solution of **5a** (0.126 g, 0.000531 mol) in $CHCl_3$–CH_3OH (1:1, 2 mL) was added pyridine (0.2 mL, 0.00248 mol), followed by thiophenol (0.2 mL, 0.00195 mol) at 25 °C. After 1 h, the pale yellow reaction mixture was evaporated and flushed with toluene, and the semicrystalline residue was slurried with ether and filtered to give 0.121 g of solid, which was purified via crystallization and preparative TLC (silica gel; $CHCl_3$–CH_3OH, 90:10) to give the diol corresponding to **4a**[2,3] [0.024 g (19%), MS M^+ = 239] and **10** [R = C_6H_5; 0.63 g (51%)]: mp 231–233 °C (acetone); NMR (CD_3OD) δ 2.25 (s, CH_3), 6.01 (br s, C_5H), 7.14 (s, 5 H). Anal. ($C_{12}H_{11}O_2NS$) C, H, N.

Treatment of the ether-soluble material with an excess of a solution of 2,4-dinitrophenylhydrazine (in H_2SO_4, H_2O, and CH_3OH) provided the di-DNPH of isobutylglyoxal (0.052 g, 21%), mp 234–238 °C. Anal. ($C_{18}H_{18}N_8O_8$) C, H, N.

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Notes

Oxytocin and Lysine-vasopressin with N^5,N^5-Dialkylglutamine in the 4 Position: Effect of Introducing Sterically Hindered Groups into the Hydrophilic Cluster of Neurohypophyseal Hormones[1]

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The synthesis and pharmacological potencies of oxytocin and lysine-vasopressin analogues are reported in which the N^5-amide of their glutaminyl residues are dialkylated. These analogues have been studied as an ongoing exploration of the biological effects on the natural hormones of substituting individually one of the amino acid residues, which has a hydrophilic side chain and which are thought to be part of the hydrophilic surface of the hormones. [4-(N^5,N^5-Dimethylglutamine)]oxytocin (**17**), [4-(N^5,N^5-di-*n*-propylglutamine)]oxytocin (**18**), and [4-(N^5,N^5-dimethylglutamine)]lysine-vasopressin (**19**) were synthesized by classical solution techniques. Potencies in the in vitro rat uterotonic, avian vasodepressor, rat pressor, and rat antidiuretic assays were determined and are as follows, respectively: for compound **17** 3.01 ± 0.14 units/mg, 4.55 ± 0.03 units/mg, tachyphylaxis and tachyphylaxis; for compound **18** <0.1, <0.1, <0.05, and <0.002 unit/mg; for compound **19** <0.05, <0.1, 1.27 ± 0.03, and 1.88 ± 0.04 units/mg. The potencies in all cases are significantly less than those of the parent hormone. The results are discussed in terms of the proposed biologically active conformations of the hormones at the uterotonic receptor and at the antidiuretic receptor.

In the proposed biologically active conformations of oxytocin at the uterine receptor[2,3] (Figure 1A) and of vasopressin at the antidiuretic receptor[4] (Figure 1B), one surface of the 20-membered antiparallel β-pleated sheet

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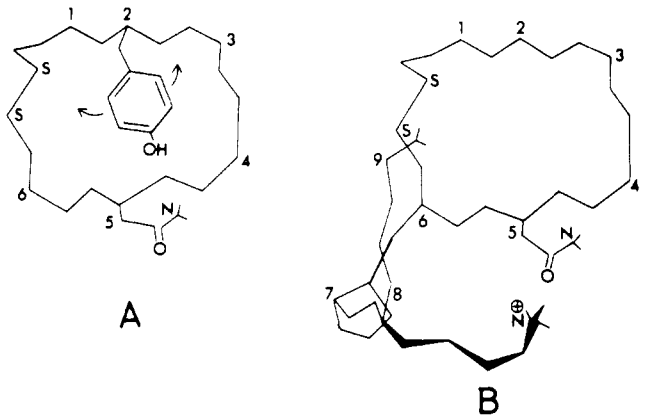

