Design of More Potent Antagonists of the Antidiuretic Responses to Arginine-vasopressin

Maurice Manning,* Aleksandra Olma, Wieslaw A. Klis, Aleksander M. Kolodziejczyk,

Department of Biochemistry, Medical College of Ohio at Toledo, Toledo, Ohio 43699

Janny Seto, and Wilbur H. Sawyer

Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, New York 10032.
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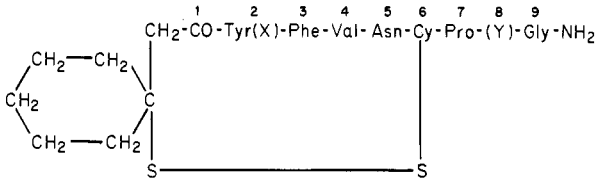
As part of a program aimed at designing more potent and selective antagonists of the antidiuretic responses to arginine-vasopressin (AVP), we substituted *O*-alkyl-D-tyrosine (where alkyl = methyl, ethyl, isopropyl, or *n*-propyl) at position 2 in our eight previously reported *O*-alkyl-L-tyrosine antagonists of antidiuretic and vasopressor responses to AVP. We also substituted D-tyrosine for L-tyrosine in two vasopressor antagonists with weak antidiuretic agonistic activity, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$VDAVP] and its L-arginine isomer [d$(CH_2)_5$VAVP]. The ten analogues, synthesized by the solid-phase method, are as follows: 1, d$(CH_2)_5$-D-Tyr(Me)VDAVP; 2, d$(CH_2)_5$-D-Tyr(Et)VDAVP; 3, d$(CH_2)_5$-D-Tyr(*i*-Pr)VDAVP; 4, d$(CH_2)_5$-D-Tyr(*n*-Pr)VDAVP; 5, d$(CH_2)_5$-D-Tyr(Me)VAVP; 6, d$(CH_2)_5$-D-Tyr(Et)VAVP; 7, d$(CH_2)_5$-D-Tyr(*n*-Pr)VAVP; 8, d-$(CH_2)_5$-D-Tyr(*i*-Pr)VAVP; 9, d$(CH_2)_5$-D-TyrVDAVP; 10, d$(CH_2)_5$-D-TyrVAVP. These analogues were tested for agonistic and antagonistic activities in rat antidiuretic and rat vasopressor systems. All ten D-tyrosine analogues possess transient weak antidiuretic activities (0.004–0.05 U/mg). Subsequent doses of AVP are reversibly antagonized for 1–3 h, depending on the dose of the antagonist. They exhibit the following antiantidiuretic pA_2 values: 1, 7.19 ± 0.11; 2, 7.59 ± 0.04; 3, 7.51 ± 0.06; 4, 7.60 ± 0.05; 5, 7.77 ± 0.07; 6, 7.81 ± 0.07; 7, 7.66 ± 0.11; 8, 7.61 ± 0.06; 9, 7.03 ± 0.05; 10, 7.51 ± 0.08. They are all effective antagonists of vasopressor responses to AVP. Analogues 1–8 are two to ten times more potent than their respective *O*-alkyl-L-tyrosine isomers as antidiuretic antagonists. Since the vasopressor potencies of the *O*-alkyl-L-tyrosine analogues have either diminished or remained virtually unchanged, these analogues exhibit a selective increase in their antiantidiuretic/antivasopressor ratios with respect to their respective *O*-alkyl-L-tyrosine analogues. The finding that the substitution of an unalkylated D-tyrosine for L-tyrosine in d$(CH_2)_5$VDAVP and d$(CH_2)_5$VAVP converts these weak antidiuretic agonists into potent antagonists of antidiuretic responses to AVP is highly significant, especially in view of the relative ease of synthesis and much higher yields of unalkylated vs. alkylated tyrosine analogues. These ten new analogues are potentially useful as pharmacological tools and as therapeutic agents. The findings presented here have also obvious potential for the design of even more potent and selective antidiuretic antagonists.

