Amino acid analysis: Tyr, 0.98; Phe, 0.98; Val, 1.03; Asp, 1.01; Pro, 1.01; Arg, 1.01; Gly, 1.00; NH_3, 2.11.

Analysis following performic acid oxidation prior to hydrolysis gave a Cys(O_3H)-Gly ratio of 1.05:1.00.

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Design of More Potent and Selective Antagonists of the Antidiuretic Responses to Arginine-vasopressin Devoid of Antidiuretic Agonism

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Substitution of D-tyrosine at position 2 of [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin, d(CH_2)$_5$VAVP, turned this weak antidiuretic agonist and antagonist of vasopressor responses into an effective antagonist of the antidiuretic response. d(CH_2)$_5$-D-TyrVAVP, however, like other reported antagonists of the antidiuretic response, retains some antidiuretic agonistic activity. It is also a relatively strong antagonist of vasopressor and oxytocic responses. In attempting (a) to increase the specificity of antagonists of the antidiuretic response, (b) to eliminate residual agonistic activity and enhance antagonistic potency, and (c) to help delineate structural features at position 2 required for antidiuretic antagonism, we have synthesized eight new analogues substituted at position 2 by the solid-phase method: **1**, d(CH_2)$_5$-D-PheVAVP; **2**, d(CH_2)$_5$-D-PheVDAVP; **3**, d-(CH_2)$_5$-D-IleVAVP; **4**, d(CH_2)$_5$-D-LeuVAVP; **5**, d(CH_2)$_5$-D-ValVAVP; **6**, d(CH_2)$_5$-D-AlaVAVP; **7**, d(CH_2)$_5$GlyVAVP; **8**, d(CH_2)$_5$-D-ArgVAVP. These analogues were tested for agonistic and antagonistic activities by antidiuretic, vasopressor, and oxytocic assays in rats. Analogues **1**, **3**, **4**, and **5** exhibit no agonistic activities in these assays. These four analogues, as well as analogue **2**, effectively antagonize antidiuretic responses to AVP. Their antiantidiuretic pA_2 values are as follows: **1**, 8.07 ± 0.09; **2**, 7.07 ± 0.1; **3**, 7.98 ± 0.05; **4**, 7.79 ± 0.12; **5**, 7.48 ± 0.06. Analogues **6**–**8** are weak antidiuretic agonists and exhibit no detectable antiantidiuretic activity. Analogues **3**–**8** show greatly reduced potencies as antagonists of vasopressor and oxytocic responses. Thus, analogues **3**–**5** show much greater specificity as antagonists of the antidiuretic response than any previously reported. Analogues **1** and **3** are also the most potent antagonists of the antidiuretic response yet reported. The combination of increased antiantidiuretic potency and specificity should make these analogues useful tools for studies on the role of AVP in causing water retention in experimental animals and in man. They may also serve as prototypes for the design of even more potent and selective antidiuretic antagonists. Potent and specific antagonists of the antidiuretic action of ADH could be valuable for treating water retention in a variety of clinical situations.

We recently reported the first known effective antagonists of in vivo antidiuretic responses to both exogenous and endogenous arginine-vasopressin (AVP).[1,2] These antagonists were designed by incorporating *O*-alkyltyrosine residues (where alkyl = methyl, ethyl, isopropyl, or *n*-propyl) at position 2 in two vasopressor antagonists, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin [d(CH_2)$_5$VDAVP][3] and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin [d(CH_2)$_5$VAVP].[4] We subsequently found that the substitution of an *O*-alkyl-D-tyrosine residue in each of these eight antidiuretic antagonists brought about substantial increases in their respective antiantidiuretic potencies.[5,6] This led to the discovery that the incorporation of an unalkylated D-tyrosine residue for L-tyrosine at position 2 in d(CH_2)$_5$VAVP and in d(CH_2)$_5$VDAVP converted these weak antidiuretic agonists into potent antagonists of the antidiuretic responses to AVP.[5,6] All of these antidiuretic antagonists exhibit transient antidiuretic agonistic activity and are also potent antagonists of the vasopressor responses to AVP.[2,5,6] The finding that the substitution of an unalkylated D-tyrosine residue in d(CH_2)$_5$VAVP and in d(CH_2)$_5$VDAVP led to peptides possessing antidiuretic antagonism raised the possibility that the incorporation of other D-amino acids at position 2 in d(CH_2)$_5$VAVP and in d-(CH_2)$_5$VDAVP might lead to more potent and/or more selective antidiuretic antagonists lacking any residual antidiuretic agonistic activity. Initially, we substituted D-phenylalanine at position 2 in d(CH_2)$_5$VAVP and in d-(CH_2)$_5$VDAVP. Preliminary data on the resulting peptides were highly encouraging; both peptides were effective antidiuretic antagonists. The L-arginine-containing peptide was, however, much more potent than the D-arginine-containing one—a result highly consistent with all of our earlier data.[1,2,5,6] Thus, we decided to restrict further explorations of position 2 with D-amino acid substitutions to d(CH_2)$_5$VAVP. Therefore, following these findings with

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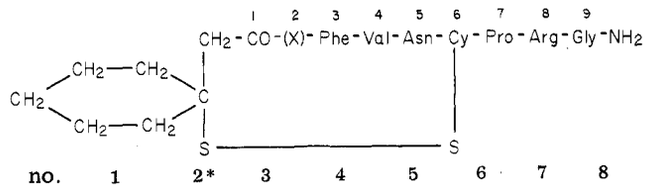
(1) Sawyer, W. H.; Pang, P. K. T.; Seto, J.; McEnroe, M.; Lammek, B.; Manning, M. *Science* **1981**, *212*, 49.
(2) Manning, M.; Lammek, B.; Kolodziejczyk, A. M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1981**, *24*, 701.
(3) Lowbridge, J.; Manning, M.; Haldar, J.; Sawyer, W. H. *J. Med. Chem.* **1978**, *21*, 313.
(4) Manning, M.; Lammek, B.; Kruszynski, M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.*, preceding paper in this issue.
(5) Manning, M.; Olma, A.; Klis, W. A.; Kolodziejczyk, A. M.; Seto, J.; Sawyer, W. H. Proceedings of the American Peptide Symposium, 7th, Rich, D.; Gross, E., Eds.; Pierce Chemical Co.: Rockford, IL, 1981, p 257.
(6) Manning, M.; Olma, A.; Klis, W. A.; Kolodziejczyk, A. M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1982**, *25*, 45.

