J. Med. Chem. 1989, 32, 244-247

2-*O*-Alkyltyrosine Derivatives of 1-Deamino-arginine-vasopressin: Highly Specific and Potent Antidiuretic Agonists[†]

Bernard Lammek,[‡] Krzysztof Bankowski,[§] Aleksandra Misicka,[§] Maurice Manning,*[,‖] J. Seto,[⊥] and W. H. Sawyer[⊥]

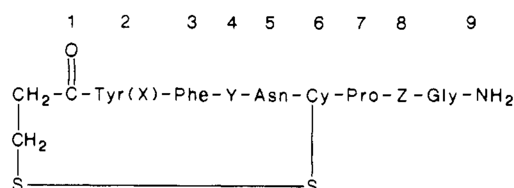
Department of Biochemistry, Medical College of Ohio, Toledo, Ohio 43699, and Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, New York 10032. *Received April 7, 1988*

We report the solid-phase synthesis of eight 2-*O*-alkyltyrosine analogues of 1-deamino-arginine-vasopressin (dAVP) with enhanced antidiuretic agonistic specificity. These peptides are as follows: 1-deamino[2-*O*-methyltyrosine]-arginine-vasopressin (dTyr(Me)AVP), 1-deamino[2-*O*-ethyltyrosine]arginine-vasopressin (dTyr(Et)AVP), 1-deamino[2-*O*-methyltyrosine,8-D-arginine]vasopressin (dTyr(Me)DAVP), 1-deamino[2-*O*-ethyltyrosine,8-D-arginine]vasopressin (dTyr(Et)DAVP), 1-deamino[2-*O*-methyltyrosine,4-valine]arginine-vasopressin (dTyr(Me)VAVP), 1-deamino[2-*O*-ethyltyrosine,4-valine]arginine-vasopressin (dTyr(Et)VAVP), 1-deamino[2-*O*-methyltyrosine,4-valine,8-D-arginine]vasopressin (dTyr(Me)VDAVP), and 1-deamino[2-*O*-ethyltyrosine,4-valine,8-D-arginine]vasopressin (dTyr(Et)VDAVP). All analogues were tested for antidiuretic, antivasopressor, and antioxytocic activities. Deamination, as was expected, significantly enhanced the antidiuretic properties of these analogues relative to their parent *N*-amino-*O*-alkyltyrosine peptides. With the exception of dTyr(Me)AVP, all of these analogues are antagonists of the vasopressor responses to AVP and of the uterine response to oxytocin. Thus they all exhibit high antidiuretic agonistic specificity. Due to its remarkable properties, dTyr(Me)VDAVP is a unique compound in this series. It appears to be the most potent antidiuretic agonist (1740 units/mg) and also a vasopressor antagonist and a potent oxytocin antagonist. It is thus a highly specific antidiuretic agonist. In general, all of these new analogues are highly specific and thus are potentially useful as pharmacological tools and clinical agents.

Among the single modifications that have been shown to enhance antidiuretic agonistic specificity in arginine–vasopressin (AVP) analogues are D-Arg^8 substitution, deamination, and substitution of the Gln residue at position 4 by more hydrophobic amino acids. These modifications, alone and in combination, have led to peptides possessing high or infinite antidiuretic/vasopressor (A/P) activity ratios, e.g., DAVP (A/P = 240), dDAVP (A/P = 3000), and dVDAVP (A/P = infinite).[1] Recently, we reported the synthesis and some pharmacological properties of new $Tyr(Me)^2$– and $Tyr(Et)^2$–substituted analogues of AVP, DAVP, VAVP, and VDAVP.[2] We found that the $Tyr(Me)^2$ modification allowed retention of antidiuretic activity, whereas the $Tyr(Et)^2$ substitution led to substantial losses of antidiuretic activity. All analogues exhibited antivasopressor and antioxytocic properties, which gave them high antidiuretic agonistic specificity with infinite antidiuretic/pressor (A/P) activity ratios. The present study was undertaken in an attempt to determine the effects of deamination on the antidiuretic, vasopressor, and oxytocic potencies and on the antidiuretic specificity of these 2-*O*-alkyltyrosine analogues of AVP, DAVP, VAVP, and VDAVP.