We recently reported the synthesis and some pharmacological properties of 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 modifying one of our previously reported antagonists of the vasopressor responses to AVP, i.e., [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$VDAVP],[3] and its L-arginine isomer, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin [d$(CH_2)_5$VAVP].[4] Both d$(CH_2)_5$VDAVP and d-$(CH_2)_5$VAVP are weak antidiuretic agonists. Although not an antagonist of in vivo responses to AVP, d$(CH_2)_5$VDAVP had been shown to be a competitive antagonist of the binding of AVP to rat renal medullary membranes and of AVP-stimulated adenylate cyclase activation in vitro.[5] The incorporation of a series of *O*-alkyl substituents, i.e., *O*-methyl, *O*-ethyl, *O*-isopropyl, and *O*-*n*-propyl, on the tyrosine residue at position 2 converted both of these peptides into antagonists of in vivo antidiuretic responses to AVP.[1,2] These modifications also led to significant enhancements of antivasopressor potencies.[1,2] The eight antagonists so designed have the following general structures:



1′–4′, X = Me, Et, *i*-Pr, *n*-Pr, Y = D-Arg
5′–8′, X = Me, Et, *i*-Pr, *n*-Pr; Y = L-Arg

The L-arginine-containing analogues are all more potent than any of the D-arginine-containing analogues.[1,2] Antiantidiuretic potencies vary with the size of the *O*-alkyl substituent at position 2.[1,2] However, in both the D-Arg and the L-Arg series the *O*-ethyl-containing analogues are the most potent.[1,2] In studies on related peptides aimed at enhancing the antiantidiuretic potencies of these analogues, we have found that the following structural features are essential for in vivo antidiuretic antagonism in theses analogues:[1,2,4] (1) The β,β-cyclopentamethylene group on the β carbon at position 1, (2) the *O*-alkyl substituents at position 2, (3) the phenylalanine residue at position 3, and (4) the valine residue at position 4. Thus, analogues lacking any one of these structural features do not antagonize in vivo antidiuretic responses to AVP.

In continuing to explore structural modifications which would lead to either retention or enhancement of antiantidiuretic potencies, we wished to determine the effects of optical inversion of the tyrosine residue in these analogues. We thus synthesized [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$-D-Tyr(Me)VDAVP]. Our preliminary studies indicated that this peptide exhibited enhanced antiantidiuretic potency relative to the L-Tyr(Me)-containing isomer. Following this promising

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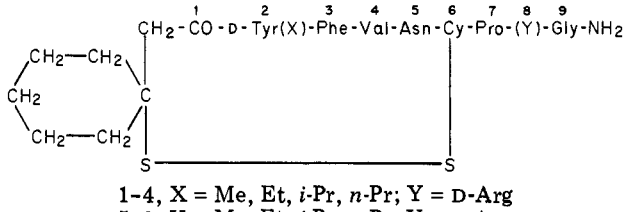
Table I. Experimental Conditions and Properties of New and Known Boc-D-Tyr(alkyl) Derivatives[a,c]

no.	substrates: D-Tyr derivatives	substrates: alkylating agent	conditions of alkylation: time, h	conditions of alkylation: solvent	product	crystn solvent system	yield, %	mp, °C	$[\alpha]^{25}_D$ (c 1, EtOH), deg
I	Boc-D-Tyr	$SO_2(OEt)_2$	1.5	benzene	Boc-D-Tyr(Et)[d]	cyclohexane	63.0	81–83	−32.0
II	Boc-D-Tyr-OMe	$MeSO_3$-*n*-Pr	3.0	benzene/DMF	Boc-D-Tyr(*n*-Pr)[b]	petroleum ether	74.0	83–85	−37.3
III	Boc-D-Tyr-OMe	$MeSO_3$-*i*-Pr	7.0	benzene/DMF	Boc-D-Tyr(*i*-Pr)[b]	petroleum ether	72.3	85–86	−55.8
IV	Boc-D-Tyr-OMe	BzlBr	4.0	benzene/DMF	Boc-D-Tyr(Bzl)	ethyl acetate/ petroleum ether	74.6	105–106	−24.3

[a] Synthesized using a crown ether as described in ref 11. [b] Satisfactory analytical data (±0.3%) for C, H, and N were reported for the two new compounds listed in the table. [c] Abbreviations used are: Boc, *tert*-butyloxycarbonyl; Me, methyl; Pr, propyl; $SO_2(OEt)_2$, diethyl sulfate; $MeSO_3$-*n*-Pr, *n*-propyl methanesulfonate; Tyr-OMe, tyrosine methyl ester; Tyr(R), tyrosine alkyl ether. [d] Literature[11] mp 90–92 °C, $[\alpha]^{26}_D$ −39.7° (*c* 1, EtOH).

lead, we synthesized the D-Tyr(alkyl)[2] isomers of the remaining 2′–8′ analogues. With the findings from these analogues on hand, we were eager to determine what contribution the unalkylated D-tyrosine residue made toward the resultant enhancements of antiantidiuretic potencies and wondered about the possibility that alkylation of D-tyrosine might not be a necessary prerequisite for antidiuretic antagonism. We thus decided to incorporate an unalkylated D-tyrosine residue at position 2 in d-$(CH_2)_5$VDAVP and in d$(CH_2)_5$VAVP.

