Potent Antagonists of the Antidiuretic Responses to Arginine-vasopressin Based on Modifications of [1-(β-Mercapto-β,β-cyclopentamethylenepropionic Acid),2-D-phenylalanine,4-valine]arginine-vasopressin at Position 4†

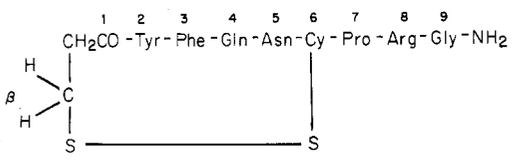
Maurice Manning,*‡ Aleksandra Olma,‡,⊥ Wieslaw A. Klis,‡,∥ Janny Seto,§ and Wilbur H. Sawyer§

Department of Biochemistry, Medical College of Ohio at Toledo, Toledo, Ohio 43699, and Department of Pharmacology, College of Physicans and Surgeons of Columbia University, New York, New York 10032. *Received January 7, 1983*

As part of a program in which we are attempting (a) to delineate the structural features at positions 1–9 in our previously reported antidiuretic antagonists required for antidiuretic antagonism and (b) to obtain analogues with enhanced antiantidiuretic potency and/or selectivity, we have synthesized 14 new analogues of the antidiuretic antagonist [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin [d-$(CH_2)_5$-D-Phe^{2}VAVP), in which the valine residue at position 4 was replaced by the following L-amino acids and glycine: Ile, Abu, Thr, Ala, Gln, Lys, Cha, Nle, Nva, Phe, Leu, Gly, Tyr, and Pro. These analogues are **1**, d-$(CH_2)_5$-D-Phe^2,Ile^{4}AVP; **2**, d$(CH_2)_5$-D-Phe^2,Abu^{4}AVP; **3**, d$(CH_2)_5$-D-Phe^2,Thr^{4}AVP; **4**, d$(CH_2)_5$-D-Phe^2,Ala^{4}AVP; **5**, d$(CH_2)_5$-D-Phe^{2}AVP; **6**, d$(CH_2)_5$-D-Phe^2,Lys^{4}AVP; **7**, d$(CH_2)_5$-D-Phe^2,Cha^{4}AVP; **8**, d$(CH_2)_5$-D-Phe^2,Nle^{4}AVP; **9**, d$(CH_2)_5$-D-Phe^2,Nva^{4}AVP; **10**, d$(CH_2)_5$-D-Phe^2,Phe^{4}AVP; **11**, d$(CH_2)_5$-D-Phe^2,Leu^{4}AVP; **12**, d$(CH_2)_5$-D-Phe^2,Gly^{4}AVP; **13**, d$(CH_2)_5$-D-Phe^2,Tyr^{4}AVP; **14**, d$(CH_2)_5$-D-Phe^2,Pro^{4}AVP. The protected intermediates required for the synthesis of all of these peptides were prepared by the solid-phase method and cleaved from the resin by ammonolysis. Following deblocking with Na in NH_3 and oxidizing with $K_3[Fe(CN)_6]$, each peptide was purified on Sephadex G-15 in a two-step procedure using 50% HOAc and 0.2 M HOAc as eluants. Analogues 1–14 were tested for agonistic and antagonistic activities by antidiuretic, vasopressor, and oxytocic assays in rats. Analogues **1**, **2**, and **4–6** exhibit no detectable antidiuretic agonistic activity. All analogues, with the exception of the Pro^4-containing analogue, are antidiuretic antagonists. Their antiantidiuretic pA_2 values are as follows: **1**, 8.24 ± 0.08; **2**, 7.96 ± 0.07; **3**, 7.62 ± 0.09; **4**, 7.52 ± 0.03; **5**, 7.21 ± 0.07; **6**, 7.22 ± 0.12; **7**, 7.19 ± 0.08; **8**, 7.12 ± 0.09; **9**, 6.99 ± 0.06; **10**, 6.07 ± 0.11; **11**, 6.07 ± 0.11; **12**, 5.85 ± 0.05; **13**, ~5.57; **14**, a weak agonist (0.004 U/mg). Analogues 1–14 also antagonize the vascular responses to arginine-vasopressin (AVP) and the in vitro oxytocic responses to oxytocin. Analogues **1**, **2**, **3**, and **5** have also been shown to antagonize the in vivo oxytocic responses to oxytocin. Five of these analogues (**1**, **2**, **3**, **6**, and **7**) exhibit enhanced antiantidiuretic/antivasopressor selectivity. d$(CH_2)_5$-D-Phe^2,Lys^{4}AVP and other position-4 analogues with side-chain functional groups may be useful covalent ligands with which to probe the structural characteristics of AVP renal and vascular receptors. With an antiantidiuretic "effective dose" of 0.46 ± 0.07 nmol/kg and a pA_2 value of 8.24 ± 0.08, d$(CH_2)_5$-D-Phe^2,Ile^{4}AVP (**1**) appears to be the most potent antidiuretic antagonist reported to date. This and some of the other analogues reported here should prove to be useful pharmacological tools for studies on the role(s) of AVP in the etiology of water retention states and may also be of value as therapeutic agents for the treatment of such conditions in humans. These findings also provide valuable insights for the design of more potent and selective antidiuretic antagonists.

Following our recent discovery of the first effective antagonists of in vivo antidiuretic responses to arginine-vasopressin (AVP),[1,2] we embarked on a program aimed at enhancing their antiantidiuretic potency and antiantidiuretic/antivasopressor selectivity and eliminating their weak transient antidiuretic agonistic activity. We selected the most potent of the original antagonists, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-ethyltyrosine,4-valine]arginine-vasopression [d-$(CH_2)_5$Tyr(Et)VAVP],[1,2] as the lead compound and focused initially on modifications at position 2.[3-5] These studies led in time to two new lead compounds, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-Phe^{2}VAVP] and its D-Ile^2 analogues, d$(CH_2)_5$-D-Ile^{2}VAVP.[5] d$(CH_2)_5$-D-Phe^2-VAVP and d$(CH_2)_2$-D-Ile^{2}VAVP are the most potent antidiuretic antagonists previously reported. They are almost 3 times more potent than d$(CH_2)_5$Tyr-(Et)VAVP. Furthermore, they are devoid of evident antidiuretic agonism and also exhibit enhanced antiantidiuretic/antivasopressor selectivity relative to that of d-