0022-2623/82/1825-0414$01.25/0

the D-Phe^2-substituted analogues, we selected additional D-amino acids which would provide some insight into the structural features of the side chains at position 2 required for antagonism. We wondered, for example, if aromatic character was a requirement. If not, how large did the side-chain have to be and could it contain an ionized grouping? The additional amino acids chosen to answer these questions were D-isoleucine, D-leucine, D-valine, D-alanine, and D-arginine. To test the possibility that a side chain was not a requirement for antidiuretic antagonism, we decided to incorporate glycine at position 2. The eight new analogues designed following the above reasoning are as follows: **1**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-PheVAVP]; **2**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$-D-PheVDAVP]; **3**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-isoleucine,4-valine]arginine-vasopressin [d-$(CH_2)_5$-D-IleVAVP]; **4**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-leucine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-LeuVAVP]; **5**, [1-(β-mercapto-β,-β-cyclopentamethylenepropionic acid),2-D-valine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-ValVAVP]; **6**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-alanine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-AlaVAVP]; **7**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-glycine,4-valine]arginine-vasopressin [d-$(CH_2)_5$GlyVAVP]; **8**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-arginine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-ArgVAVP]. These eight analogues have the following general structure:



no. 1 2* 3 4 5 6 7 8

X = D-Phe, D-Phe, D-Ile, D-Leu, D-Val, D-Ala, Gly, D-Arg

* D-Arg at position 8

We now present the synthesis and some pharmacological properties of these eight analogues.

Peptide Synthesis. The protected peptide precursors required for the synthesis of all eight peptide antagonists were prepared by the solid-phase method of peptide synthesis.[7-10] *p*-Nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate[11] was used in each final coupling step. All active ester couplings[12,13] were facilitated by the addition of *N*-hydroxybenzotriazole (HOBT).[14] All of the protected peptide amides were obtained by ammonolytic cleavage[9] from the respective acyl octapeptide resins. Each protected precursor was deblocked with Na in NH_3[15] using a previously described yield-enhancing modification of the standard workup procedure.[2] The yields of free peptides were 2–3 times greater than those of the *O*-alkyltyrosine analogues.[2,6] The deblocked disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$.[16] The analogues were desalted and purified by gel filtration on Sephadex G-15 as previously described.[17]

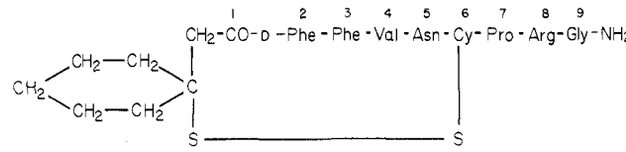
Bioassay Methods. The agonistic and antagonistic potencies of these analogues were measured using previously described methods.[1,2,18-20] These included intravenous vasopressor assays in phenoxybenzamine-treated rats under urethane anesthesia, antidiuretic assays in rats under ethanol anesthesia, and oxytocic assays on isolated rat uteri and rat uteri in situ. The USP posterior pituitary reference standard was used in assays for agonistic and antagonistic activities, except for the rat uterus in situ. Synthetic oxytocin (Syntocinon, Sandoz) was used as the agonist in these assays. Agonistic activities are expressed in units per milligram. Antagonistic potencies were determined and expressed as "effective doses" and as pA_2 values.[21] The effective dose is defined as the dose (in nanomoles per kilogram) that reduces the response seen from 2*x* units of agonist to the response with 1*x* units of agonist. Estimated in vivo "pA_2" values represent the negative logarithms of the effective doses divided by the estimated volume of distribution (67 mL/kg). Inhibition of antidiuretic responses was tested by injecting the standard 20 min after injecting the antagonist, to allow for recovery from the initial antidiuretic responses to some of the antagonists. For those analogues showing no agonistic activity (compounds **1**, **3**, **4**, and **5**) the standard could be injected 10 min after the antagonist. Each antagonist was administered in two doses, a high dose which reduced the response to 2*x* units of agonist to less than the response to 1*x* units of agonist, and a low dose which did not fully reduce the response to that given by 1*x* units of agonist. The effective dose in each case was obtained by interpolation on a logarithmic scale between the two doses of antagonist.[1]

Results and Discussion

The antiantidiuretic, antivasopressor, and antioxytocic potencies of analogues **1–8** are presented as effective doses and as pA_2 values in Tables I and III. The effects of some of the D-amino acid substitutions at position 2 on antiantidiuretic/antivasopressor and antiantidiuretic/antioxytocic selectivities are presented in Table II and IV. Four of these analogues exhibit no detectable antidiuretic agonistic activity. These are d$(CH_2)_5$-D-PheVAVP, d-$(CH_2)_5$-D-IleVAVP, d$(CH_2)_5$-D-LeuVAVP, and d$(CH_2)_5$-D-ValVAVP. These four analogues and d$(CH_2)_5$-D-Phe VDAVP reversibly antagonize subsequent doses of AVP for 1–3 h depending on the dose of antagonist. Two of these peptides, d$(CH_2)_5$-D-PheVAVP and d$(CH_2)_5$-D-IleVAVP are more potent than any previously reported from these laboratories.[1,2,5,6] All, except the Gly^2 peptide, are antagonists of the vasopressor responses to AVP. However,