Figure 1. Panel A is the schematic representation of the active elements of the biologically active conformation of oxytocin (the hydroxyl group of the tyrosine residue in position 2 and the side chain of the carboxamide group of the asparagine residue in position 5) for the rat uterotonic activity; the COOH-terminal tripeptide has been deleted for simplification. Panel B is the schematic representation of the active elements of lysine-vasopressin (the side-chain carboxamide of Asn^5 and the side-chain amino group of Lys^8) for the activation of the antidiuretic receptor. Numbers indicate residue positions.

structure of both molecules is characterized by a cluster of hydrophilic side chains. For oxytocin, the cluster is comprised of the hydroxyl group of the tyrosine residue and the carboxamide groups of the asparagine, glutamine, and glycine residues. The hydroxyl group and the asparagine carboxamide group appear to be the active elements initiating the oxytocic response.[2,3] For vasopressin, the composition of the hydrophilic cluster is similar to that of oxytocin. The important exception is that it includes the basic moiety on the side chain of residue 8 of the antidiuretic hormone rather than the tyrosine hydroxyl moiety in position 2. The asparagine carboxamide group and the basic moiety of the side chain of residue 8 appear to be the active elements initiating the antidiuretic response.[4] A feature common to the biologically active conformation of both hormones is the presence of the side chain of the amino acid residue in position 4 that can influence the orientation of the active elements[2,5] in the hydrophilic cluster. To further study the effect of modifications to the side chain of the 4-position residue on the biological activities of neurohypophyseal hormones, we have synthesized oxytocin and lysine-vasopressin analogues which have the N^5 of the glutaminyl carboxamide dialkylated.

The protected nonapeptides for [4-(N^5,N^5-dimethylglutamine)]oxytocin ([Glu[$N(CH_3)_2$]^4]OT), [4-(N^5,N^5-di-*n*-propylglutamine)]oxytocin ([Glu[$N(n\text{-}C_3H_7)_2$]^4]OT), and [4-(N^5,N^5-dimethylglutamine)]lysine-vasopressin ([Glu[$N(CH_3)_2$]^4]LVP) were prepared in solution by stepwise elongation using active esters[6] (*p*-nitrophenyl[6,7] or *N*-hydroxysuccinimide[8]) of the appropriate *N*-(*tert*-butyloxycarbonyl) (*N*-Boc) protected amino acid, except in the case of the NH_2-terminal cysteinyl residue which was incorporated as *N*-(benzyloxycarbonyl)-*S*-benzylcysteine *N*-hydroxysuccinimide ester [Z-Cys(Bzl)-OSu]. The protecting groups were removed from the nonapeptides by sodium in liquid ammonia,[9] and conversion to the disulfide was achieved by oxidation with 1,2-diiodoethane.[10] The resulting analogues were purified by gel filtration[11] on Sephadex G-15 and by partition chromatography[12] on Sephadex G-25.

The analogues were assayed for some of the pharmacological activities characteristic of neurohypophyseal hormones, and the results are summarized in Table I. In addition, in the rat uterotonic assay in vitro all three analogues exhibited a reduced ability to maximally stimulate uterine contraction as compared to oxytocin. An interpretation of these results on the basis of the biologically active conformation of oxytocin[2,3] suggests that alkyl groups on the N^5 of the glutaminyl carboxamide sterically interfere with one or both of the active elements, Tyr^2 and Asn^5, in the oxytocin and vasopressin analogues under study. On the other hand, replacement by methyl groups of both hydrogens on the asparagine carboxamide active element does not decrease the intrinsic activity of [5-(N^4,N^4-dimethylasparagine)]oxytocin on the rat uterus.[13] Thus, it appears that methylation directly on this active element does not cause a detrimental reorientation of the active elements for interaction with the uterotonic receptor.[14]

Whether the decreased antidiuretic potency of the analogues (and particularly the vasopressin derivative), which have an N^5,N^5-dialkylated glutamine residue in position 4, is due to a similar effect on the biologically active conformation of vasopressin at the antidiuretic receptor, as has been discussed above for the biologically active conformation of oxytocin at the uterotonic receptor, cannot be ascertained from our present data. To reach a conclusion one has to await comparative results between these kind of analogues and the natural hormones in in vitro antidiuretic assay systems.