† Symbols and abbreviations are in accordance with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 977). All amino acids are in the L configuration unless otherwise noted. Other abbreviations used are: Abu, α-aminobutyric acid; Cha, cyclohexylalanine; Nle,norleucine; Nva, norvaline; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; BOC, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; HOAc, acetic acid; HOBT, *N*-hydroxybenzotriazole, NPE, nitrophenyl ester; AVP, arginine-vasopressin; VAVP, [4-valine]arginine-vasopressin; d, 1-deamino; $(CH_2)_5$, cyclopentamethylene; d$(CH_2)_5$, 1-deamino-cyclopentamethylene. The d$(CH_2)_5$ abbreviation, rather than the more cumbersome 1-β-Mpa(β-$CH_2)_5$[12] abbreviation, is used as the designation for the 1-(β-mercapto-β,β-cyclopentamethylene-propionic acid) residue at position 1 in analogues of [1-deamino]arginine-vasopressin (dAVP) and [1-deamino]oxytocin. dAVP has the following structure:



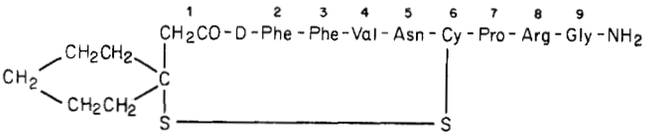
Thus, the d$(CH_2)_5$ abbreviation as employed in this and in many previous publications from these[1-6,19,20,22] and numerous other laboratories (see, for example, ref 7–9 cited in Manning, M.; Lammek, B.; Kruszynski, M.; Seto, J.; Sawyer, W. H., *J. Med. Chem.* **1982**, *25*, 408) specifies that the two hydrogens on the β-carbon of the β-mercaptopropionic acid (β-Mpa) residue at position 1 in [1-deamino]arginine-vasopressin and analogues are replaced by a cyclopentamethylene, $(CH_2)_5$, substituent.

‡ Medical College of Ohio at Toledo.
§ College of Physicians and Surgeons of Columbia University.
⊥ Visiting investigator from Technical of Lodz, Poland.
∥ Visiting investigator from Univesity of Wroclaw, Poland.

(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) 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., Ed.; Pierce Chemical Co.: Rockford, IL, 1981; p 257.
(4) Manning, M.; Olma, A.; Klis, W. A.; Kolodziejczyk, A. M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1982**, *25*, 45.
(5) Manning, M.; Klis, W. A.; Olma, A.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1982**, *25*, 414.

0022-2623/83/1826-1607$01.50/0

$(CH_2)_5$Tyr(Et)VAVP. Their ease of synthesis relative to d$(CH_2)_5$Tyr(Et)VAVP[5] was another decisive reason in favor of using d$(CH_2)_5$-D-Phe^{2}VAVP and d$(CH_2)_5$-D-Ile$_2$VAVP to explore modifications at other positions. d$(CH_2)_5$-D-Phe^{2}VAVP has the following structure:

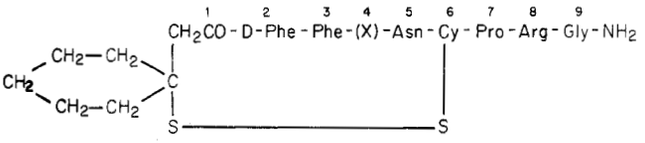


In this report we describe a series of analogues of d-$(CH_2)_5$-D-Phe^{2}VAVP in which the valine residue at position 4 has been replaced by a number of other amino acids. We will present our findings on a related series of position-4-modified analogues of d$(CH_2)_5$-D-Ile^{2}VAVP separately.

Our reasons for selecting position 4 for study are as follows: We had clear evidence for a critical requirement for valine at position 4 in our original antidiuretic antagonists, i.e., those in which position 2 is occupied by an L-Tyr(alk) residue (where alk = Me, Et, *i*-Pr and *n*-Pr).[1,2] Thus, for example, although d$(CH_2)_5$Tyr(Me)AVP[6] and d$(CH_2)_5$Tyr(Et)AVP,[7] both of which have glutamine at position 4, are potent vasopressor antagonists, they are also weak antidiuretic agonists. Valine was thus incorporated as a matter of course in all subsequently designed antidiuretic antagonists aimed at determining how chiral, steric, and electronic changes at the remaining positions would effect antiantidiuretic potency. In the light of our preliminary findings from these studies, we decided to reassess the requirement for valine and were particularly intrigued by the possibility that valine might not be an essential requirement in those antagonists that have a D-amino acid at position 2. In a preliminary investigation in which valine in d$(CH_2)_5$-D-Phe^{2}VAVP was replaced by isoleucine, we were gratified to find that the resulting compound, d$(CH_2)_5$-D-Phe^2,Ile^{4}AVP, was a highly potent antidiuretic antagonist. Encouraged by this finding and by the possibility of obtaining even more potent antidiuretic antagonists, we decided to further examine the structural features of the amino acid side chain at position 4 in d$(CH_2)_5$-D-Phe^{2}X^{4}AVP required for antiantidiuretic potency and selectivity. In addition to isoleucine, we selected the following L-amino acids as replacements for the valine residue at position 4 in d$(CH_2)_5$-D-Phe^{2}VAVP: Abu, Thr, Ala, Gln, Lys, Cha, Nle, Nva, Phe, Leu, Gly, Tyr, and Pro.