(7) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.
(8) Merrifield, R. B. *Biochemistry* **1964**, *3*, 1385.
(9) Manning, M. *J. Am. Chem. Soc.* **1968**, *90*, 1348.
(10) Manning, M.; Coy, E.; Sawyer, W. H. *Biochemistry* **1970**, *9*, 3925.
(11) Nestor, Jr., J. J.; Ferger, M. F.; du Vigneaud, V. *J. Med. Chem.* **1975**, *18*, 284.
(12) Bodanszky, M.; du Vigneaud, V. *J. Am. Chem. Soc.* **1959**, *81*, 5688.
(13) Bodanszky, M.; Kondo, M.; Lin, C. Y.; Sigler, G. F. *J. Org. Chem.* **1974**, *39*, 44.
(14) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.
(15) du Vigneaud, V.; Ressler, C.; Swan, J. M.; Katsoyannis, P. G.; Roberts, C. W. *J. Am. Chem. Soc.* **1954**, *76*, 3115.
(16) Hope, D. B.; Murti, V. V. S.; du Vigneaud, V. *J. Biol. Chem.* **1962**, *237*, 1563.
(17) Manning, M.; Wuu, T. C.; Baxter, J. W. M. *J. Chromatogr.* **1968**, *38*, 396.
(18) Manning, M.; Lowbridge, L.; Stier, Jr., C. T.; Haldar, J.; Sawyer, W. H. *J. Med. Chem.* **1977**, *20*, 1228.
(19) Kruszynski, M.; Lammek, B.; Manning, M.; Seto, J.; Haldar, J.; Sawyer, W. H. *J. Med. Chem.* **1980**, *23*, 364.
(20) Sawyer, W. H.; Haldar, J.; Gazis, D.; Seto, J.; Bankowski, K.; Lowbridge, J.; Turan, A.; Manning, M. *Endocrinology* **1980**, *106*, 81.
(21) Schild, H. O. *Br. J. Pharmacol. Chemother.* **1947**, *2*, 189.

Table I. Antiantidiuretic and Antivasopressor Potencies of Arginine-vasopressin (AVP) Antagonists Containing Some D-Amino Acids and Glycine at Position 2



$d(CH_2)_5$-D-Phe VAVP

		antiantidiuretic		antivasopressor	
no.	peptide[g]	effective dose,[a] nmol/kg	pA_2[b]	effective dose,[a] nmol/kg	pA_2[b]
	$d(CH_2)_5$VAVP[d]	agonist		0.76 ± 0.10	7.97 ± 0.06 (8)[c]
	$d(CH_2)_5$-D-TyrVAVP[e]	2.2 ± 0.2	7.51 ± 0.08 (4)[c]	0.29 ± 0.09	8.41 ± 0.11 (4)
	$d(CH_2)_5$-D-TyrVDAVP[e]	6.3 ± 0.8	7.03 ± 0.05 (4)	0.60 ± 0.04	8.05 0.03 (4)
1	$d(CH_2)_5$-D-PheVAVP	0.67 ± 0.13[f]	8.07 ± 0.09 (8)	0.58 ± .04	8.06 ± 0.03 (4)
2	$d(CH_2)_5$-D-PheVDAVP	6.9 ± 1.3	7.07 ± 0.10 (9)	0.73	7.98 ± 0.07 (4)
3	$d(CH_2)_5$-D-IleVAVP	0.70 ± 0.0[f]	7.98 ± 0.05 (4)	8.2 ± 1.4	6.94 ± 0.08 (5)
4	$d(CH_2)_5$-D-LeuVAVP	1.2 ± 0.3[f]	7.79 ± 0.12 (4)	26 ± 5	6.45 ± 0.09 (4)
5	$d(CH_2)_5$-D-ValVAVP	2.3 ± 0.3[f]	7.48 ± 0.06 (4)	27 ± 3	6.41 ± 0.05 (4)
6	$d(CH_2)_5$-D-AlaVAVP	agonist		177 ± 31	5.79 ± 0.08 (4)
7	$d(CH_2)_5$GlyVAVP	agonist		agonist	
8	$d(CH_2)_5$-D-ArgVAVP	>90	<5.9	~260	~5.4

[a] The effective dose is defined as the dose (in nanomoles per kilogram) that reduces the response seen with $2x$ units of agonist to equal the response seen with $1x$ units of agonist administered before antagonist. [b] Estimated in vivo pA_2 values represent the negative logarithms of the "effective dose" divided by the estimated volume of distribution (67 mL/kg). [c] Means ± SE; number of assay groups in parentheses. [d] From Manning et al.[4] [e] From Manning et al.[4-6] [f] No evident agonistic activity. [g] The abbreviations and their full names are as follows: $d(CH_2)_5$VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin; $d(CH_2)_5$-D-TyrVAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-tyrosine,4-valine]arginine-vasopressin; $d(CH_2)_5$-D-PheVAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin; $d(CH_2)_5$-D-PheVDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine,8-D-arginine]vasopressin; $d(CH_2)_5$-D-IleVAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-isoleucine,4-valine]arginine-vasopressin; $d(CH_2)_5$-D-LeuVAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-leucine,4-valine]arginine-vasopressin; $d(CH_2)_5$-D-ValVAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-valine,4-valine]arginine-vasopressin; $d(CH_2)_5$-D-AlaVAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-alanine,4-valine]arginine-vasopressin; $d(CH_2)_5$-GlyVAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-glycine,4-valine]arginine-vasopressin; $d(CH_2)_5$-D-ArgVAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-arginine,4-valine]arginine-vasopressin.

those containing aliphatic D-amino acids at position 2 exhibit significantly decreased antivasopressor and antioxytocic potencies relative to the D-Tyr^2- and D-Phe^2-containing peptides.