To date, only a few 1-deamino analogues of tyrosine-(alkyl)-lysine-vasopressin ($dTyr(alk)^2LVP$, alk = ethyl propyl, butyl, hexyl) have been reported.[3] Only one such of analogue of dAVP (dTyr(Me)DAVP) has been described to date.[4]

We now describe the synthesis and some pharmacological properties of seven new analogues designed according to the above rationale. These peptides are as follows: 1-deamino[2-*O*-methyltyrosine]arginine-vasopressin (dTyr(Me)AVP), 1-deamino[2-*O*-ethyltyrosine]arginine-vasopressin (dTyr(Et)AVP), 1-deamino[2-*O*-ethyltyrosine,8-D-arginine]vasopressin (dTyr(Et)DAVP), 1-deamino[2-*O*-methyltyrosine,4-valine]arginine-vasopressin (dTyr(Me)VAVP), 1-deamino[2-*O*-ethyltyrosine,4-valine]arginine-vasopressin (dTyr(Et)VAVP), 1-deamino[2-*O*-methyltyrosine,4-valine,8-D-arginine]vasopressin (dTyr(Me)VDAVP), and 1-deamino[2-*O*-ethyltyrosine,4-valine,8-D-arginine]vasopressin (dTyr(Et)VDAVP). We also resynthesized the previously reported 1-deamino[2-*O*-methyltyrosine,8-D-arginine]vasopressin (dTyr(Me)-DAVP).[4] All analogues have the following general structure:



where X = methyl or ethyl, Y = Gln or Val, Z = L- or D-Arg.

Peptide Synthesis

The protected peptide precursors required for the synthesis of the 1-deamino-2-*O*-alkyl analogues were prepared by the Merrifield method of solid-phase synthesis[5,6] using

* Address correspondence to Maurice Manning, Ph.D., D.Sc., Department of Biochemistry, Medical College of Ohio, C.S. 10008, Toledo, Ohio 43699.

[‡] Visiting investigator from the University of Gdansk.

[§] Visiting investigators from the University of Warsaw.

[‖] Medical College of Ohio.

[⊥] College of Physicians and Surgeons of Columbia University.

[†] Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* 1984, *138*, 9). All amino acids are in the L configuration unless otherwise noted. Other abbreviations used are: Tyr(Me), *O*-methyltyrosine; Tyr(Et), *O*-ethyltyrosine; AVP, arginine-vasopressin; dAVP, 1-deamino-arginine-vasopressin; DAVP, [8-D-arginine]vasopressin; VAVP, [4-valine]arginine-vasopressin; VDAVP [4-valine,8-D-arginine]-vasopressin; dDAVP, 1-deamino[8-D-arginine]vasopressin; dVAVP, 1-deamino[4-valine]arginine-vasopressin; dVDAVP, 1-deamino[4-valine,8-D-arginine]vasopressin; DMF, dimethylformamide; DCCI, dicyclohexylcarbodiimide; BOC, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; AcOH, acetic acid; TFA, trifluoroacetic acid; HOBT, *N*-hydroxybenzotriazole; Et_3N, triethylamine.

(1) Manning, M.; Grzonka, T.; Sawyer, W. H. In *The Pituitary*; Beardwell, C.; Robinson, D., Eds.; Butterworth: Kent, England, 1981; p 265.
(2) Bankowski, K.; Lammek, B.; Kruszynski, M.; Manning, M.; Seto, J.; Sawyer, W. H. *Collect. Czech. Chem. Commun.*, in press.
(3) Larsson, L. E.; Lindeberg, G. *J. Med. Chem.* **1978**, *21*, 352.
(4) Krchnak, V.; Zaoral, M. *Collect. Czech. Chem. Commun.* **1979**, *44*, 1642.
(5) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.