Experimental Section

Capillary melting points were determined on a Buchi SMP-20 apparatus and are reported uncorrected. Optical rotations were measured with a Carl Zeiss precision polarimeter (0.005°). For

(1) All optically active amino acids are of the L configuration. Abbreviations used follow the recommendations of IUPAC–IUB as found in *Biochemistry* **1975**, *14*, 449, and *Biochem. J.* **1972**, *126*, 773. Other abbreviations used are: Glu[$N(CH_3)_2$], N^5,N^5-dimethylglutamine; Boc, *tert*-butyloxycarbonyl; OT, oxytocin; LVP, lysine-vasopressin; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DMF, dimethylformamide; Z, benzyloxycarbonyl; -OSu, *N*-hydroxysuccinimide ester; -ONp, *p*-nitrophenol ester.

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(14) Deaminooxytocinoic acid dimethylamide has been synthesized (Takashima, H.; Fraefel, W.; du Vigneaud, V. *J. Am. Chem. Soc.* **1969**, *91*, 6182) but no dose–response studies in the in vitro uterotonic assay were reported for this compound. Therefore, the effect on intrinsic activity of dimethylation of the carboxamide in position 9 is not known.

TLC, loads of 10–50 μg were applied to precoated plates of silica gel G, F-254 (E. Merck or Riedel de Haen), and were developed until the solvent ascended 10–15 cm. The following systems (all by volume) were used for TLC: (A) 1-butanol–acetic acid–water (4:1:1), (B) benzene–2-butanol (7:3), (C) chloroform–methanol–acetic acid (85:10:5), (D) 1-butanol–pyridine–acetic acid–water (15:10:3:6), (E) 1-butanol–pyridine–water (20:10:11), (F) ethyl acetate–acetic acid–pyridine–water (5:1:5:3), (G) 1-butanol–acetic acid–water (4:1:5, upper phase). The products were detected by UV or chlorination, followed by either *o*-tolidine or 1% KI–starch solutions. Elemental analyses were done by the Microanalytical Laboratory of the National Hellenic Research Foundation and the data (C, H, N) fall within ±0.4% of theory. Amino acid analyses[15] were performed on aliquots of the samples after hydrolysis in deaerated ampules with 6 N HCl (110 °C, 16–22 h) on a Durrum D-500 amino acid analyzer.[16] Cystine was determined as cysteic acid.[17] The amino acid derivatives were supplied by Fluka A. G. and Beckman, Inc., while the other reagents and solvents were analytical grade.

N^2-(*tert*-Butyloxycarbonyl)-N^5,N^5-dimethylglutamine (1). Palladium oxide (0.5 g) was added to a warm solution of N^2-(*tert*-butyloxycarbonyl)-N^5,N^5-dimethylglutamine benzyl ester[18] (4.8 g, 13 mmol) in 2-propanol–water (14:1, 150 mL), and catalytic hydrogenation was carried out. After 4 h, the catalyst was filtered off, the filtrate was evaporated under reduced pressure, and the residue was triturated with ethyl acetate–petroleum ether (1:3) with cooling. The product was recrystallized from ethyl acetate: yield 2.85 g (80%); mp 124–125 °C; $[\alpha]^{20}_D$ +2.2° (*c* 1, ethanol); TLC R_f (A) 0.73, R_f (C) 0.88. Anal. ($C_{12}H_{22}N_2O_5$) C, H, N.

N^2-(*tert*-Butyloxycarbonyl)-N^5,N^5-dimethylglutamine *p*-Nitrophenyl Ester (Boc-Glu[N(CH$_3$)$_2$]-ONp) (2). To a sample of 1 (3.42 g, 12.5 mmol) dissolved in DMF (20 mL) were added successively *p*-nitrophenol (2.08 g, 20% excess) and DCC (2.58 g, 12.5 mmol). The reaction mixture was stirred for 2 h at 0 °C and then at 4 °C for 15 h. The separated DCU was removed by filtration and washed with ethyl acetate. The solvent was evaporated from the combined filtrate and washings under reduced pressure, the resulting oily residue was dissolved in ethanol (15 mL), petroleum ether (50 mL) was added, and the solution was cooled. The crystalline product (3.35 g, 68%) had mp 109–111 °C. After recrystallization of an aliquot from a minimal amount of ethanol, it had mp 112.5–113.5 °C; $[\alpha]^{20}_D$ −20° (*c* 5, ethyl acetate); TLC R_f (A) 0.89, R_f (B) 0.94. Anal. ($C_{18}H_{25}N_3O_7$) C, H, N.