Effects of D-Phe/D-Tyr Interchange on Antiantidiuretic Potencies. The replacement of the D-Tyr residues at position 2 in $d(CH_2)_5$-D-TyrVAVP and in $d(CH_2)_5$-D-TyrVDAVP by D-Phe resulted in a substantial improvement in the antiantidiuretic potency of the L-Arg^8-containing peptides and virtually no change in potency of the D-Arg^8-containing peptide (Table I). Thus, $d(CH_2)_5$-D-PheVAVP, with an antiantidiuretic pA_2 of 8.07, is almost 3 times more potent than $d(CH_2)_5$-D-TyrVAVP (pA_2 = 7.51). Whereas, with antiantidiuretic pA_2 values of 7.03 and 7.07 ($d(CH_2)_5$-D-TyrVDAVP and $d(CH_2)_5$-D-PheVDAVP are virtually equipotent. These findings are further testimony to the difficulty of predicting the effects of a given structural change in different peptides. In one peptide, D-Phe effected a substantial increase in potency, whereas in the other, it effected virtually no increase in potency. It may be noted also that L-Arg^8-containing peptide, $d(CH_2)_5$-D-PheVAVP, is 10 times more potent as an antiantidiuretic than its D-Arg^8 isomer, $d(CH_2)_5$-D-PheVDAVP.

Effects of Aliphatic D-Amino Acids at Position 2 on Antidiuretic Antagonism. The replacement of D-Phe in $d(CH_2)_5$-D-PheVAVP by either D-Ile, D-Leu, or D-Val resulted in peptides which retained antiantidiuretic properties. In fact, $d(CH_2)_5$-D-IleVAVP (pA_2 = 7.98) is almost equipotent with $d(CH_2)_5$-D-PheVAVP (pA_2 = 8.07). Thus, aromatic character is not a requirement of the side chain of a D-amino acid at position 2 for endowing these peptides with antiantidiuretic properties. However, the size and polar character of the side chain appear to be critical. Thus, the incorporation of D-Ala or Gly at position 2 in $d(CH_2)_5$-D-PheVAVP resulted in a complete loss of antiantidiuretic potency. Both $d(CH_2)_5$-D-AlaVAVP and $d(CH_2)_5$GlyVAVP are weak antidiuretic agonists (Table I). Also the D-Val^2-containing peptide, i.e., $d(CH_2)_5$-D-ValVAVP, although a potent antiantidiuretic peptide, is only one-half as potent as $d(CH_2)_5$-D-LeuVAVP and only one-third as potent as $d(CH_2)_5$-D-IleVAVP, another indication that size is a critical factor at position 2. A D-Arg/D-Phe interchange in this peptide also resulted in a peptide with no evident antiantidiuretic properties.

Effects of D-Amino Acid Substituents at Position 2 on Antiantidiuretic/Antivasopressor Selectivity. Three of the peptides reported here, $d(CH_2)_5$-D-LeuVAVP, $d(CH_2)_5$-D-IleVAVP, and $d(CH_2)_5$-D-ValVAVP, exhibit enhanced antiantidiuretic potencies relative to their antivasopressor potencies. Their respective antivasopressor/antiantidiuretic effective dose ratios are 22, 12 and 12 (Table II). Thus, these three peptides, as well as d-$(CH_2)_5$-D-PheVAVP, exhibit marked increases in antiantidiuretic/antivasopressor selectivity relative to d-$(CH_2)_5$-D-TyrVAVP. Replacement of the D-Tyr residue in $d(CH_2)_5$-D-TyrVAVP by D-Phe brought about a 50% reduction in antivasopressor potency, i.e., from a pA_2 of 8.41 for $d(CH_2)_5$-D-TyrVAVP to a pA_2 of 8.06 for $d(CH_2)_5$-D-PheVAVP. Because this interchange had resulted in over a threefold enhancement of antiantidiuretic potency, the gain in antiantidiuretic/antivasopressor selectivity is sevenfold (Table II). Replacement of the D-Tyr residue at position 2 in $d(CH_2)_5$-D-TyrVAVP by D-Ile, D-Leu, and

Table II. Enhancement of Antiantidiuretic Potency Relative to Antivasopressor Potency of AVP Antagonists

no.	peptide[d]	antivasopressor: effective dose (ED),[a] nmol/kg	anti-antidiuretic: effective dose (ED),[a] nmol/kg	ED ratio[b]	selectivity[c]
	d(CH_2)$_5$-D-TyrVAVP	0.29	2.2	0.13	1
1	d(CH_2)$_5$-D-PheVAVP	0.58	0.67	0.87	7
3	d(CH_2)$_5$-D-IleVAVP	8.2	0.70	12	92
4	d(CH_2)$_5$-D-LeuVAVP	26	1.2	22	170
5	d(CH_2)$_5$-D-ValVAVP	27	2.3	12	92

[a] See Table I. [b] ED ratio = antivasopressor ED/antiantidiuretic ED. [c] Selectivity ratio is calculated from ED ratios with the value for d(CH_2)$_5$-D-TyrVAVP arbitrarily given a value of 1.0 = ED ratio/0.13. [d] Abbreviations as in Table I.