0022-2623/89/1832-0244$01.50/0 © 1988 American Chemical Society

Table I. Effects of Deamination on the Antidiuretic and Vasopressor Potencies of 2-*O*-Alkyltyrosine Analogues of AVP, DAVP, VAVP, and VDAVP

			antivasopressor		antidiuretic/ pressor ratio:
no.	peptide	antidiuretic act., units/mg	effective dose,[b] nmol/kg	pA_2[c,g]	A/P[d]
	dAVP	1745 ± 385 [323 ± 16][a]	agonist (346 units/mg) [370 units/mg][a]		5
1	dTyr(Me)AVP	830 ± 70 [386 ± 36]	agonist (4.1 ± 0.3 units/mg) [9.7 units/mg]		202
2	dTyr(Et)AVP	242 ± 43 [152 ± 13]	11 ± 2	6.90 ± 0.09+ [7.17][a]	infinite
	dDAVP	1200 ± 126 [257 ± 35]	agonist (0.39 units/mg) [1.1 units/mg]		3037
3	dTyr(Me)DAVP[e]	236 ± 24 [309 ± 63]	9.8 ± 2.3	6.97 ± 0.10 [6.74]	infinite
4	dTyr(Et)DAVP	30 ± 4 [17.1 ± 0.9]	8.8 ± 1.2	6.89 ± 0.05+ [7.31]	infinite
	dVAVP	1150 ± 110 [738 ± 65]	agonist (51 units/mg) [32 units/mg]		225
5	dTyr(Me)VAVP	789 ± 91 [443 ± 3]	3.7 ± 0.4	7.26 ± 0.04* [6.63]	infinite
6	dTyr(Et)VAVP	187 ± 43 [29 ± 2]	1.9 ± 0.2	7.54 ± 0.04* [7.10]	infinite
	dVDAVP	1230 ± 170 [653 ± 51]	7.2 ± 1.4 [0.037 units/mg]	7.03 ± 0.11*	infinite
7	dTyr(Me)VDAVP	1740 ± 430[f] [350]	8.4 ± 2.1	7.01 ± 0.13* [6.63]	infinite
8	dTyr(Et)VDAVP	132 ± 20 [106 ± 11]	6.3 ± 1.4	7.07 ± 0.11* [6.54]	infinite

[a] Potency for each of the corresponding amino group containing derivatives.[2] [b] The effective dose (ED) is defined as the dose (in nanomoles/kilogram) that reduces the response seen with 2*x* units of agonist to equal the response seen with 1*x* unit of agonist administered before antagonist. [c] Estimated in vivo pA_2 values represent the negative logarithms of the "effective dose" divided by the estimated volume of distribution (6.7 mL/kg). [d] Ratio of antidiuretic to vasopressor agonistic activities. [e] This analogues was originally reported[4] to have only 10 units/mg ADH activity. [f] The slopes of the log dose–response regressions for this analogue varied and deviated significantly from that for the standard. This value represents the mean of ten estimates of relative potency derived from comparing the ED_{50} values of the analogue and standard curves from the same individual rats. [g] (*) These pA_2 values were significantly ($P < 0.05$) greater than those reported[2] for the corresponding amino analogues. (+) These analogues showed significantly less antagonistic activity on these assays than did their aminated counterparts.[2]

previously described modifications.[7-10] Coupling reactions were mediated either by the active ester method[11] or by the DCCl/HOBT method.[12] β-(benzylthio)propionic acid was used in all final coupling steps. The protected acyl octapeptide amides were obtained by ammonolytic cleavage[10,13] from the respective acyl octapeptide resin. Na in NH_3 was used to deblock each protected precursor as previously described[7-10,14] and the resulting disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$.[15] The free peptides were desalted and purified by gel filtration[16] on Sephadex G-15 as previously described.[17]