The ten new analogues designed by the above rationale are as follows: **1**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$-D-Tyr(Me)VDAVP]; **2**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid,2-(*O*-ethyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$-D-Tyr(Et)VDAVP]; **3**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-isopropyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$-D-Tyr(*i*-Pr)VDAVP]; **4**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-*n*-propyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$-D-Tyr(*n*-Pr)-VDAVP]; **5**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)-D-tyrosine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-Tyr(Me)VAVP]; **6**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-ethyl)-D-tyrosine,4-valine]arginine-vasopressin [d-$(CH_2)_5$-D-Tyr(Et)VAVP]; **7**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-isopropyl)-D-tyrosine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-Tyr(*i*-Pr)-VAVP]; **8**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-*n*-propyl)-D-Tyrosine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-Tyr(*n*-Pr)VAVP]; **9**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-tyrosine,4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$-D-TyrVDAVP]; **10**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-tyrosine,4-valine]arginine-vasopressin [d$(CH_2)_5$-D-TyrVAVP]. These ten analogues have the following general structures:



1-4, X = Me, Et, *i*-Pr, *n*-Pr; Y = D-Arg
5-8, X = Me, Et, *i*-Pr, *n*-Pr; Y = L-Arg
9, X = H; Y = D-Arg
10, X = H; Y = L-Arg

We now present the synthesis and some pharmacological properties of these ten analogues, all of which have been found to antagonize effectively antidiuretic and vasopressor responsees by rats to exogenous AVP. A preliminary report on the properties of these antagonists has been presented.[6]

Peptide Synthesis. The protected peptide precursors required for the synthesis of all ten peptides antagonists were prepared by the solid-phase method of peptide synthesis[7-10] entirely on the resin. Boc-D-Tyr(Me), Boc-D-Tyr(Et), Boc-D-Tyr(Bzl), Boc-D-Tyr(*i*-Pr), and Boc-D-Tyr(*n*-Pr) were synthesized using a crown ether,[11] the latter two for the first time. The experimental conditions used for their preparations, as well as for Boc-D-Tyr(Bzl), together with their properties are given in Table I. *p*-Nitrophenyl-β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropioniate[12] was used in each final coupling step. All active ester couplings[13,14] were facilitated by the addition of *N*-hydroxybenzotriazole (HOBT).[15] All of the protected peptide amides were obtained by ammonolytic cleavage[2,9] from the respective acyl octapeptide resins. Each protected precursor was deblocked with Na in NH_3[16] using a previously described yield-enhancing modification of the standard workup procedure.[2] Even with this modification, the yields of free peptides obtained from the *O*-alkyl-D-tyrosine peptides did not match those obtained for the corresponding *O*-alkyl-L-tyrosine peptides.[2] However, they were far superior to yields obtained without the modification. The yields of free peptides from the two unalkylated D-tyrosine peptides were 2–3 times greater than those of the *O*-alkyl-D-tyrosine analogues. The deblocked disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$.[17] The analogues were desalted and purified by gel filtration on Sephadex G-15 as previously described.[18]

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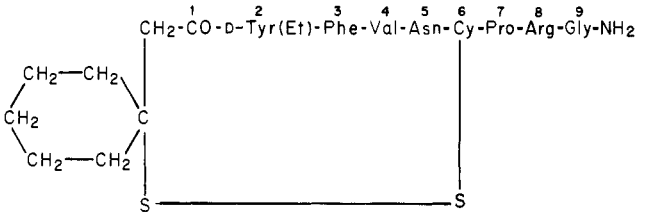
Table II. Effects of D-Tyr(alkyl)[2] Substitution on Potencies of Antidiuretic and Vasopressor Antagonists of Arginine-vasopressin (AVP)