Table III. Antioxytocic Activities of Some Arginine-vasopressin (AVP) Antagonists Containing D-Amino Acids and Glycine at Position 2

		antioxytocic activities (rat uterus)			
		in vitro		in vivo	
no.	peptide[e]	no Mg^{2+}: pA_2[b]	0.5 mM Mg^{2+}: pA_2[b]	effective dose,[a] nmol/kg	pA_2[b]
	d(CH_2)$_5$VAVP	7.34 ± 0.07[c]	7.31 ± 0.03[c]	26 ± 7[d]	6.47 ± 0.11[d]
	d(CH_2)$_5$-D-TyrVAVP	8.11 ± 0.12[d]	7.78 ± 0.12[d]	6.9 ± 3[d]	7.01 ± 0.08[d]
	d(CH_2)$_5$-D-TyrVDAVP	7.99 ± 0.12[d]	7.78 ± 0.08[d]	8.1 ± 1.2[d]	6.93 ± 0.06[d]
1	d(CH_2)$_5$-D-PheVAVP	7.74 ± 0.06	8.29 ± 0.05	8.9 ± 1.9	6.92 ± 0.10 (5)
2	d(CH_2)$_5$-D-PheVDAVP	7.70 ± 0.05	8.21 ± 0.06		
3	d(CH_2)$_5$-D-IleVAVP	7.98 ± 0.10	7.53 ± 0.07	44 ± 10	6.21 ± 0.09 (4)
4	d(CH_2)$_5$-D-LeuVAVP	7.32 ± 0.11	7.38 ± 0.08	61 ± 9	6.05 ± 0.09 (4)
5	d(CH_2)$_5$-D-ValVAVP	7.90 ± 0.08	6.89 ± 0.04	220 ± 30	5.49 ± 0.04 (4)
6	d(CH_2)$_5$-D-AlaVAVP	6.16 ± 0.05	5.01 ± 0.09		
7	d(CH_2)$_5$GlyVAVP	5.54 ± 0.15	<4.7		
8	d(CH_2)$_5$-D-ArgVAVP	<5.0			

[a,b] See Table I. [c] Manning et al.[4] [d] Unpublished data. [e] Abbreviations as in Table I.

Table IV. Enhancement of Antiantidiuretic Potency Relative to in Vivo Antioxytocic Potency of AVP Antagonists

no.	peptide[d]	antioxytocic: effective dose (ED),[a] nmol/kg	antiantidiuretic: effective dose (ED),[a] nmol/kg	ED ratio[b]	selectivity[c]
	d(CH_2)$_5$-D-TyrVAVP	6.9	2.2	3.1	1
1	d(CH_2)$_5$-D-PheVAVP	8.9	0.67	13	4
3	d(CH_2)$_5$-D-IleVAVP	44	0.70	63	20
4	d(CH_2)$_5$-D-LeuVAVP	61	1.2	51	16
5	d(CH_2)$_5$-D-ValVAVP	220	2.3	96	31

[a] See Table I. [b] ED ratio = antioxytocic ED/antiantidiuretic ED. [c] Selectivity ratio is calculated from ED ratio with value for d(CH_2)$_5$-D-TyrVAVP arbitrarily given a value of 1.0 = 1.0 ED ratio/3.1. [d] Abbreviations as in Table I.

D-Val resulted in even more dramatic reductions of antivasopressor potencies. For example, the antivasopressor pA_2 values of d(CH_2)$_5$-D-IleVAVP, d(CH_2)$_5$-D-LeuVAVP, and d(CH_2)$_5$-D-ValVAVP are, respectively, only 6.94, 6.45, and 6.41. Thus, these peptides have retained only 3.5, 1.1, and 1.1%, respectively, of the antivasopressor potency of d(CH_2)$_5$-D-TyrVAVP. Since they each possess more potent antiantidiuretic activities, they exhibit 92-fold, 170-fold, and 92-fold gains in antiantidiuretic/antivasopressor selectivity relative to that of d(CH_2)$_5$-D-TyrVAVP. These findings are in striking contrast to those obtained for our earlier antagonists—all of which exhibited more potent antivasopressor than antiantidiuretic potencies.[2,5,6] These results are encouraging as an illustration of the possibility of designing even more potent and selective antidiuretic antagonists.

Effects of Aliphatic D-Amino Acid Substituents at Position 2 on Antiantidiuretic/Antioxytocic Selectivity. The substitution of D-Ile, D-Leu, or D-Val for D-Tyr or D-Phe in this series of analogues decreased antioxytocic potency (Table III). Since these analogues remain potent antiantidiuretic agents, there was a marked gain in antiantidiuretic/antioxytocic selectivity (Table IV). This further indicates that it is possible to design selective antidiuretic antagonists.

Conclusion

We have shown that replacement of the D-Tyr residue at position 2 in the potent antidiuretic antagonist d-(CH_2)$_5$-D-TyrVAVP[5,6] by D-Phe and by a variety of aliphatic D-amino acids results in peptides, d(CH_2)$_5$-(X)^{2}VAVP, which exhibit either enhanced antidiuretic antagonism (X = D-Phe, D-Ile, D-Leu) and/or enhanced antiantidiuretic/antivasopressor and antiantidiuretic/antioxytocic selectivities (X = D-Leu, D-Ile, D-Val, D-Phe). There is a lower limit to the size of the aliphatic D-amino acid at position 2 required for antidiuretic antagonism. Thus, d(CH_2)$_5$-D-AlaVAVP and d(CH_2)$_5$GlyVAVP are antidiuretic agonists. A positively charged D-amino acid residue at position 2 seems to lead to loss of antagonistic potency, since d(CH_2)$_5$-D-ArgVAVP has virtually no antagonistic potency. Four of these peptides, i.e., d(CH_2)$_5$-(X)^{2}VAVP, where X = D-Phe, D-Ile, D-Leu, and D-Val, exhibit no antidiuretic agonistic activity. These four

peptides by virtue of their enhanced antiantidiuretic/antivasopressor and antiantidiuretic/antioxytocic selectivities are potentially useful for studies which require discrimination between antidiuretic, vasopressor, and oxytocic receptors. Two of the antiantidiuretic peptides presented here, d$(CH_2)_5$-D-PheVAVP and d$(CH_2)_5$-D-IleVAVP, are more potent than any previously reported. These properties, plus the fact that all but one of the new antidiuretic antagonists were obtained in much higher yields than our original *O*-alkyltyrosine-containing antagonists,[2,6] make these attractive candidates for development as potentially useful drugs for the treatment of hyponatremia due to water retention, as in the syndrome of inappropriate secretion of the antidiuretic hormone (SIADH or the Schwartz–Bartter syndrome). The data presented here also provide promising clues for the design of more potent and selective antidiuretic antagonists.