Bioassay Methods

Analogues were assayed for antidiuretic activities by intravenous injection into ethanol-anesthetized and water-loaded rats[18] and for vasopressor activities by intravenous injection into phenoxybenzamine-treated rats under urethane anesthesia.[19] The USP posterior pituitary reference standard was used in all assays. Antagonistic activities were estimated with the same bioassay preparations. The "effective dose" (ED) of an antagonist was estimated as the dose, in nanomoles/kilogram, that reduces the response to 2*x* units of agonist to equal the response to 1*x* units given in the absence of antagonist. In vivo pA_2 values were calculated as the negative logarithms of the ED values divided by an arbitrarily chosen volume of distribution (67 mL/kg).[20] Oxytocic agonism and antagonism were estimated by assays on uteri isolated from estrogen-treated female rats and suspended in media containing no Mg^{2+} or 0.5 mM Mg^{2+}.[21] Agonistic activities were estimated by the method of Holton[22] and antagonistic pA_2 values were determined as described by Schild.[23] The in vivo antioxytocic pA_2 was also estimated for one analogue by methods described by Sawyer et al. in ref 24.

Table II. Antioxytocic Potencies of 2-*O*-Alkyltyrosine Analogues of AVP, DAVP, VAVP, and VDAVP

		antioxytocic (in vitro),[a] pA_2	
no.	peptide	no Mg^{2+}	0.5 mM Mg^{2+}
	dAVP	agonist (54 units/mg)	agonist (167 units/mg)
1	dTyr(Me)AVP	7.41 ± 0.18	agonist (1.1 ± 0.3+)
2	dTyr(Et)AVP	7.37 ± 0.06	7.40 ± 0.06*
	dDAVP	agonist (1.5 units/mg)	agonist (2.9 units/mg)
3	dTyr(Me)DAVP	7.93 ± 0.06*	7.40 ± 0.05*
4	dTyr(Et)DAVP	7.28 ± 0.04+	7.05 ± 0.03*
	dVAVP	agonist (31 units/mg)	agonist (26 units/mg)
5	dTyr(Me)VAVP	7.93 ± 0.10	7.71 ± 0.07*
6	dTyr(Et)VAVP	7.90 ± 0.07*	7.64 ± 0.03*
	dVDAVP	agonist (8 units/mg)	agonist (3 units/mg)
7	dTyr(Me)VDAVP	7.77 ± 0.17	8.02 ± 0.11 (7.38 ± 0.10)[b]
8	dTyr(Et)VDAVP	7.55 ± 0.09	7.64 ± 0.05*

[a] (*) These pA_2 values were significantly ($P < 0.05$) greater than those reported[2] for the corresponding amino analogues. (+) These analogues showed significantly less antagonistic activity on these assays than did their aminated counterparts.[2] [b] In vivo.

(6) Merrifield, R. B. *Biochemistry* **1964**, *2*, 1385.
(7) Manning, M.; Lammek, B.; Kruszynski, M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1982**, *25*, 408.
(8) Manning, M.; Lowbridge, J.; Stier, C. T., Jr.; Haldar, J.; Sawyer, W. H. *J. Med. Chem.* **1977**, *20*, 1228.
(9) Kruszynski, M.; Lammek, B.; Manning, M.; Seto, J.; Haldar, J.; Sawyer, W. H. *J. Med. Chem.* **1980**, *23*, 364.
(10) Manning, M. *J. Am. Chem. Soc.* **1968**, *90*, 1348.
(11) (a) Bodanszky, M.; du Vigneaud, V. *J. Am. Chem. Soc.* **1959**, *81*, 5688. (b) Bodanszky, M.; Kondo, M.; Lin, C. Y.; Sigler, G. F. *J. Org. Chem.* **1974**, *39*, 444.
(12) (a) Sheehan, J. C.; Hess, G. P. *J. Am. Chem. Soc.* **1955**, *77*, 1067. (b) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.
(13) Bodanszky, M.; Sheehan, J. T. *Chem. Ind. (London)* **1964**, 1423.
(14) (a) du Vigneaud, V.; Ressler, C.; Swan, J. M.; Roberts, C. W.; Katsoyannis, P. G.; Gordon, S. J. *J. Am. Chem. Soc.* **1953**, *75*, 4879. (b) du Vigneaud, V.; Ressler, C.; Swan, J. M.; Roberts, C. W.; Katsoyannis, P. G. *Ibid.* **1954**, *76*, 3115.
(15) Hope, D. V.; Murti, V. V. S.; du Vigneaud, V. *J. Biol. Chem.* **1962**, *237*, 1563.
(16) Porath, J.; Flodin, P. *Nature (London)* **1959**, *183*, 1657.
(17) Manning, M.; Wuu, T. C.; Baxter, J. W. M. *J. Chromatogr.* **1968**, *38*, 396.
(18) Sawyer, W. H. *Endocrinology* **1958**, *63*, 694.
(19) Dekanski, J. *Br. J. Pharmacol.* **1952**, *7*, 567.
(20) Dyckes, D. F.; Nestor, J. J., Jr.; Ferger, M. F.; du Vigneaud, V. *J. Med. Chem.* **1974**, *17*, 250.
(21) Munsick, R. A. *Endocrinology* **1960**, *66*, 451.
(22) Holton, P. *Br. J. Pharmacol.* **1948**, *3*, 328.
(23) Schild, H. O. *Br. J. Pharmacol.* **1947**, *2*, 189.