The 14 new analogues designed following the above reasoning are as follows: **1**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-isoleucine]arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Ile^{4}AVP]; **2**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-(α-aminobutyric acid)]arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Abu^{4}AVP]; **3**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-threonine]arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Thr^{4}AVP]; **4**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine, 4-alanine]-arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Ala^{4}AVP]; **5**, [1-(β-mercapto-β,β- cyclopentamethylenepropionic acid),2-D-phenylalanine]arginine-vasopressin [d$(CH_2)_5$-D-Phe^{2}AVP]; **6**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-lysine]arginine-vasopressin [d-$(CH_2)_5$-D-Phe^2,Lys^{4}AVP]; **7**, [1-(β-mercapto,β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-cyclohexylalanine]arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Cha^{4}AVP]; **8**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-norleucine]arginine-vasopressin [d$(CH_2)_5$-D-Nle^{4}AVP]; **9**, [1-(β-mercapto,β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-norvaline]arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Nva^{4}AVP]; **10**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-phenylalanine]arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Phe^{4}AVP]; **11**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-leucine]arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Leu^{4}AVP]; **12**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-glycine]arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Gly^{4}AVP]; **13**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-tyrosine]-arginine-vasopressin [d$(CH_2)_5$-D-Phe$_2$,Tyr^{4}AVP]; **14**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-proline]arginine-vasopressin [d$(CH_2)_5$-D-Phe^2,Pro^{4}AVP].

These 14 analogues have the following general structure:



X = Ile (**1**), Abu (**2**), Thr (**3**), Ala (**4**), Gln (**5**), Lys (**6**), Cha (**7**), Nle (**8**), Nva (**9**), Phe (**10**), Leu (**11**), Gly (**12**), Tyr (**13**), Pro (**14**).

We now present the synthesis and some pharmacological properties of these 14 analogues.

Peptide Synthesis. The protected peptide precursors required for the synthesis of the 14 analogues of d-$(CH_2)_5$-D-Phe^{2}VAVP were prepared by the solid-phase method of peptide synthesis[8,9] using previously described modifications.[6,10,11] *p*-Nitrophenyl β-(benzylmercapto)-β,β-cyclopentamethylenepropionate[12] was used in each final coupling step. All active ester couplings[13,14] were facilitated by the addition of *N*-hydroxybenzotriazole (HOBT).[15] The 14 protected peptide amides were obtained by ammonolytic cleavage from the respective acyl octapeptide resins.[10,11] Each protected precursor was deblocked with Na in NH_3[16] by a previously described yield-enhancing modification of the standard workup procedure.[2] The deblocked disulfhydryl compounds were oxidatively cyclized with dilute potassium ferricyanide.[17] The analogues were desalted and purified by gel filtration on Sephadex G-15 as previously described.[2,5,6,18]

Bioassay Methods. The agonistic and antagonistic potencies of these analogues were measured by previously described methods.[1,2,6,19,20] These included intravenous

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

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 $2x$ units of agonist to the response with $1x$ unit 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**, **2**, and **4–6**), the standard could be injected 10 min after the antagonist. Each peptide was administered in two doses, a high dose, which reduced the response to $2x$ units of agonist to less than the response to $1x$ unit of agonist, and a low dose, which did not fully reduce the response to that given by $1x$ unit of agonist. The effective dose in each case was obtained by interpolation on a logarithmic scale between the two doses of antagonist.[21]

Results and Discussion

The antiantidiuretic, antivasopressor, and antioxytocic (in vitro for all analogues, in vivo only for some) potencies of the 14 d$(CH_2)_5$-D-Phe^{2}X^{4}AVP analogues, where X = Ile, Abu, Thr, Ala, Gln, Lys, Cha, Nle, Nva, Phe, Leu, Gly, Tyr, and Pro, together with their antivasopressor/antiantidiuretic and antioxytocic/antiantidiuretic "effective dose" ratios, are presented in Tables I and II. With the exception of the Pro^4 analogue, all of these peptides antagonize in vivo antidiuretic responses to AVP. However, the range of antiantidiuretic potencies as reflected by their effective doses and by their pA_2 values is very broad; from a high of 0.46 nmol/kg and a pA_2 = 8.24 for d$(CH_2)_5$-D-Phe^2,Ile^{4}AVP to a low of ~178 nmol/kg and a pA_2 = ~5.57 for d$(CH_2)_5$-D-Phe^2,Tyr^{4}AVP. d$(CH_2)_5$-D-Phe^2,Ile^{4}AVP appears to be more potent as an antiantidiuretic than its parent analogue, d$(CH_2)_5$-D-Phe^{2}VAVP (pA_2 = 8.06) and, thus, the most potent antidiuretic antagonist reported to date.

Val^4 Requirement for Antidiuretic Antagonism Is No Longer Valid. On the basis of earlier findings[1,2,6,7] we had assumed that the presence of a valine residue at position 4 is critical for antidiuretic antagonism. Thus, although d$(CH_2)_5$Tyr(alk)AVP (where alk = Me, Et), which have Gln at position 4, are potent vasopressor antagonists, they are also weak antidiuretic agonists.[6,7] Replacement of Gln by Val at position 4 converted these into potent antidiuretic antagonists.[1,2] The surprising findings reported here illustrate very vividly the need for caution in drawing conclusions regarding structural requirements on the basis of single changes at a given position, for it is clear that when position 2 in d$(CH_2)_5$Y^{2}X^{4}AVP is occupied by D-Phe, position 4 can tolerate a wide variety of aliphatic amino acids with retention of high antiantidiuretic potency.

Antagonists That Lack Apparent Agonistic Activity. As indicated earlier, d$(CH_2)_5$-D-Phe^{2}VAVP appears to be a pure in vivo antidiuretic antagonist; i.e., it has no evident agonistic activity.[5] Remarkably, of the 13 new antagonists presented here, only 5 have retained this feature. These analogues are d$(CH_2)_5$-D-Phe^2,Ile^4-AVP; d$(CH_2)_5$-D-Phe^2,Abu^4-AVP; d$(CH_2)_5$-D-Phe^2,Ala^{4}AVP; d-$(CH_2)_4$-D-Phe^2-AVP, and d$(CH_2)_5$-D-Phe^2,Lys^4-AVP. We cannot explain why all the remaining analogues have lost this characteristic.