Experimental Section

The procedure of solid-phase synthesis followed that previously published.[7-10] Chloromethylated resin (Chemalog, 1% cross-linked S-DVB, 200–400 mesh, 0.75–1.00 mequiv/g) was esterified[22] with Boc-Gly to an incorporation of 0.5 mmol/g. Amino acid derivatives were supplied by Bachem Inc. or Chemalog Inc. Boc-D-Ile and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate[11] were synthesized. Dimethylformamide (DMF) was distilled under reduced pressure. Other solvents and reagents were analytical grade. Thin-layer chromatography (TLC) was on silica gel (0.25 mm, Brinkman Silplate). The following solvent systems were used: (A) 1-butanol–acetic acid–water (4:1:1, v/v); (B) chloroform–methanol (7:3, v/v); (C) 1-butanol–acetic acid–water–pyridine (15:3:3:10, 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,[23] peptides (~0.7 mg) were hydrolyzed with constant-boiling hydrochloric acid (400 μL) containing phenol (10 μL) in evacuated and sealed ampules for 18 h at 118 °C. The analyses were performed on Model MM-100 Glencoe and on Model 121 Beckman automatic amino acid analyzers. Molar ratios were referred to Val = 1.00. The cysteine content of the free peptides was estimated as $^1/_2$-Cys. Elemental analyses were performed by Integral Microanalytical Laboratories, Inc., Raleigh, NC. The analytical results for elements indicated by their symbols were within 0.4% of the theoretical values. Optical rotations were measured with a Rudolph polarimeter Model 80.

Boc-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (A). Boc-Gly-resin (20 g, 10 mmol of Gly) was subjected to six cycles of deprotection, neutralization, and coupling[7-10] to yield the protected heptapeptide resin (30 g, 10 mmol).