Table III. Physicochemical Properties of the Protected Peptides: [β-(Benzylthio)propionyl]-X²-Phe-Y⁴-Asn-Cys(Bzl)-Pro-Z⁸-Gly-NH₂ (I-VIII)

no.	structure			formula	yield,[a,b] %	mp, °C	$[\alpha]^{25}_D$, deg (c = 1, DMF)	R_f		
	X^2	Y^4	Z^8					A	B	C
I	Tyr(Me)	Gln	Arg(Tos)	$C_{68}H_{86}N_{14}O_{14}S_3$	79.8	215–217	–31.2	0.51		0.61
II	Tyr(Et)	Gln	Arg(Tos)	$C_{69}H_{88}N_{14}O_{14}S_3$	76.0	215–218	–37.6	0.52		0.62
III	Tyr(Me)	Gln	D-Arg(Tos)	$C_{68}H_{86}N_{14}O_{14}S_3$	81.2	214–215	–23.4	0.70	0.46	0.81
IV	Tyr(Et)	Gln	D-Arg(Tos)	$C_{69}H_{88}N_{14}O_{14}S_3$	78.0	213–214	–24.2	0.72	0.41	0.85
V	Tyr(Me)	Val	Arg(Tos)	$C_{68}H_{87}N_{13}O_{13}S_3$	83.1	229–230	–28.4	0.74	0.76	0.84
VI	Tyr(Et)	Val	Arg(Tos)	$C_{69}H_{89}N_{13}O_{13}S_3$	68.7	232–234	–37.8	0.58	0.50	0.82
VII	Tyr(Me)	Val	D-Arg(Tos)	$C_{68}H_{87}N_{13}O_{13}S_3$	76.4	234–235	–22.3	0.69	0.80	0.78
VIII	Tyr(Et)	Val	D-Arg(Tos)	$C_{69}H_{89}N_{13}O_{13}S_3$	79.9	235–236	–24.6	0.65	0.82	0.76

[a] Yields were calculated on the basis of the glycine content of the starting resin. [b] All the protected peptides gave the expected amino acid ratios after hydrolysis ± 3%.[26]

Results and Discussion

The antidiuretic, antivasopressor, and antioxytocic properties of the seven new Tyr(Me)- and Tyr(Et)-substituted analogues of dAVP, dDAVP, dVAVP, and dVDAVP with those for dTyr(Me)DAVP, together with these properties of each parent peptide, are given in Tables I and II.

Effect of Deamination on Antidiuretic Activities. Comparing the activities of the presently reported 1-deamino analogues with the corresponding amino peptides,[2] we observed that, as expected, deamination appeared to enhance the antidiuretic properties of all analogues with the exception of dTyr(Me)DAVP. This analogue exhibited somewhat diminished antidiuretic activity relative to its NH_2-containing precursor (236 units/mg versus 309 units/mg). This is a rather puzzling finding which illustrates yet another clear instance of predictability gone somewhat awry. It should be noted that the antidiuretic potency originally reported for this compound was 10 units/mg.[4]

Three of the analogues presented here, dTyr(Me)AVP, dTyr(Me)VAVP, and dTyr(Me)VDAVP, exhibit the high antidiuretic activities of 830, 789, and 1740 units/mg, respectively.