d(CH_2)$_5$-D-Tyr(Et)VAVP

		antiantidiuretic effective dose[a]		antivasopressor effective dose[a]	
no.	antagonist[e]	nmol/kg	pA_2[b]	nmol/kg	pA_2[b]
1	d(CH_2)$_5$-D-Tyr(Me)VDAVP	4.9 ± 1.3	7.19 ± 0.12 (4)[c] [6.68][d]	0.54 ± 0.07	8.10 ± 0.04 (4)[c] [8.44][d]
2	d(CH_2)$_5$-D-Tyr(Et)VDAVP	1.8 ± 0.2	7.59 ± 0.04 (4) [7.10]	0.88 ± 0.12	7.88 ± 0.05 (4) [8.31]
3	d(CH_2)$_5$-D-Tyr(*i*-Pr)VDAVP	2.2 ± 0.3	7.51 ± 0.06 (4) [6.88]	0.46 ± 0.08	8.19 ± 0.08 (5) [8.41]
4	d(CH_2)$_5$-D-Tyr(*n*-Pr)VDAVP	1.7 ± 0.2	7.60 ± 0.05 (4) [6.67]	0.82 ± 0.10	7.92 ± 0.05 (4) [7.86]
5	d(CH_2)$_5$-D-Tyr(Me)VAVP	1.2 ± 0.3	7.77 ± 0.07 (6) [7.35]	0.23 ± 0.04	8.48 ± 0.08 (4) [8.32]
6	d(CH_2)$_5$-D-Tyr(Et)VAVP	1.1 ± 0.2	7.81 ± 0.07 (5) [7.57]	0.45 ± 0.11	8.22 ± 0.12 (4) [8.16]
7	d(CH_2)$_5$-D-Tyr(*i*-Pr)VAVP	1.6 ± 0.4	7.66 ± 0.11 (4) [7.32]	0.28 ± 0.04	8.40 ± 0.06 (4) [8.36]
8	d(CH_2)$_5$-D-Tyr(*n*-Pr)VAVP	1.7 ± 0.2	7.61 ± 0.06 (4) [7.29]	0.51 ± 0.10	8.14 ± 0.08 (4) [8.22]

[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 x 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] pA_2 values for each of the corresponding *O*-alkyl-L-tyrosine antagonists reported in ref 2. [e] The abbreviations and their full names are as follows: d(CH_2)$_5$-D-Tyr(Me)VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin; d(CH_2)$_5$-D-Tyr(Et)VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-ethyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin; d(CH_2)$_5$-D-Tyr(*i*-Pr)VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-isopropyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin; d(CH_2)$_5$-D-Tyr(*n*-Pr)VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-propyl)-D-tyrosine,2-(*O*-methyl)-D-tyrosine,4-valine]arginine-vasopressin; d(CH_2)$_5$-D-Tyr(Et)VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(*O*-ethyl)-D-tyrosine, 4-valine]arginine-vasopressin; d(CH_2)$_5$-D-Tyr(*i*-Pr)VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-isopropyl)-D-tyrosine,4-valine]arginine-vasopressin; d(CH_2)$_5$-D-Tyr(*n*-Pr)VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-propyl)-D-tyrosine,4-valine]arginine-vasopressin.

Bioassay Methods. The agonistic and antagonistic potencies of these analogues were measured using previously described methods.[1,2,19-21] These included intravenous vasopressor assays in phenoxybenzamine-treated rats under urethane anesthesia and antidiuretic assays in rats under ethanol anesthesia. The USP posterior pituitary reference standard was used in all assays for agonistic and antagonistic activities. Agonistic activities are expressed in units per milligram. Antagonistic potencies were determined and expressed as "effective doses" and as pA_2 values.[22] 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 $1x$ 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). For the measurement of antiantidiuretic potencies, the agonist was injected 20 and 50 min after the antagonist. All of the antagonists except analogues **5** and **6** produced maximal inhibition of antidiuretic responses to agonist after 20 min, and responses at this time were used to estimate effective doses and pA_2's. Analogues **5** and **6** usually showed more inhibition of responses at 50 than at 20 min. For these analogues, responses to agonist administered 50 min after antagonist were used in estimating effective doses and pA_2's. Each antagonist was administered in two doses, a high dose which reduced the response to $2x$ units of agonist to less than the response to $1x$ units of agonist, and a low dose which did not fully reduce the response to that given by $1x$ 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 and antivasopressor potencies of the *O*-alkyl-D-tyrosine analogues, i.e., analogues **1–8**, together with the pA_2 values for the corresponding L-tyrosine analogues[2] are presented in Table II. The same potencies for the unsubstituted D-tyrosine analogues, i.e., analogues **9** and **10**, together with those of their respective L-tyrosine parent analogues, d(CH_2)$_5$VDAVP[3] and d(CH_2)$_5$VAVP,[4] are presented in Table III. None of the ten new analogues exhibit any pressor activity. All ten analogues possess transient weak antidiuretic activities (0.004–0.05 U/mg). Subsequent doses of AVP are reversibly antagonized for 1–3 h, depending on the dose of antagonist.