N^2-(*tert*-Butyloxycarbonyl)-N^5,N^5-dipropylglutamine Benzyl Ester (3). Coupling of N^2-(*tert*-butyloxycarbonyl)-glutamic acid α-benzyl ester (3.37 g, 10 mmol) with 1.51 g (15 mmol) of dipropylamine was effected in the usual manner via the mixed anhydride procedure in THF solution.[18] After 2 h, the reaction mixture was filtered and the solvent was evaporated in vacuo. The remaining residue was taken up with ethyl acetate, washed with 5% $NaHCO_3$ and water, and dried over Na_2SO_4. The solvent was removed and the residue crystallized upon addition of petroleum ether and cooling: yield 2.92 g (69%); mp 48 °C; $[\alpha]^{20}_D$ −35° (*c* 1, ethanol); TLC R_f (A) 0.94, R_f (C) 0.91. Anal. ($C_{23}H_{36}N_2O_5$) C, H, N.

N^2-(*tert*-Butyloxycarbonyl)-N^5,N^5-dipropylglutamine (4). To a 50-mL solution of 3 (2.8 g, 6.66 mmol) in 50 mL of 2-propanol was added 10 mL of water, and catalytic hydrogenation was carried out over palladium oxide (0.3 g). After 4 h, the catalyst was filtered off, the solvent was removed in vacuo, and the remaining residue was crystallized by trituration with ethyl acetate–ether–petroleum ether (1:2:5) while cooling. The product was recrystallized from 2-propanol–water (1:1): yield 2.13 g (97%); mp 77–79 °C; $[\alpha]^{20}_D$ −11.7° (*c* 1, $CHCl_3$); TLC R_f (A) 0.91, R_f (C) 0.7. Anal. ($C_{16}H_{30}N_2O_5$) C, H, N.

Stepwise Syntheses. Boc-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2[19] served as the starting material for the syntheses of the oxytocin analogues. The synthesis of Boc-Glu[N(CH$_3$)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (**5**) is typical of the process of stepwise elongation. A sample of the protected pentapeptide (1.4 g, 2 mmol) was dissolved in CF_3CO_2H (4 mL). After 45 min, the CF_3CO_2H was evaporated under reduced pressure; the residue was triturated to a solid with Et_2O, collected by filtration, washed with Et_2O, and dried in vacuo. The solid was dissolved in DMF (7 mL), the pH of the solution was adjusted to 7.5 with *N*-methylmorpholine, and **2** (0.92 g, 2.3 mmol) was added. The progress of the reaction was followed by a combination of TLC and Kaiser test.[20] After 48 h at room temperature, the solvent was removed under reduced pressure and the residue solidified by trituration with ethyl acetate. The solid was filtered off, washed with cold water, and dried over P_2O_5. It was then dissolved in hot methanol and precipitated with ether (1:2, v/v). The yields, physical properties, and other analytical data for all intermediates are given in Table II.

Instead of using an active ester, Boc-Glu[N(*n*-C_3H_7)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (**9**) was prepared by adding a DMF solution of **4** preactivated[21] with dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole to a DMF solution of Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2. After the reaction was complete, isolation of the product was performed as described above.

Z-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2[22] (2.69 g, 3 mmol) was the starting material for the vasopressin analogue. After decarbobenzoxylation with 2.5 N HBr–HOAc, the HBr salt was precipitated with Et_2O, isolated by filtration, washed with Et_2O, and dried in vacuo. The HBr salt was then dissolved in DMF, neutralized with *N*-methylmorpholine, and coupled with **2** (1.58 g, 4 mmol) as described above.