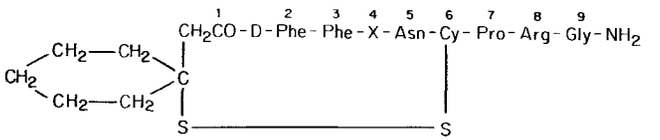
Effects of Aliphatic vs. Aromatic Character at Position 4 on Antiantidiuretic Potencies. Aliphatic amino acids are clearly more effective than aromatic amino acids in endowing these position-4-modified peptides with potent antidiuretic antagonism. Thus, the Phe^4 and Tyr^4 analogues are much weaker antidiuretic antagonists than nearly all of the antagonists that have aliphatic amino acids at position 4 (Table I). This could be due to a combination of steric and electronic, i.e., aromaticity, factors. The possibility that aromatic character may play a more deleterious role than steric bulk may be ascertained by the fact that the Cha^4-containing antagonist is over 10 times more potent than the Phe^4 antagonist, although both amino acid side chains are of approximately equal size. These findings are in dramatic contrast to our previous observations on the structural requirements for aliphatic vs. aromatic D-amino acid substitutions at position 2 in antidiuretic antagonists.[5] Thus, d$(CH_2)_5$-D-Phe^{2}VAVP and its D-Ile^2 analogue have virtually identical antiantidiuretic potencies, indicating virtually no differences between aliphatic and aromatic side chains at position 2 in these analogues.[5]

Effects of β-Branching, Steric Factors, and Ionization at Position 4 on Antiantidiuretic Potencies. The potencies of these new antidiuretic antagonists correlate reasonably well with the presence or absence of branching on the β-carbon of the side chain at position 4. Branching on the γ-carbon is clearly most deleterious. Thus, the Ile^4 analogue is 20 times more potent than the Nle^4 analogue, which in turn is 10 times more potent than the Leu^4 analogue. Similarly, the Val^4 analogue is over 10 times more potent than the Nva^4 analogue. The need for a minimum aliphatic side chain at position 4 is illustrated by the fact that the Ala^4 antagonist is over 600 times more potent than the Gly^4 antagonist. The presence of a positively charged side chain at position 4 has been well tolerated. Thus, with an antiantidiuretic pA_2 = 7.22, the Lys^4 analogue is equipotent with the Gln^4 and Nle^4-containing analogues. d$(CH_2)_5$-D-Phe^2,Lys^{4}AVP and related analogues, e.g., Orn^4 or one containing some other functional or photoreactive group, could be useful tools with which to explore renal or vascular AVP receptors by covalent attachment via the δ- or ϵ-amino group at position 4.

Effects of Position-4 Modifications on Antivasopressor Potencies. The structural features at position 4 in d$(CH_2)_5$-D-Phe^{2}X^{4}AVP required for vasopressor antagonism appear to be even less rigid than those required for antidiuretic antagonism. Analysis of the data in Table I reveals some interesting points. (1) The Phe^4, Tyr^4, Leu^4, and Gly^4 substitutions are not nearly as deleterious to antivasopressor potencies as they are to antiantidiuretic potencies. In fact, with an antivasopressor pA_2 = 7.70, the Leu^4-containing analogue is one of the more potent vasopressor antagonists in the entire series. (2) In this series, aliphatic amino acids are clearly superior to aromatic amino acids at position 4 for preserving antivasopressor potenty. (3) With an antivasopressor pA_2 = 8.35, the

(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 Based on Modifications of $d(CH_2)_5$-D-Phe^{2}VAVP at Position 4



$d(CH_2)_5$-D-Phe^{2}X^{4}AVP

			antiantidiuretic		antivasopressor		
no.	peptide[g]	X^4 side chain	ED,[a,c] nmol/kg	pA_2[b,c]	ED,[a,c] nmol/kg	pA_2[b,c]	ED ratio[d]
	$d(CH_2)_5$-D-Phe^{2}VAVP[e]	$CH(CH_3)_2$	0.67 ± 0.13[f]	8.07 ± 0.09	0.58 ± 0.04	8.06 ± 0.03	0.9
1	$d(CH_2)_5$-D-Phe^2,Ile^{4}AVP	$CH(CH_3)CH_2CH_3$	0.46 ± 0.07[f]	8.24 ± 0.08	0.99 ± 0.12	7.86 ± 0.05	2.2
2	$d(CH_2)_5$-D-Phe^2,Abu^{4}AVP	CH_2CH_3	0.77 ± 0.12[f]	7.96 ± 0.07	1.4 ± 0.3	7.70 ± 0.08	1.8
3	$d(CH_2)_5$-D-Phe^2,Thr^{4}AVP	$CH(OH)CH_3$	1.7 ± 0.4	7.62 ± 0.09	2.9 ± 0.5	7.38 ± 0.07	1.7
4	$d(CH_2)_5$-D-Phe^2,Ala^{4}AVP	CH_3	2.2 ± 0.7[f]	7.52 ± 0.13	1.9 ± 0.2	7.55 ± 0.05	0.9
5	$d(CH_2)_5$-D-Phe^{2}AVP	$CH_2CH_2{\cdot}CONH_2$	4.3 ± 0.6[f]	7.21 ± 0.07	0.30 ± 0.03	8.35 ± 0.04	0.07
6	$d(CH_2)_5$-D-Phe^2,Lys^{4}AVP	$CH_2CH_2CH_2{\cdot}NH_3^+$	4.5 ± 1.9[f]	7.22 ± 0.12	7.9 ± 2.6	6.93 ± 0.10	1.7
7	$d(CH_2)_5$-D-Phe^2,Cha^{4}AVP	CH_2-C_6H_{11}	4.6 ± 0.9	7.19 ± 0.08	9 ± 4	6.70 ± 0.10	2.0
8	$d(CH_2)_5$-D-Phe^2,Nle^{4}AVP	$CH_2CH_2CH_2CH_3$	5.6 ± 1.3	7.12 ± 0.09	2.1 ± 0.3	7.52 ± 0.05	0.4
9	$d(CH_2)_5$-D-Phe^2,Nva^{4}AVP	$CH_2CH_2CH_3$	6.9 ± 0.09	6.99 ± 0.06	1.4 ± 0.4	7.73 ± 0.12	0.2
10	$d(CH_2)_5$-D-Phe^2,Phe^{4}AVP	CH_2-C_6H_5	65 ± 15	6.07 ± 0.11	25 ± 4	6.45 ± 0.07	0.5
11	$d(CH_2)_5$-D-Phe^2,Leu^{4}AVP	$CH_2CH(CH_3)CH_3$	66 ± 19	6.07 ± 0.77	1.3 ± 0.1	7.70 ± 0.03	0.02
12	$d(CH_2)_5$-D-Phe^2,Gly^{4}AVP	H	97 ± 10	5.85 ± 0.05	11 ± 2	6.79 ± 0.07	0.11
13	$d(CH_2)_5$-D-Phe^2,Tyr^{4}AVP	p-CH_2-C_6H_4-OH	~178	~5.57	22 ± 6	6.58 ± 0.11	0.12
14	$d(CH_2)_5$-D-Phe^2,Pro^{4}AVP	c-$(CH_2)_3$	weak agonist (0.004 U/mg)		weak agonist (<0.01 U/mg)		