Boc-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-resin (B). The protected heptapeptide resin B (15.0 g, 5 mmol) was prepared from 10 g (5 mmol) of Boc-Gly-resin using solid-phase methodology.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Phe-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [Pr-d$(CH_2)_5$-D-PheVAVP, I]. The Boc heptapeptidyl resin A (1.2 g, 0.4 mmol) was convertd to protected acyl octapeptidyl resin in two cycles of solid-phase peptide synthesis[7-10] using as the carboxy component Boc-D-Phe and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate,[11-13] respectively, the latter coupling being facilitated by the addition of *N*-hydroxybenzotriazole monohydrate (HOBT).[14] The protected acyl octapeptidyl resin was ammonolyzed,[9] and the amide was extracted with hot DMF and precipitated by addition of water. The crude product was reprecipitated from DMF–ethanol–ethyl ether to give I: yield 450 mg (78.7% based on initial Gly content of the resin); mp 213–214 °C; $[\alpha]^{23}_D$ −24.2° (*c* 1, DMF); TLC R_f (A) 0.61, R_f (B) 0.73, R_f (C) 0.79. Anal. ($C_{71}H_{90}N_{13}O_{12}S_3 \cdot 2H_2O$) C, H, N. Amino acid analysis: Phe, 2.06; Val, 1.00; Asp, 0.85; Pro, 0.90; Cys(Bzl), 0.98; Arg, 0.88; Gly, 1.02; NH_2, 2.13.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Phe-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 [Pr-d$(CH_2)_5$-D-PheVDAVP, II]. The heptapeptidyl resin B (1.2 g, 0.4 mmol) was converted to acyl octapeptidyl resin and then ammonolyzed, and the product was isolated and purified as described for I to give II: yield 500 mg (88.4% based on initial Gly content of the resin); mp 210–212 °C; $[\alpha]^{25}_D$ −12.7° (*c* 1, DMF); TLC R_f (A) 0.78, R_f (B) 0.77, R_f (C) 0.88. Anal. ($C_{71}H_{90}N_{13}O_{12}S_3 \cdot H_2O$) C, H, N. Amino acid analysis: Phe, 2.04; Val, 1.00; Asp, 0.90; Cys(Bzl), 0.97; Pro, 0.93; Arg, 0.92; NH_3, 2.15.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Ile-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [Pr-d$(CH_2)_5$-D-IleVAVP, III]. The Boc heptapeptidyl resin A (1.2 g, 0.4 mmol) was converted to protected acyl octapeptidyl resin using Boc-D-Ile and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate in successive couplings and then ammonolyzed, and the product was extracted and purified as described for I to give III: yield 525 mg (94.1% based on initial Gly content on the resin); mp 229–230 °C; $[\alpha]^{25}_D$ −28.9° (*c* 0.8, DMF); TLC R_f (A) 0.61, R_f (B) 0.77, R_f (C) 0.82. Anal. ($C_{69}H_{95}N_{13}O_{12}S_3 \cdot 2H_2O$) C, H, N. Amino acid analysis: Ile, 0.93; Phe, 0.97; Val, 1.00; Asp, 1.07; Cys(Bzl), 0.91; Pro, 1.07; Arg, 1.18; Gly, 1.09; NH_3, 1.84.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Leu-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [Pr-d$(CH_2)_5$-D-LeuVAVP, IV]. The heptapeptidyl resin A (1.2 g, 0.4 mmol) was converted to acyl octapeptidyl resin using Boc-D-Leu and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate in successive couplings and then ammonolyzed, and the product was extracted and purified to give IV: yield 450 mg (80.8% based on initial Gly content of the resin); mp 216–218 °C; $[\alpha]^{24}_D$ −20.4° (*c* 0.5, DMF); TLC R_f (A) 0.49, R_f (B) 0.76, R_f (C) 0.90. Anal. ($C_{69}H_{95}O_{12}N_{13}S_3 \cdot 2H_2O$) C, H, N. Amino acid analysis: Leu, 1.09; Phe, 0.98; Val, 1.00; Asp, 1.13; Cys(Bzl), 1.05; Pro, 1.08; Arg, 0.97; NH_3, 1.86.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Val-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [Pr-d$(CH_2)_5$-D-ValVAVP, V]. The Boc heptapeptidyl resin A (1.2 g, 0.4 mmol) was converted to protected acyl octapeptidyl resin using Boc-D-Val and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate in successive couplings and then ammonolyzed, and the product was extracted and purified as described for I to give V: yield 480 mg (86.6% based on initial Gly content of the resin); mp 225–227 °C; $[\alpha]^{25}_D$ −16.6° (*c* 0.8; DMF); TLC R_f (A) 0.70, R_f (B) 0.80, R_f (C) 0.85. Anal. ($C_{68}H_{93}N_{13}O_{12}S_3 \cdot 2H_2O$) C, H, N. Amino acid analysis: Val, 2.00; Phe, 0.92; Asp, 1.01; Cys(Bzl), 0.98; Pro, 1.01; Arg, 1.17; Gly, 1.04; NH_3, 1.92.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Ala-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [Pr-d$(CH_2)_5$-D-AlaVAVP, VI]. The Boc heptapeptidyl resin A (1.2 g, 0.4 mmol) was converted to protected acyl octapeptidyl resin using Boc-D-Ala and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate in successive couplings and then ammonolyzed, and the products were extracted and purified as described for I to give VI: yield 447 mg (86.6% based on initial Gly content of the resin); mp 214–215 °C; $[\alpha]^{25}_D$ −27.8° (*c* 0.8, DMF); TLC R_f (A) 0.65; R_f (B) 0.74; R_f (C) 0.77. Anal. ($C_{66}H_{89}N_{13}O_{12}S_3$) C, H, N. Amino acid analysis: Ala, 1.03; Phe, 0.97; Val, 1.00; Asp, 1.05; Cys(Bzl), 0.95; Arg, 0.92; Gly, 0.95; NH_3, 2.11.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-Gly-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [Pr-d$(CH_2)_5$GlyVAVP, VII]. The Boc heptapeptidyl resin A (1.2 g, 0.4 mmol) was converted to protected acyl octapeptidyl resin using Boc-Gly and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate in successive couplings and then ammonolyzed, and the product was extracted and purified as for I to give VII: yield 350 mg (65.0% based on initial Gly content of the resin); mp 184–186 °C; $[\alpha]^{24}_D$ −59.2° (*c* 1, DMF); TLC R_f (A) 0.54, R_f (B) 0.65, R_f (C) 0.83. Anal. ($C_{65}H_{87}O_{12}N_{13}S_3 \cdot 2H_2O$) C, H, N. Amino acid analysis: Phe, 1.08; Val, 1.00; Asp, 1.12; Cys(Bzl), 1.10; Pro, 1.13; Arg, 0.96; Gly, 2.21; NH_3, 1.80.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylene-D-Arg-(Tos)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [Pr-d-

(22) Gisin, B. F. *Helv. Chim. Acta* **1973**, *56*, 1476.
(23) Spackman, D. H.; Stein, W. H.; Moore, S. *Anal. Chem.* **1958**, *30*, 1190.