Effect of D-Tyr(alkyl) vs. L-Tyr(alkyl) on Antidiuretic Antagonism. Comparison of the antiantidiuretic pA_2 values of each of the D-Tyr(alkyl) antagonists **1–8** in Table II with those for each of the respective L-Tyr(alkyl) antagonists reported earlier[1,2] (also shown in parentheses in Table II) shows clearly that each antagonist containing D-Tyr(alkyl) is more potent than the corresponding L-Tyr(alkyl)-substituted antagonist. In the D-Arg-containing series, i.e., analogues **1–4**, the enhancements are quite striking, ranging from a twofold increase in antidiuretic potency for the D-Tyr(Me)/L-Tyr(Me) pair to almost a tenfold increase for the D-Tyr(*n*-Pr)/L-Tyr(*n*-Pr) pair. In

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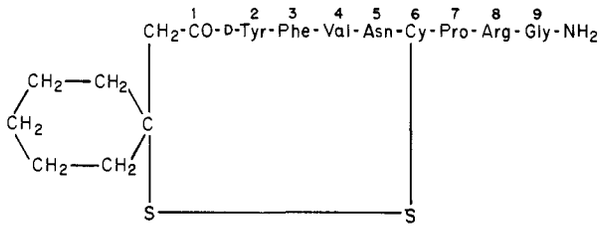
Table III. Unalkylated D-Tyr[2] Analogues of Two Weak Antidiuretic Agonists Are Potent Antidiuretic Antagonists[f]



d(CH$_2$)$_5$-D-TyrVAVP

no.	peptide	antiantidiuretic effective dose[a] nmol/kg	antiantidiuretic effective dose[a] pA$_2$[b]	antivasopressor effective dose[a] nmol/kg	antivasopressor effective dose[a] pA$_2$[b]
	d(CH$_2$)$_5$VDAVP	agonist[d]		1.50 ± 0.2	7.68 ± 0.05[c] (11)
9	d(CH$_2$)$_5$-D-TyrVDAVP	6.3 ± 0.8	7.03 ± 0.05[c] (4)	0.60 ± 0.04	8.05 ± 0.03 (4)
	d(CH$_2$)$_5$VAVP	agonist[e]		0.76 ± 0.10	7.97 ± 0.06 (8)
10	d(CH$_2$)$_5$-D-TyrVAVP	2.2 ± 0.2	7.51 ± 0.08 (4)	0.29 ± 0.09	8.41 ± 0.11 (4)

[a-c] See Table II. [d] 0.10 unit/mg (ref 3). [e] 0.2 unit/mg (ref 4). [f] The abbreviations and their full names are as follows: d(CH$_2$)$_5$VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin; d(CH$_2$)$_5$-D-TyrVDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-tyrosine,4-valine,8-D-arginine]vasopressin, 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.

the L-Arg series, i.e., analogues **5–8**, the enhancement is neither as variable nor as great. Thus, the enhancement of antiantidiuretic potency is two- to threefold in all D-Tyr(alkyl)/L-Tyr(alkyl) pairs in this series.

Relative Effects of the *O*-Alkyl-D-tyrosine Substituents on Antidiuretic Antagonism. In contrast to the effects of size in the L-Tyr(alkyl) series, where differences in size effected clear differences in antiantidiuretic potencies and where the *O*-ethyl-containing analogues were clearly more potent in both the D-Arg- and L-Arg-containing antagonists, size differences in the D-Tyr(alkyl) series are not so clearly differentiated. Thus, in the D-Arg series the D-Tyr(Me) analogue has an antiantidiuretic pA_2 of 7.19. Replacement of the *O*-methyl group by *O*-ethyl, *O*-isopropyl, or *O*-*n*-propyl effects virtually the same twofold enhancement in antidiuretic potency in all cases. In the L-Arg series, the *O*-methyl and *O*-ethyl analogues are almost equipotent as are the *O*-isopropyl and *O*-*n*-propyl analogues, although the latter pair is somewhat less potent. Thus, in this series there appears to be a drop-off in antiantidiuretic potency with increasing size of the *O*-alkyl substituents. With an antiantidiuretic pA_2 of 7.81, d(CH$_2$)$_5$-D-Tyr(Et)VAVP is the most potent of these D-Tyr(alkyl)-containing antagonists. It is thus the most potent antagonist of in vivo antidiuretic responses to AVP reported to date.