[a] The effective dose (ED) 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 $1x$ unit 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 (6.7 mL/kg). [c] Means ± SE. [d] ED ratio = antivasopressor ED/antiantidiuretic ED. [e] From Manning et al.[5] [f] No evident agonistic activity. [g] The abbreviations and their full names are as follows: $d(CH_2)_5$-D-Phe^{2}VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Ile^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-isoleucine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Abu^{4}AVP), [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-(α-aminobutyric acid)]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Thr^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-threonine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Ala^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-alanine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^{2}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid],2-D-phenylalanine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Lys^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-lysine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Cha^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-cyclohexylalanine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Nle^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-norleucine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Nva^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-norvaline]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Phe^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-phenylalanine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Leu^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-leucine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Gly^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-glycine]arginine vasopressin; $d(CH_2)_5$-D-Phe^2,Tyr^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-tyrosine]arginine-vasopressin; $d(CH_2)_5$-D-Phe^2,Pro^{4}AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-proline]arginine-vasopressin.

Table II. In Vitro and Some in Vivo Antioxytocic Potencies of AVP Antagonists Modified at Position 4

		antioxytocic activities (rat uterus)				
		in vitro		in vivo		
no.	peptide[f]	no Mg^{2+}: pA_2[b,c]	0.5 mM Mg^{2+}: pA_2[b,c]	ED,[a,c] nmol/kg	pA_2	ED ratio[d]
	d(CH$_2$)$_5$-D-Phe^{2}VAVP[e]	7.74 ± 0.06	8.29 ± 0.05	8.9 ± 1.9	6.92 ± 0.10 (5)	13
1	d(CH$_2$)$_5$-D-Phe^2,Ile^{4}AVP	8.04 ± 0.09	8.19 ± 0.06	5.8 ± 1.7	7.11 ± 0.12	13
2	d(CH$_2$)$_5$-D-Phe^2,Abu^{4}AVP	7.73 ± 0.07	7.76 ± 0.08	2.4 ± 0.6	7.52 ± 0.10	3.1
3	d(CH$_2$)$_5$-D-Phe^2,Thr^{4}AVP	8.04 ± 0.08	8.01 ± 0.11	3.2 ± 0.4	7.35 ± 0.07	1.9
4	d(CH$_2$)$_5$-D-Phe^2,Ala^{4}AVP	7.68 ± 0.12	7.73 ± 0.05			
5	d(CH$_2$)$_5$-D-Phe^{2}AVP	8.59 ± 0.08	8.43 ± 0.07	4.0 ± 0.6	7.25 ± 0.06	0.9
6	d(CH$_2$)$_5$-D-Phe^2,Lys^{4}AVP	7.60 ± 0.06	7.47 ± 0.07			
7	d(CH$_2$)$_5$-D-Phe^2,Cha^{4}AVP	7.78 ± 0.09	7.59 ± 0.07			
8	d(CH$_2$)$_5$-D-Phe^2,Nle^{4}AVP	7.54 ± 0.07	7.78 ± 0.10			
9	d(CH$_2$)$_5$-D-Phe^2,Nva^{4}AVP	8.03 ± 0.08	7.72 ± 0.05			
10	d(CH$_2$)$_5$-D-Phe^2,Phe^{4}AVP	7.57 ± 0.07	7.67 ± 0.06			
11	d(CH$_2$)$_5$-D-Phe^2,Leu^{4}AVP	7.76 ± 0.05	7.88 ± 0.03			
12	d(CH$_2$)$_5$-D-Phe^2,Gly^{4}AVP	7.76 ± 0.08	7.33 ± 0.09			
13	d(CH$_2$)$_5$-D-Phe^2,Tyr^{4}AVP	7.68 ± 0.10	7.43 ± 0.06			
14	d(CH$_2$)$_5$-D-Phe^2,Pro^{4}AVP	5.76 ± 0.07	5.43 ± 0.05			

[a,b,c,e] See Table I. [d] ED ratio = antioxytocic ED/antiantidiuretic ED. [f] Abbreviation as in Table I.

Gln^4-containing analogue is the most potent of the entire series.

Enhancement of Antiantidiuretic/Antivasopressor Selectivity. Only five of these analogues exhibit enhanced antiantidiuretic/antivasopressor selectivity, as reflected by their effective dose ratios. These are the Ile^4, Abu^4, Thr^4, Lys^4, and Cha^4 analogues. With an antiantidiuretic/antivasopressor effective dose ratio of 2.2, d(CH$_2$)$_5$-D-Phe^4,Ile^{4}AVP is the most selective of the series. It may be recalled that three of our earlier analogues, d(CH$_2$)$_5$-D-Leu^{2}VAVP, d(CH$_2$)$_5$-D-Ile^{2}VAVP, and d(CH$_2$)$_5$-D-Val^{2}VAVP, exhibited effective does ratios of 22, 12, and 12, respectively.[5] Thus, the incorporation of Ile at position 4 in each of these three peptides might be expected to result in further enhancements of their antiantidiuretic/antivasopressor selectivities.

Effects of Position-4 Substituents on in Vitro and in Vivo Antioxytocic Potencies. All of these peptides inhibit in vitro responses to oxytocin on the rat uterus. Four of the most potent of the in vitro antagonists were also tested in vivo. These are d(CH$_2$)$_5$-D-Phe^2,Ile^{4}AVP; d(CH$_2$)$_5$-D-Phe^2,Abu^{4}AVP; d(CH$_2$)$_5$-D-Phe^2,Thr^{4}AVP, and d(CH$_2$)$_5$-D-Phe^{2}AVP. All four are potent antioxytocics in vivo. With a pA_2 of 7.52, d(CH$_2$)$_5$-D-Phe^2,Abu^{4}AVP compares very favorably to some of our previously reported potent in vivo oxytocic antagonists[22] and is, in fact, the most potent in vivo antioxytocic compound reported to date.