Effect of Deamination on Antivasopressor Properties. Except for dTyr(Me)AVP, which still possesses some weak agonistic activity (4.1 units/mg), all of the 1-deamino analogues are antagonists of the vasopressor response to AVP. However the effects of deamination are inconsistent; in some cases this modification leads to decreases, in others to increases of antivasopressor potency.

Effect of Deamination on Antidiuretic Specificity. The recently reported Tyr(Me)- and Tyr(Et)-substituted analogues of AVP, DAVP, VAVP, and VDAVP exhibited high antidiuretic agonistic specificity.[2] Deamination of these peptides resulted in further increases of antidiuretic activity. In fact, dTyr(Me)VDAVP appears to be one of the most potent and specific antidiuretic agonists reported to date.

Effect of Deamination on Antioxytocic Activity. All analogues listed in Table II are antagonists of the in vitro oxytocic responses to oxytocin in the absence and in the presence of magnesium. Only dTyr(Me)AVP shows weak agonistic activity in the presence of magnesium. In general, antioxytocic activities of these deamino analogs were comparable to, or greater than, those of their aminated counterparts. Remarkably, the most potent antagonist appears to be dTyr(Me)VDAVP. This has an antioxytocic pA_2 (0.5 mM Mg^{2+}) = 8.02. This peptide is also a potent antagonist of oxytocic responses in vivo with a pA_2 = 7.38 ± 0.10.

(24) Sawyer, W. H.; Haldar, J.; Gazis, D.; Seto, J.; Bankowski, K.; Lowbridge, J.; Turan, A.; Manning, M. *Endocrinology* **1980**, *106*, 81.

Conclusion

We have reported the synthesis and some pharmacological properties of eight 1-deamino-2-*O*-alkyltyrosine analogues of AVP, DAVP, VAVP, and VDAVP. Deamination, as was expected, significantly enhanced the antidiuretic properties of most of these analogues relative to their parent amino-*O*-alkyltyrosine peptides.[2] With the exception of dTyr(Me)AVP, all of the new analogues are antagonists of the vasopressor responses to AVP and uterine responses to oxytocin. Thus they all exhibit high antidiuretic agonistic specificity. Due to its remarkable properties, dTyr(Me)VDAVP is a unique compound in the series. It appears to be the most potent antidiuretic agonist (1740 units/mg) and it is also a vasopressor antagonist and a potent oxytocic antagonist in vitro and in vivo. It is thus a highly selective antidiuretic agonist, possibly even more potent an antidiuretic agent than dDAVP but devoid of detectable vasopressor or oxytocic agonism. In general, all of these new analogues are highly selective and thus are potentially useful as pharmacological tools and clinical agents. It should be noted that dTyr(Me)VDAVP (donated as an unpublished compound) has been utilized as a selective V_2 agonist in two published studies.[28,29]