Effects of D-Tyr vs. L-Tyr on in Vivo Antidiuretic Antagonism. Both d(CH$_2$)$_5$VDAVP[3] and d(CH$_2$)$_5$VAVP[4] are weak antidiuretic agonists and do not exhibit antidiuretic antagonism in vivo. The data given in Table III for analogues **9** and **10**, i.e., d(CH$_2$)$_5$-D-Tyr-VDAVP and d-(CH$_2$)$_5$-D-Tyr-VAVP, clearly show that the substitution of D-tyrosine for L-tyrosine in both of these molecules converts these antidiuretic agonists to potent antagonists of in vivo antidiuretic responses. This is a striking finding, for it shows clearly that alkylation of the tyrosine residue at position 2 per se is not a prerequisite for antidiuretic antagonism.

With regard to their antiantidiuretic potencies, it is interesting to note that d(CH$_2$)$_5$-D-Tyr-VDAVP (pA_2 = 7.03) and d(CH$_2$)$_5$-D-Tyr-VAVP (pA_2 = 7.51) are each more potent than all but one of the L-Tyr(alkyl)-substituted antagonists of the D-Arg and L-Arg series, respectively (Table II).[2] From this one can conclude that the substitution of a D-tyrosine residue at positoin 2 in either d-(CH$_2$)$_5$VDAVP or d(CH$_2$)$_5$VAVP has virtually the same effect in terms of their antiantidiuretic properties as alkylation of the L-tyrosine residue in these molecules with methyl, ethyl, isopropyl, or *n*-propyl groups. It is also noteworthy that the L-Arg-containing D-Tyr analogue, i.e., d(CH$_2$)$_5$-D-TyrVAVP, is clearly much more potent that its D-arginine isomer, i.e., d(CH$_2$)$_5$-D-TyrVDAVP. This finding is highly consistent with our previous findings[1,2] and with those for the other D-Tyr(alkyl)/L-Arg antagonists reported here.

Effects of D-Tyr(alkyl) and D-Tyr on Antivasopressor Potencies. The D-Tyr(alkyl) substitutions have differing effects on antivasopressor potencies depending on whether the arginine residue at position 8 is in the D or the L configuration. In the D-Arg-containing peptides (analogues 1–4, Table II), the substitution of *O*-Me-, *O*-Et-, and *O*-*i*-Pr-D-Tyr brought about a twofold decrease of antivasopressor potencies. Curiously in this series the *O*-*n*-propyl-D-tyrosine substitution effected virtually no change of antivasopressor potency. With one exception, substitutions of all the D-Tyr(alkyl) residues in the L-Arg series (analogues **5–8**, Table II) effected virtually no change in antivasopressor potencies, the exception being the *O*-methyl-D-tyrosine derivative, which exhibited a modest increase in antivasopressor potency. Thus, since the D-Tyr(alkyl)-containing analogues are all more potent antagonists of in vivo antidiuretic responses to AVP than their respective L-Tyr(alkyl) isomers, all are more selective than their L-Tyr(alkyl) isomers with respect to their antiantidiuretic/antivasopressor ratios. By contrast with the effects of the *O*-alkyl-D-tyrosine substitutions, the replacement of L-Tyr by D-Tyr in d(CH$_2$)$_5$VDAVP and d-(CH$_2$)$_5$VAVP brought about significant enhancements of antivasopressor potencies in the respective resultant peptides (analogues **9** and **10**, Table III). Thus, the substitution of D-Tyr in both of these peptides not only converted them to potent antidiuretic antagonists but also enhanced their antivasopressor potencies.