Conclusion

We have shown that replacement of the Val^4 residue in the potent antidiuretic antagonist d(CH$_2$)$_5$-D-Phe^{2}VAVP[5] by a series of 14 different amino acids leads to retention of potent antidiuretic antagonism in a majority of the resulting analogues. This demonstrates that when D-Phe is substituted in position 2 in our original antagonists d(CH$_2$)$_5$Tyr(alk)VAVP (where alk = Me, Et),[1,2] position 4 can tolerate a wide variety of structural changes without loss of antidiuretic antagonism. This is all the more surprising in view of our earlier finding that Val^4 plays a critical role in endowing d(CH$_2$)$_5$Tyr(Et)VAVP with antiantidiuretic properties.[1,2,6,7] Short-chain and β-branched aliphatic amino acids at position 4 in d(CH$_2$)$_5$-D-Phe^{2}X^{4}AVP are clearly superior to either long-chain or γ-branched aliphatic amino acids in leading to enhancement of antiantidiuretic potencies. The substitution of aromatic amino acids at position 4 leads to drastic losses of antiantidiuretic antagonism. Five of the new antagonists, d(CH$_2$)$_5$-D-Phe^2,Ile^{4}AVP, d(CH$_2$)$_5$-D-Phe^{2}Abu^{4}AVP, d(CH$_2$)$_5$-D-Phe^2,Ala^{4}AVP, d(CH$_2$)$_5$-D-Phe^{2}AVP, and d-(CH$_2$)$_5$-D-Phe^2,Lys^{4}AVP, exhibit no evident antidiuretic agonistic activity. With an antiantidiuretic pA_2 = 8.24, d(CH$_2$)$_5$-D-Phe^2,Ile^{4}AVP appears to be the most potent antidiuretic antagonist reported to date. It also exhibits enhanced antiantidiuretic/antivasopressor selectivity relative to d(CH$_2$)$_5$-D-Phe^{2}VAVP. The findings reported here on the structural flexibility at position 4 in d-(CH$_2$)$_5$-D-Phe^{2}X^{4}AVP analogues, together with our earlier findings that position 2 in d(CH$_2$)$_5$X^{2}VAVP can tolerate a wide variety of D-amino acids with retention of antidiuretic antagonism,[3-5] offer very valuable clues for the design of even more potent and/or selective antidiuretic antagonists. Some of the highly potent antidiuretic antagonists reported here, in particular, d(CH$_2$)$_5$-D-Phe^{2}Ile^{4}AVP and d(CH$_2$)$_5$-D-Phe^2,Abu^{4}AVP, are potentially useful as pharmacological tools for studies on AVP-induced water-retention states and might also be of therapeutic value for the treatment of AVP-induced hyponatremias of diverse etiologies, as, for example, in the Schwartz–Bartter syndrome.[23] The prospect of probing the structural characteristics of AVP renal and vascular receptors using d(CH$_2$)$_5$-D-Phe^2,Lys^{4}AVP, and related position-4-modified analogues possessing other active groups, as covalent ligands is now also a distinct possibility.

Experimental Section

The procedure of "solid-phase" synthesis followed that previously published.[8-11] Chloromethylated resin (Chemalog, 1% cross-linked S-DVB, 200–400 mesh, 0.75–1.00 mequiv/g) was esterified with Boc-Gly by the cesium salt method[24] to an incorporation of 0.5 mmol/g. Amino acid derivatives were supplied by Bachem Inc. or Chemalog Inc. *p*-Nitrophenyl β-(benzylmercapto)-β,β-cyclopentamethylenepropionate[12] was 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, Brinkmann Silpate). The following solvent systems were used: (A) butan-1-ol–acetic acid–water (4:1:1, v/v); (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 (4:1:5, v/v, uppper phase). Loads

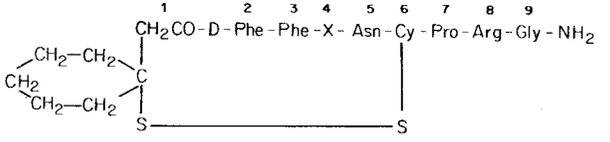
(22) Bankowski, K.; Manning, M.; Seto, J.; Haldar, J.; Sawyer, W. H. *Int. J. Pept. Protein Res.* **1980**, *16*, 382.
(23) Bartter, F. C.; Schwartz, W. H. *Am. J. Med.* **1967**, *42*, 790.
(24) Gisin, B. F. *Helv. Chim. Acta* **1973**, *56*, 1476.

Table III. Physicochemical Properties of the Protected Peptides: β-(Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Phe-Phe-X-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (I–XIV)