[4-(N^5,N^5-Dimethylglutamine)]oxytocin (17). A sample of the protected nonapeptide **8** (110 mg, 77 μmol) was deprotected by sodium in liquid ammonia.[9] After evaporation of the ammonia by a nitrogen stream, the residue was dissolved in deaerated 50% aqueous methanol, and the disulfide was formed by oxidation with 1 equiv of 1,2-diiodoethane.[10] The product was subjected to gel filtration on a 117 × 2.5 cm column of Sephadex G-15 (fine) with 50% acetic acid[23] (elution volume = 145 mL). It was further purified by partition chromatography[12] on a 65 × 2.8 cm column of Sephadex G-25 (100–200 mesh, block polymerizate) which had been equilibrated with both phases of the solvent system 1-butanol–benzene–aqueous 3.5% acetic acid containing 1.5% pyridine (9:1:10, v/v). The column was eluted with the upper phase and the product emerged at an R_f of 0.20, as determined by the method of Lowry et al.[24] Final purification was effected by gel filtration on a 68 × 2.8 cm column of Sephadex G-25 (block polymerizate, 200–270 mesh) with 0.2 M acetic acid: yield following lyophilization from 0.2 M acetic acid, 46 mg; TLC gave a single spot, R_f (A) 0.30, R_f (B) 0.61, R_f (C) 0.72, R_f (D) 0.25; $[\alpha]^{26}_D$ −15° (*c* 0.8, 1 M acetic acid). Amino acid analysis: Cys(O_3H), 1.95; Asp, 1.01; Glu, 1.02; Pro, 1.01; Gly, 1.00; Ile, 0.96; Leu, 1.02; Tyr, 0.90; NH_3, 1.97.

[4-(N^5,N^5-Di-*n*-propylglutamine)]oxytocin (18). A sample of **16** (103 mg, 68.7 μmol) was deprotected, oxidized, and desalted as in **17**. Partition chromatography on the isolated product (63.7 mg) was performed on Sephadex G-25 using the system 1-butanol–benzene–acetic acid–aqueous 1.5% pyridine (10:15:12:18; R_f 0.38) and, following isolation of the material, gel filtration was

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Table I. Biological Activities of Oxytocin and Lysine-vasopressin Compared with Their N^5,N^5-Dialkylated Glutamine Analogues

compd	uterotonic[a] (rat)	avian[a] vasodepressor	pressor[a] (rat)	antidiuretic[a] (rat)	% max uterotonic resp ± SEM
OT	546 ± 18[c]	507 ± 15[d]	2.7 ± 0.2[d]	3.1 ± 0.1[d]	100 (n =8)
[Glu[N(CH_3)$_2$]^4]OT	3.01 ± 0.14	4.55 ± 0.03	nd[b]	nd[b]	75 ± 5 (n = 8)
[Glu[N(n-C_3H_7)$_2$]^4]OT	<0.1	<0.1	<0.05	<0.002	8 ± 5 (n = 3)
[Glu[N(CH_3)$_2$]^4]LVP	<0.05	<0.1	1.27 ± 0.03	1.88 ± 0.01	44 ± 13 (n = 3)
LVP	4.8 ± 0.3[e]	48 ± 2[e]	243 ± 3[e,f]	203 ± 7[f]	nd

[a] Agonist activities are expressed in units/mg ± SEM. [b] In the pressor and antidiuretic assays, the compound was found to be tachyphylactic; no inhibitory activity to oxytocin or arginine-vasopressin induced pressor responses were noted. [c] See ref 32. [d] See ref 33. [e] See ref 34. [f] See ref 35.

Table II. Intermediates in the Stepwise Synthesis of Analogues Containing N^5,N^5-Dialkylglutamine