Experimental Section

The protected peptide intermediates I–VIII (Table III) were synthesized by the solid-phase method[5,6] by previously described procedures.[7-10] Chloromethylated resin (Bio-Rad Bio-Beads S × 1) was esterified with BOC-Gly to a load of 0.4–0.65 mmol/g according to Gisin.[25] Amino acid derivatives, including BOC-Tyr(Me) and BOC-Tyr(Et), were supplied by Bachem, Inc. Triethylamine (TEA) and *N*-methylmorpholine (NMM) were distilled from ninhydrin. Dimethylformamide (DMF) was distilled under reduced pressure. Methanol was dried with magnesium methoxide and distilled. Other solvents and reagents were of analytical grade. Thin-layer chromatography (TLC) was on silica gel (0.25 mm, Brinkmann Silplate). The following solvent systems were used: (A) butan-1-ol–acetic acid–water (4:1:5, v/v, upper phase), (B) chloroform–methanol (7:3, v/v), (C) butan-1-ol–acetic acid–water–pyridine (15:3:3:10; v/v), (D) butan-1-ol–acetic acid–water (4:1:1, v/v). Loads of 10–50 μg were applied and chromatograms were a minimum length of 10 cm. Iodine vapor was used for detection. For amino acid analysis,[26] peptides (approximately 0.5 mg) were hydrolyzed with constant-boiling hydrochloric acid (500 μL) containing phenol (10 μL) in evacuated and sealed ampules for 18 h at 120 °C. The analyses were performed on a Model 121 M Beckman automatic amino acid analyzer. Molar ratios were referred to Gly = 1.00. The cysteine

(25) Gisin, B. F. *Helv. Chim. Acta* **1973**, *56*, 1476.
(26) Spackman, D. H.; Stein, W. H.; Moore, S. *Anal. Chem.* **1958**, *30*, 1190.
(27) Moore, S. J. *Biol. Chem.* **1963**, *238*, 235.
(28) Kiraly, M.; Audigier, S.; Tribollet, E.; Barberis, C.; Dolivo, M.; Dreifuss, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 5335.
(29) Tribollet, E.; Barberis, C.; Jard, S.; DuBois-Dauphim, M.; Dreifuss, J. J. *Brain Res.* **1988**, *442*, 105.

Table IV. Physicochemical Properties of Agonists 1-8

```
CH2-CO-X2-Phe-Y4-Asn-Cy-Pro-Z8-Gly-NH2
|                     |
CH2                   |
|                     |
S---------------------S
```

no.	structure			yield,[a,b] (%)	$[\alpha]^{25}{}_D$, deg (c = 0.3, 1 N AcOH)	R_f		
	X^2	Y^4	Z^8			A	C	D
1	Tyr(Me)	Gln	Arg	49.8	−81.5	0.19	0.34	0.13
2	Tyr(Et)	Gln	Arg	60.8	−80.5	0.18	0.33	0.12
3	Tyr(Me)	Gln	D-Arg	55.0	−62.1	0.25	0.19	0.07
4	Tyr(Et)	Gln	D-Arg	65.0	−65.7	0.17	0.30	0.08
5	Tyr(Me)	Val	Arg	49.1	−89.0	0.26	0.62	0.16
6	Tyr(Et)	Val	Arg	53.2	−93.1	0.26	0.60	0.15
7	Tyr(Me)	Val	D-Arg	60.1	−71.5	0.27	0.56	0.24
8	Tyr(Et)	Val	D-Arg	58.5	−67.3	0.29	0.59	0.25

[a] Yields are based on the amount of protected peptide used in the reduction–reoxidation step in each case. [b] All the free peptides gave the expected amino acid analysis ratios after hydrolysis ± 3%.

content of the free peptides was estimated on the $Cy(SO_3H)$ to Gly ratio from analyses following performic acid oxydation.[27] All peptides (protected and free) gave the expected amino acid ratios (±3%). Elemental analyses on the protected peptides (Table III) were performed by Galbraith Laboratories, Inc., Knoxville, TN. The analytical results for elements indicated by their symbols were within 0.4% of theoretical values. Optical rotations were measured with a Model 80 Rudolph polarimeter.

[β-(Benzylthio)propionyl]-Tyr(Et)-Phe-Val-Asn-Cys-(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (Pr VIII). Boc-Gly-resin (1.56 g, 1 mmol) was converted to protected acyl octapeptidyl resin in eight cycles of solid-phase peptide synthesis using as the carboxy component BOC-D-Arg(Tos), BOC-Pro, BOC-Cys(Bzl), BOC-Asn *p*-nitrophenyl ester, BOC-Val, BOC-Phe, BOC-Tyr(Et), and finally β-(benzylthio)propionic acid, respectively. The protected acyl octapeptide was cleaved by ammonolysis.[10] The crude product was extracted with hot DMF and, after removal of the resin, precipitated by the addition of hot water. The precipitate was collected, dried in vacuo, and reprecipitated from DMF–ethanol–ethyl ether to give the required protected peptide amide (0.854 g, 79.9%) (based on the Gly content of the starting resin). The physicochemical properties of this and the remaining protected peptides I–VIII, which were prepared in essentially the same manner, are given in Table III.