no.	X	formula	wt, mg	yield, %	mp, °C	$[\alpha]_D^{25}$, deg	TLC R_f (A)	TLC R_f (B)	TLC R_f (C)	amino acid analyses ratios: Phe	Asp	Cys(Bzl)	Pro	Arg	Gly	NH_3	X
I	Ile	$C_{73}H_{95}N_{13}O_{12}S_3 \cdot 2H_2O$	380	65.9	211–213	−27.1[a]	0.52	0.77	0.80	2.11	1.07	1.08	0.95	0.96	1.00	1.89	0.96
II	Abu	$C_{71}H_{91}N_{13}O_{12}S_3 \cdot 2H_2O$	510	72.0	205–206	−12.6[b]	0.50	0.94	0.81	2.03	1.07	1.02	1.03	0.87	1.00	1.92	0.95
III	Thr(Bzl)	$C_{78}H_{97}N_{13}O_{13}S_3 \cdot 2H_2O$	340	57.5	170–173	−9.7[b]	0.60	0.98	0.85	1.86	1.06	0.98	0.88	1.08	1.00	1.87	1.19
IV	Ala	$C_{70}H_{89}N_{13}O_{12}S_3 \cdot 3H_2O$	350	62.0	186–188	−13.3[b]	0.52	0.68	0.80	2.01	0.96	1.07	1.03	0.98	1.00	2.06	0.98
V	Gln	$C_{72}H_{92}N_{14}O_{13}S_3 \cdot H_2O$	430	59.1	185–187	−17.6[a]	0.34	0.67	0.77	1.98	1.02	1.07	0.97	1.09	1.00	2.88	1.01
VI	Lys(Tos)	$C_{80}H_{102}N_{14}O_{14}S_4 \cdot 2H_2O$	410	63.0	163–165	−19.5[b]	0.54	0.83	0.79	1.96	0.98	1.02	0.89	0.99	1.00	2.05	1.05
VII	Cha	$C_{79}H_{98}N_{13}O_{12}S_3 \cdot 2H_2O$	420	71.0	194–195	−19.2[b]	0.51	0.84	0.91	2.01	1.02	1.13	0.87	1.12	1.00	1.96	1.17
VIII	Nle	$C_{73}H_{95}N_{13}O_{12}S_3 \cdot 2H_2O$	390	67.5	207–208	−19.4[b]	0.49	0.67	0.84	1.98	1.03	1.11	0.98	1.04	1.00	1.96	0.87
IX	Nva	$C_{71}H_{95}N_{13}O_{12}S_3 \cdot 2H_2O$	340	60.2	206–207	−17.6[a]	0.55	0.90	0.87	2.01	1.07	0.98	1.02	0.97	1.00	1.82	0.98
X	Phe	$C_{76}H_{93}N_{13}O_{12}S_3$	370	62.0	200–202	−27.1[a]	0.55	0.70	0.84	2.02	1.07	1.14	0.89	1.06	1.00	1.82	1.01
XI	Leu	$C_{73}H_{95}N_{13}O_{12}S_3$	380	66.7	210–211	−24.4[b]	0.56	0.69	0.79	2.01	0.96	0.98	0.90	1.07	1.00	1.93	1.08
XII	Gly	$C_{69}H_{87}N_{13}O_{12}S_3 \cdot 2H_2O$	433	77.5	134–137	−20.3[b]	0.47	0.50	0.80	1.94	0.97	0.98	0.96	0.95	1.00	1.89	1.00
XIII	Tyr(Bzl)	$C_{83}H_{99}N_{13}O_{13}S_3 \cdot 2H_2O$	410	64.7	199–202	−22.5[b]	0.59	0.92	0.85	1.98	1.10	1.03	0.98	0.93	1.00	2.03	0.96
XIV	Pro	$C_{72}H_{91}N_{13}O_{12}S_3 \cdot 2H_2O$	380	67.5	134 dec	−30.1[b]	0.44	0.89	0.81	1.93	1.03	1.03	0.99	0.97	1.00	1.87	1.00

[a] *c* 0.9, DMF. [b] *c* 1, DMF.

Table IV. Physicochemical Properties of $d(CH_2)_5$-D-Phe^2X^4AVP Antagonists 1–14



$d(CH_2)_5$-D-Phe^2,X^4AVP

no.	peptide[a]	X^4	wt, mg	yield, %	$[\alpha]^{25}_D$, deg	TLC R_f (A)	TLC R_f (C)	TLC R_f (D)	amino acid analyses ratios: Phe	Asp	½Cys	Pro	Arg	Gly	NH_3	X^4
1	$d(CH_2)_5$-D-Phe^2,Ile^4AVP	Ile	35	38.1	−116.4[b]	0.33	0.58	0.39	2.10	1.06	0.92	0.92	0.94	1.00	1.94	0.92
2	$d(CH_2)_5$-D-Phe^2,Abu^4AVP	Abu	40.2	43.1	−106.8[f]	0.28	0.53	0.35	2.08	0.99	1.03	1.10	1.00	1.00	1.86	0.99
3	$d(CH_2)_5$-D-Phe^2,Thr^4AVP	Thr	46.1	53.3	−118.3[c]	0.22	0.48	0.34	1.94	0.94	0.92	0.98	0.99	1.00	1.86	0.92
4	$d(CH_2)_5$-D-Phe^2,Ala^4AVP	Ala	46.5	51.1	−118.2[b]	0.21	0.50	0.35	1.97	0.94	0.93	1.02	0.91	1.00	1.92	1.01
5	$d(CH_2)_5$-D-Phe^2AVP	Gln	62.7	66.4	−136.4[c]	0.13	0.40	0.32	2.01	1.03	1.11	0.98	1.09	1.00	1.90	1.00
6	$d(CH_2)_5$-D-Phe^2,Lys^4AVP	Lys	41	49.4	−102.5[b]	0.02	0.19	0.28	1.95	0.93	0.97	1.10	0.94	1.00	1.83	0.98
7	$d(CH_2)_5$-D-Phe^2,Cha^4AVP	Cha	8.9	9.6	−70.9[d]	0.36	0.62	0.41	1.89	1.01	0.92	0.89	0.97	1.00	2.00	1.18
8	$d(CH_2)_5$-D-Phe^2,Nle^4AVP	Nle	24.2	26.4	−121.0[c]	0.34	0.58	0.39	1.95	0.95	0.90	0.90	0.99	1.00	1.91	0.96
9	$d(CH_2)_5$-D-Phe^2,Nva^4AVP	Nva	44	50.3	−101.8[c]	0.32	0.57	0.38	1.94	0.94	1.03	0.91	0.98	1.00	1.95	0.96
10	$d(CH_2)_5$-D-Phe^2,Phe^4AVP	Phe	21	22.8	−106.6[c]	0.33	0.55	0.38	1.90	0.95	0.99	0.95	0.98	1.00	1.99	0.97
11	$d(CH_2)_5$-D-Phe^2,Leu^4AVP	Leu	20.1	21.9	−114.0[f]	0.34	0.59	0.39	2.13	0.93	0.94	0.87	1.09	1.00	1.95	1.00
12	$d(CH_2)_5$-D-Phe^2,Gly^4AVP	Gly	46.2	51.2	−100.0[g]	0.19	0.47	0.34	1.81	0.87	0.96	0.98	0.96	1.00	1.88	1.00
13	$d(CH_2)_5$-D-Phe^2,Tyr^4AVP	Tyr	11.6	13.2	−58.8[f]	0.31	0.53	0.37	1.82	0.89	1.12	0.92	0.98	1.00	1.84	0.93
14	$d(CH_2)_5$-D-Phe^2,Pro^4AVP	Pro	34.4	38.9	−125.6[c]	0.16	0.46	0.33	1.87	0.92	0.88	0.96	0.95	1.00	2.10	0.92

[a] Abbreviations as in Table I. [b] *c* 0.4, 1 N AcOH. [c] *c* 0.3, 1 N AcOH. [d] *c* 0.11, 1 N AcOH. [e] *c* 0.15, 1 N AcOH. [f] *c* 0.25, 1 N AcOH. [g] *c* 0.5, 1 N AcOH.