no.	compd	coupling agent	yield, %	mp, °C	$[\alpha]^{20}_D$, deg (c 1, DMF)	TLC R_f[a]	anal. calcd[b]
5	Boc-Glu[N(CH_3)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	Boc-Glu[N(CH_3)$_2$]-ONp	87	163–166	−50.9	0.60 (A), 0.64 (D)	$C_{36}H_{61}N_9O_{10}S$
6	Boc-Ile-Glu[N(CH_3)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	Boc-Ile-OSu	74	193–196	−55.1	0.63 (A), 0.68 (D)	$C_{37}H_{72}N_{10}O_{11}S$
7	Boc-Tyr(Bzl)-Ile-Glu[N(CH_3)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	Boc-Tyr(Bzl)-OSu	80	211–213	−35.9	0.60 (A), 0.90 (D)	$C_{61}H_{85}N_{11}O_{13}S$
8[c]	Z-Cys(Bzl)-Tyr(Bzl)-Ile-Glu[N(CH_3)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	Z-Cys(Bzl)-OSu	37	225–227	−54.5[d]	0.80 (A), 0.88 (E)	$C_{71}H_{96}N_{12}O_{14}S_2$
9	Boc-Glu[N(n-C_3H_7)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	DCC-HBT[e]	66	184–186	−45.9	0.70 (A), 0.71 (D)	$C_{43}H_{69}N_9O_{10}S$
10	Boc-Ile-Glu[N(n-C_3H_7)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	Boc-Ile-OSu	50	203–205	−50.0[f]	0.73 (A), 0.79 (D), 0.88 (E)	$C_{49}H_{80}N_{10}O_{11}S$
11	Boc-Tyr(Bzl)-Ile-Glu[N(n-C_3H_7)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	Boc-Tyr(Bzl)-OSu	81	210–212	−40.9	0.76 (A), 0.95 (D)	$C_{73}H_{97}N_{11}O_{13}S$
12[g]	Z-Cys(Bzl)-Tyr(Bzl)-Ile-Glu[N(n-C_3H_7)$_2$]-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	Z-Cys(Bzl)-OSu	53	220–222	−50.8	0.87 (A), 0.86 (G)	$C_{83}H_{104}N_{12}O_{14}S_2$
13	Boc-Glu[N(CH_3)$_2$]-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	Boc-Glu[N(CH_3)$_2$]-ONp	76	179–180	−37.1	0.54 (A)	$C_{46}H_{68}N_{10}O_{12}S_2$
14	Boc-Phe-Glu[N(CH_3)$_2$]-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	Boc-Phe-OSu	96	160–161	−39.0	0.59 (A)	$C_{53}H_{74}N_{11}O_{13}S_2$
15	Boc-Tyr(Bzl)-Phe-Glu[N(CH_3)$_2$]-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	Boc-Tyr(Bzl)-OSu	85	188–190	−36.8	0.70 (A)	$C_{71}H_{92}N_{12}O_{15}S_2$
16[h]	Z-Cys(Bzl)-Tyr(Bzl)-Phe-Glu[N(CH_3)$_2$]-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	Z-Cys(Bzl)-OSu	71	198–200	−36.5	0.78 (A), 0.76 (E)	

[a] Only single spots were detected for loads of at least 50 μg. Letters in parentheses indicate solvents system given in Experimental Section. [b] C, H, N were within ±0.4% of theory. [c] Amino acid analysis: Asp, 1.00; Glu, 1.02; Pro, 1.00; Gly, 1.00; Ile, 0.97; Leu, 1.04; Tyr, 0.90; Cys(Bzl), 1.84; NH_3, 1.86. [d] c 1, HOAc. [e] See Experimental Section. [f] c 2, DMF. [g] Compound was purified by gel filtration on a 1.4 × 80 cm column of Sephadex LH-20 using DMF. Amino acid analysis: Asp, 1.01; Glu, 0.94; Pro, 1.02; Gly, 1.00; Ile, 0.88; Leu, 1.03; Tyr, 0.82; Cys(Bzl), 1.82; NH_3, 2.05. [h] Amino acid analysis: Asp, 1.00; Glu, 1.01; Pro, 1.00; Gly, 1.00; Tyr, 0.94; Phe, 1.04; Cys(Bzl), 1.96; Lys, 0.20; NH_3, 1.83; Lys(Tos), 0.61.

performed on Sephadex G-25 (200–270 mesh) with 0.2 M acetic acid (elution volume ~340 mL): yield following lyophilization, 50.0 mg; TLC R_f (A) 0.26, R_f (D) 0.63, R_f (F) 0.89; $[\alpha]^{20}_D$ +3° (c 1.2, 50% acetic acid). Amino acid analysis: Cys(O_3H), 1.92; Asp, 1.01; Glu, 1.00; Pro, 1.02; Gly, 1.00; Ile, 0.96; Leu, 1.02; Tyr, 0.85; NH_3, 2.08.