$(CH_2)_5$-D-ArgVAVP, VIII]. The heptapeptidyl resin A (1.2 g, 0.4 mmol) was converted to the acyl octapeptidyl resin using Boc-D-Arg(Tos) and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate in successive couplings and then ammonolyzed, and the product was extracted and purified as described for I to give VIII: yield 370 mg (57.5% based on initial Gly content of the resin); mp 161–163 °C; $[\alpha]^{24}_D$ −13.8° (*c* 0.5, DMF); TLC R_f (A) 0.59, R_f (B) 0.70, R_f (C) 0.79. Anal. ($C_{76}H_{102}O_{14}N_{16}S_4 \cdot 2H_2O$) C, H, N. Amino acid analysis: Arg, 2.04; Phe, 0.97; Val, 1.00; Asp, 1.02; Cys(Bzl) 0.93; Pro, 0.94; Gly, 1.07; NH_3, 2.11.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin [d-$(CH_2)_5$-D-PheVAVP, IX]. A solution of the protected acyl octapeptidyl amide I (120 mg, 0.085 mmol) in sodium-dried and redistilled ammonia (500 mL) was treated at the boiling point and with stirring with sodium[15] from a stick of the metal contained in a small-bore glass tube until a light-blue color persisted in the solution for 30 s.[2,4,10] Dry glacial acetic acid (0.4 mL) was added to discharge the color. The solution was evaporated, and N_2 was passed through the flask. After 5 min, the residue was dissolved in degassed aqueous acetic acid (20%, 50 mL) and quickly poured into ice-cold water (~1500 mL).[2] The pH was adjusted to ~7 with concentrated ammonium hydroxide. During the neutralization, an excess of a solution of potassium ferricyanide[16] (0.01 M, 15 mL) was added gradually with stirring. The yellow solution was stirred for an additional 20 min and then for 10 min with the anion-exchange resin (Bio-Rad AG-3, Cl^- form, 40 g damp weight). The suspension was slowly filtered through a bed of resin (40 g damp weight). The bed was washed with water (100 mL), and the combined filtrate and washings were lyophilized. The resulting powder (2.1 g) was desalted on a Sephadex G-15 column (110 × 2.7 cm) eluting with aqueous acetic acid (50%)[17] with a flow rate of 5 mL/h. The eluate was fractioned and monitored for absorbance at 254 nm. The fractions comprising the major peak were checked by TLC (A), pooled, and lyophilized, and the residue was further subjected to gel filtration on a Sephadex G-15 column (100 × 1.5 cm) eluting with aqueous acetic acid (0.2 M)[17] with a flow rate of 4 mL/h. The peptide was eluted in a single peak (absorbance 254 nm). Lyophilization of the pertinent fractions gave the vasopressin analogue IX as a white powder: yield 51.1 mg (54.9%); $[\alpha]^{23}_D$ −102.3° (*c* 0.30, 1 M AcOH); TLC R_f (A) 0.17, R_f (C) 0.52. Amino acid analysis: Phe, 2.17; Val, 1.00; Asp, 1.04; Cys, 0.97; Pro, 1.04; Arg, 1.07; Gly, 1.14; NH_3, 1.96.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$-D-PheVDAVP, X]. The protected acyl octapeptide amide II (120 mg, 0.085 mmol) was reduced by sodium in liquid NH_3, reoxidized, deionized, and purified as for IX to give X: yield 16.4 mg (17.7%); $[\alpha]^{22}_D$ −53.6° (*c* 0.3, 1 M AcOH); R_f (A) 0.27, R_f (C) 0.72. Amino acid analysis: Phe, 2.10; Val, 1.00; Asp, 1.02; Cys, 0.98; Pro, 1.02; Arg, 1.10; Gly, 1.10; NH_3, 2.10.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-D-leucine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-LeuVAVP, XI]. The analogue XI was prepared from the intermediate III (120 mg, 0.086 mmol) in the manner detailed above for IX: yield 55.0 mg (63.9%); $[\alpha]^{25}_D$ −87.1° (*c* 0.3, 1 M AcOH); TLC R_f (A) 0.17, R_f (C) 0.53. Amino acid analysis: Leu, 1.01; Phe, 0.95; Val, 1.00; Asp, 1.01; Cys, 0.97; Pro, 1.01; Arg, 1.12; Gly, 1.04; NH_3, 2.20.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-D-isoleucine,4-valine]arginine-vasopressin [d-$(CH_2)_5$-D-Ile VAVP, XII]. The analogue XII was prepared from intermediate IV (120 mg, 0.086 mmol) in the manner detailed above for IX: yield 37.1 mg (41.2%); $[\alpha]^{25}_D$ −117.2° (*c* 0.2, 1 M AcOH); TLC R_f (A) 0.17, R_f (C) 0.51. Amino acid analysis: Ile, 0.95; Phe, 0.99; Val, 1.00; Asp, 1.01; Cys, 0.99; Pro, 1.01; Arg, 0.97; Gly, 1.01; NH_3, 2.10.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-D-valine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-ValVAVP, XIII]. The protected acyl octapeptide amide V (120 mg, 0.087 mmol) was reduced by sodium in liquid NH_3, reoxidized, deionized, and purified as for IX to give XIII: yield 47 mg (52.2%); $[\alpha]^{25}_D$ −121.8° (*c* 0.3, 1 M AcOH); TLC R_f (A) 0.17; R_f (B) 0.49. Amino acid analysis: Val, 2.00; Phe, 1.05; Asp, 1.03; Cys, 0.90; Pro, 1.00; Arg, 1.06; Gly, 1.05; NH_3, 1.74.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-D-alanine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-AlaVAVP, XIV]. The analogue XIV was prepared from intermediate VI (120 mg, 0.093 mmol) in the manner detailed above for IX: yield 57 mg (60.3%); $[\alpha]^{25}_D$ −135.3° (*c* 0.4, 1 M AcOH); TLC R_f (A) 0.16, R_f (C) 0.49. Amino acid analysis: Ala, 1.04; Phe, 1.00; Val, 1.00; Asp, 1.03; Cys, 0.96; Pro, 1.04; Arg, 0.99; Gly, 1.07; NH_3, 2.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-glycine,4-valine]arginine-vasopressin [d-$(CH_2)_5$GlyVAVP, XV]. The protected acyl octapeptidyl amide VII (120 mg, 0.089 mmol) was reduced by sodium in liquid NH_3, reoxidized, deionized, and purified as for IX to give XV: yield 56.6 mg (63.5%); $[\alpha]^{24}_D$ −137.6° (*c* 0.25, 1 M AcOH); TLC R_f (A) 0.15, R_f (C) 0.48. Amino acid analysis: Gly, 2.04; Phe, 0.96; Val, 1.00; Asp, 1.12; Cys, 0.92; Pro, 1.03; Arg, 0.97; NH_3, 2.10.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-D-arginine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-ArgVAVP, XVI]. The protected acyl octapeptidyl amide VIII (120 mg, 0.075 mmol) was reduced by sodium in liquid NH_3, reoxidized, desalted, and purified as for IX to give XVI: yield 52.7 mg (63.7%); $[\alpha]^{23}_D$ −97.8° (*c* 0.3, 1 M AcOH); TLC R_f (A) 0.08, R_f (C) 0.30. Amino acid analysis: Arg, 2.03; Phe, 0.94; Val, 1.00; Asp, 1.09; Cys, 0.97; Pro, 1.02; Gly, 0.93; NH_3, 1.98.

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