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,[25] 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 Models 121M and 119CL Beckman automatic amino acid analyzers. Molar ratios were referred to Gly (= 1.00). The cysteine content of the free peptides was estimated as $^1/_2$-cystine. 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 theoretical values. Optical rotations were measured with a Rudolph polarimeter Model 80.

Boc-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (A). The protected pentapeptide resin A (27.15 g, 10 mM) was prepared from 20 g (10 mM) of Boc-Gly-resin by the solid-phase peptide synthesis methodology,[8-11] i.e., four cycles of deprotection with 1 M HCl/HOAc, neutralization with 10% Et_3N in $MeCl_2$, and coupling with Boc-Arg(Tos), Boc-Pro, Boc-Cys(Bzl) mediated by DCC and Boc-Asn NPE (with HOBT as additive), respectively.

β-(Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Phe-Phe-Ile-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (I). The pentapeptidyl resin (A; 1.1 g, 0.4 mmol) was converted to protected acyl octapeptidyl resin in four cycles of solid-phase peptide synthesis by using as the carboxy component Boc-L-Ile, Boc-L-Phe, Boc-D-Phe, and *p*-nitrophenyl β-(benzylmercapto)-β,β-cyclopentamethylenepropionate,[12] respectively, the latter coupling being facilitated by the additon of *N*-hydroxybenzotriazole (HOBT).[15] The protected acyl octapeptidyl resin was ammonolyzed.[10] The crude product was extracted with hot (60 °C) DMF, precipitated by the addition of water, collected, and dried. It was taken up in hot DMF and reprecipitated with ethanol–ethyl ether to give the required protected peptide amide I (Table III).

β-(Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Phe-Phe-X-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [II–XIV, X = Abu (II), Thr(Bzl) (III), Ala (IV), Gln (V), Lys(Tos) (VI), Cha (VII), Nle (VII), Nva (IX), Phe (X), Leu (XI), Gly (XII), Tyr(Bzl) (XIII), and Pro (XIV)]. Each of the protected peptides II–XIV was synthesized from the pentapeptidyl resin (A; 1.1 g, 0.4 mmol) in the same manner as for I, except that in the first coupling, Boc-L-Ile was replaced by the Boc derivative of X in each case. The physiochemical properties of all of the protected peptides I–XIV are given in Table III.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid)-2-D-phenylalanine,4-isoleucine]arginine-vasopressin [d(CH_2)$_5$-D-Phe^2,Ile^{4}AVP] (1). A solution of the protected acyl octapeptide amide I (120 mg, 0.083 mmol) in sodium-dried and redistilled ammonia (500 mL) was treated at the boiling point and with stirring with sodium[16] 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. Dry acetic acid (0.4 mL) was added to discharge the color. The ammonia was evaporated, and nitrogen 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 ~7.0 with concentrated ammonium hydroxide. Following the neutralization, an excess of a solution of potassium ferricyanide (0.01 M, 15 mL)[17] was added gradually with stirring. The yellow solution was stirred for an additional 20 min and for 10 min with anion-exchange resin (Bio-Rad AG-3, Cl^- forms, 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 power (1.9 g) was desalted on a Sephadex G-15 column (110 × 2.7cm) eluting with aqueous acetic acid (50%)[18] 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)[18] 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 1 as a white powder (Table IV).

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-phenylalanine,4-amino acid X]arginine-vasopressins [d(CH_2)$_5$-D-Phe^2,X^4,AVP (2–14), X = Abu (2), Thr (3), Ala (4), Gln (5), Lys (6), Cha (7), Nle (8), Nva (9), Phe (10), Leu (11), Gly (12), Tyr (13), Pro (14). Each of these cyclized free peptides (2–14) was obtained from their respective protected precursors II–XIV by the procedure followed in obtaining the free peptide 1 from the protected intermediate I. Their physiochemical properties are given in Table IV. The pharmacological properties of peptides 1–14 are presented in Tables I and II.

Acknowledgment. This work was supported in part by research grants from the National Institute of General Medical Sciences (No. GM-25280), the National Institute of Arthritis, Metabolism and Digestive Diseases (No. AM-01940), and the National Heart, Lung and Blood Institute (No. HL-12738). The authors thank Dr. Johannes Meienhofer, Hoffman-LaRoche, Inc., Nutley, NJ, and Dr. Roger Roeske, Indiana University School of Medicine, Indianapolis, IN, for the amino acid analyses and Ms. Beverly Cifelli for expert assistance in the preparation of the manuscript.

Registry No. 1, 86785-86-6; 2, 86802-61-1; 3, 86785-87-7; 4, 86785-88-8; 5, 86785-89-9; 6, 86785-90-2; 7, 86785-91-3; 8, 86785-92-4; 9, 86785-93-5; 10, 86785-94-6; 11, 86785-95-7; 12, 86785-96-8; 13, 86785-97-9; 14, 86785-98-0; I, 86802-69-9; II, 86785-99-1; III, 86786-00-7; IV, 86786-01-8; V, 86786-02-9; VI, 86786-03-0; VII, 86786-04-1; VIII, 86786-05-2; IX, 86802-70-2; X, 86786-06-3; XI, 86786-07-4; XII, 86786-08-5; xIII, 86786-09-6; XIV, 86786-10-9; Boc-L-Ile, 13139-16-7; Boc-L-Phe, 13734-34-4; Boc-D-Phe, 18942-49-9; *p*-nitrophenyl β-(benzylmercapto)-β,β-cyclopentamethylene propionate, 55154-81-9; AVP, 113-79-1; oxytocin, 50-56-6.

(25) Spackman, D. H.; Stein, W. H.; Moore, S. *Anal. Chem.* **1958**, *30*, 1190.