[1-(β-Mercaptopropionic acid),2-*O*-ethyltyrosine,4-valine,8-D-arginine]vasopressin (dTyr(Et)VDAVP). A solution of the protected nonapeptide amide VIII (0.120 g, 0.11 mmol) in sodium-dried and redistilled ammonia (400 mL) was treated at the boiling point and with stirring with sodium[14] from a stick of metal contained in a small-bore glass tube until a light blue color persisted in the solution for 30 s. Dry glacial acetic acid (0.4 mL) was added to discharge the color. The solution was evaporated, the residue was dissolved in aqueous acetic acid (0.2%, 800 mL), and this solution was treated with 2 M ammonium hydroxide solution to give a solution of pH 6.5. An excess of a solution of potassium ferricyanide (0.01 M, 16 mL)[15] was added gradually with stirring. The yellow solution was stirred for a further 10 min and for 10 min with anion-exchange resin (Bio-Rad AG-3, Cl^- form, 10 g damp weight). The suspension was slowly filtered through a bed of resin (30 g damp weight). The bed was washed with aqueous acetic acid (0.2%, 200 mL), and the combined filtrate and washings were lyophilized. The resulting powder (1.5 g) was desalted on a Sephadex G-15 column (110 × 2.7 cm), eluting with aqueous acetic acid (50%)[17] with a flow rate 5 mL/h. The eluate was fractioned and monitored for absorbance at 280 nm. The fractions comprising the major peak were pooled and lyophilized, and the residue (65 mg) was further subjected to gel filtration on a Sephadex G-15 column (100 × 1.5 cm), eluting with aqueous acetic acid (0.2 M) with a flow rate of 4 mL/h.[17] The peptide was eluted in a single peak (absorbance at 280 nm). Lyophilization of the pertinent fractions yielded the vasopressin analogue [53.4 mg, 58.4% (based on the amount of protected peptide used in the reduction–reoxidation procedure)]. The physicochemical properties of this and of the remaining free peptides, **1–7**, which were prepared in the same way as for **8**, are given in Table IV. Their pharmacological properties are presented in Tables I and II.

Acknowledgment. This work was supported in part by research grants from the National Institutes of General Medical Sciences (Grant No. GM-25280) and the National Institute of Diabetes and Digestive and Kidney Diseases (Grant No. DK-01940). We thank Dr. Marian Kruszynski for help in the preparation of this manuscript, Dr. T. C. Wuu for generous use of amino acid analysis facilities, and Valerie Murphy for expert assistance in the preparation of this manuscript.

Registry No. 1, 73168-21-5; **2**, 114318-02-4; **3**, 71525-50-3; **4**, 117603-85-7; **5**, 84723-85-3; **6**, 117526-28-0; **7**, 85114-98-3; **8**, 117604-45-2; I, 117604-46-3; II, 117526-29-1; III, 71525-49-0; IV, 117604-47-4; V, 117526-30-4; VI, 117526-31-5; VII, 117604-48-5; VIII, 117604-49-6; BOC-Gly-OH, 4530-20-5; BOC-D-Arg(Tos)-OH, 61315-61-5; BOC-Pro-OH, 15761-39-4; BOC-Cys(Bzl)-OH, 5068-28-0; BOC-Asn-OC_6H_4-*p*-NO_2, 4587-33-1; BOC-Val-OH, 13734-41-3; BOC-Phe-OH, 13734-34-4; BOC-Tyr(Et)-OH, 76757-91-0; $PhCH_2SCH_2CH_2COOH$, 2899-66-3; BOC-Tyr(Me)-OH, 53267-93-9; oxytocin, 50-56-6.