[4-(N^5,N^5-Dimethylglutamine),8-lysine]vasopressin (19). An aliquot of **16** (143 mg, 87 μmol) was deprotected and oxidized to the disulfide and desalted as described for **17**. The product was further purified by gel filtration on the same Sephadex G-15 column with 0.2 M acetic acid, by partition chromatography on Sephadex G-25 using the system 1-butanol–ethanol–pyridine–acetic acid–water (4:1:1:0.4:6.4): R_f 0.22; yield following lyophilization, 69.2 mg. A final gel filtration on Sephadex G-25 (block polymerizate, 200–270 mesh) using 0.2 M acetic acid (~338 mL elution volume) yielded following lyophilization 67.7 mg (76%); TLC R_f (D) 0.25, R_f (F) 0.40; $[\alpha]^{23}_D$ −30° (c 0.6, 1 N acetic acid). Amino acid analysis: Cys(O_3H), 1.91; Asp, 1.02; Glu, 1.04; Pro, 1.06; Gly, 1.00; Tyr, 0.92; Phe, 0.98; Lys, 0.99; NH_3, 1.90.

Biological Assays. Rat uterotonic assays were performed on isolated horns from virgin rats in natural estrus according to the method of Holton as modified by Munsick with the use of Mg^{2+}-free van Dyke–Hastings solution as bathing fluid.[25] For dose–response determinations on the rat uterus in vitro, the conditions were those of above. The individual injection method[26] was used with doses being increased geometrically according to a 0.5 log 10 procedure until a maximal response was reached. Details of the experimental procedure have been previously published.[27] Avian vasodepressor assays were performed on conscious White Leghorn roosters by the method of Coon as described with modifications.[28] Antidiuretic[29] and pressor[30] assays were performed on anesthetized Sprague–Dawley male rats. Whenever measurable activity was detected, the four-point design of Schild[31] was used with at least ten determinations on three animals. Compounds without measurable activity were tested in at least two animals in which posterior pituitary reference standard gave normal responses.

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[5-(N^4,N^4-Dimethylasparagine),8-lysine]vasopressin: The First 5-Position Neurohypophyseal Hormone Analogue to Retain Significant Antidiuretic Potency[1]

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In the proposed biologically active conformation of vasopressin at the antidiuretic receptor, the side-chain carboxamide group of the 5-position asparaginyl residue has been previously suggested to be the key active element in the hormone for its initiation of the antidiuretic response. [5-(N^4,N^4-Dimethylasparagine),8-lysine]vasopressin, the analogue in which the hydrogen atoms of the $-NH_2$ portion of the primary carboxamide have been replaced by methyl groups, has been synthesized and found to retain about 3% of the antidiuretic potency of lysine-vasopressin (i.e., 5.5 ± 0.3 units/mg). This result suggests that the hydrogen atoms of the carboxamide moiety are not essential for antidiuretic activity. In addition, the analogue possesses rat pressor, avian vasodepressor, and rat uterotonic potencies of 2.55 ± 0.05, 0.39 ± 0.03, and less than 0.05 units/mg, respectively.

The 5-position asparaginyl residue plays a crucial role in the proposed "biologically active" conformation of vasopressin (Figure 1) at the antidiuretic receptor.[2] The side-chain carboxamide group acting cooperatively with the basic moiety of the 8-position residue have been suggested to be the "active elements"[3] of the hormone. That the side-chain carboxamide of the asparaginyl residue may be the key active element is demonstrated by the lack of antidiuretic activity of [5-alanine]lysine-vasopressin[4] and

(1) All optically active amino acids are of the L configuration. Abbreviations used follow the recommendations of IUPAC–IUB as found in *Biochemistry* **1975**, *14*, 449, and *Biochem. J.* **1972**, 126, 773. Other abbreviations used are: Asn[$N(CH_3)_2$], N^4,N^4-dimethylasparagine; Boc, *tert*-butyloxycarbonyl; OT, oxytocin; LVP, lysine-vasopressin; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DMF, dimethylformamide; Z, benzyloxycarbonyl; –OSu, *N*-hydroxysuccinimide ester; –ONp, *p*-nitrophenol ester; HBT, 1-hydroxybenzotriazole.

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