Conclusion

We have shown that replacement of L-Tyr(alkyl) by D-Tyr(alkyl) (where alkyl = Me, Et, *i*-Pr and *n*-Pr) in our previously reported antagonists of in vivo antidiuretic and vasopressor responses to AVP leads to enhancements of their antidiuretic potencies in all cases. Since antivaso-

pressor potencies were either reduced or remained virtually unchanged, all eight new analogues exhibit a selective increase in antiantidiuretic/antivasopressor potencies with respect to their respective L-Tyr(alkyl) analogues. In addition, we have shown that an unalkylated D-Tyr/L-Tyr interchange converted two weak antidiuretic agonists, $d(CH_2)_5VDAVP$ and $d(CH_2)_5VAVP$, into potent antidiuretic antagonists, while also enhancing their antivasopressor potencies. All of these antidiuretic antagonists are potentially useful as models for conformational analysis, pharmacological tools, and as clinical agents. Furthermore, these findings have obvious potential for the design of even more potent 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, 0.75–1.0 mequiv/g was esterified[23] with Boc-Gly to an incorporation of 0.5 mmol/g. Amino acid derivatives were supplied by Bachem Inc. or Chemalog Inc. Boc-D-Tyr(Me), Boc-D-Tyr(Et), Boc-D-Tyr(*n*-Pr), Boc-D-Tyr(*i*-Pr), and Boc-D-Tyr(Bzl) were resynthesized or synthesized for the first time using a crown ether[11] under conditions outlined in Table I. *p*-Nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate was synthesized.[12] Dimethylformamide (DMF) was distilled under reduced pressure immediately prior to its use. 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) Butan-1-ol–acetic acid–water, 4:1:1; (B) chloroform–methanol, 7:3, v/v; (C) butan-1-ol–acetic acid–water–pyridine, 15:3:3:10, v/v. Loads of 10–50 μg were applied and chromatograms were a minimum of 10-cm long. Iodine vapor was used for detection. For amino acid analysis,[24] 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 either 110 or 120 °C.[2] The analyses were performed on a Glencoe Model MM-100 and on a Beckman Model 121 automatic amino acid analyzer. 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 heptapeptidyl resin A (30 g, 10 mmol).

Boc-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-resin (B). The protected heptapeptidyl resin B (15 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-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (I). The heptapeptidyl resin A (1.2 g, 0.4 mmol) was converted to the protected acyl octapeptide resin in two cycles of solid-phase peptide synthesis using as the carboxy component Boc-D-Tyr(Bzl) and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate,[12,13] respectively, the latter coupling being facilitated by the addition of *N*-hydroxybenzotriazole monohydrate (HOBT).[15] The protected acyl octapeptidyl resin was ammonolyzed,[9] and the amide was extracted with hot DMF and precipitated by the addition of water. The crude product was reprecipitated from DMF–ethanol–ethyl ether to give I (0.544 g, 88.6% based on initial Gly content of the resin): mp 214–216 °C; $[\alpha]^{24}_D$ −18.8° (c 0.5 DMF); TLC R_f (A) 0.57, R_f (B) 0.72, R_f (C) 0.88. Anal. ($C_{79}H_{99}N_{13}O_{13}S_{13}$) C, H, N. Amino acid analysis: Tyr, 0.86; Phe, 0.84; Val, 1.00; Asp, 1.06; Cys(Bzl), 1.03; Arg, 1.03; Gly, 1.03; NH_3, 2.2.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Tyr(Me)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (II). The Boc heptapeptidyl resin A (1.2 g, 0.4 mmol) was converted to the protected acyl octapeptidyl resin and ammonolyzed, and the product was extracted and purified as described for I to give II (0.32 g, 54.8% based on initial Gly content of the resin): mp 214–216 °C; $[\alpha]^{23}_D$ −21.1° (c 1.0, DMF); TLC R_f (B) 0.82, R_f (C) 0.57. Anal. ($C_{73}H_{95}O_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 0.98; Phe, 1.00; Val, 1.00; Asp, 0.97; Cys(Bzl), 0.97; Pro, 1.00; Arg, 0.94; Gly, 0.98; NH_3, 2.15.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (III). The heptapeptidyl resin A (1.63 g, 0.62 mmol) was converted to the acyl octapeptidyl resin and ammonolyzed, and the product was extracted and purified as for I to give III as a white powder (0.57 g, 62.8%, based on initial Gly content of the resin): mp 212–214 °C; $[\alpha]^{25}_D$ −34.0° (c 0.5 DMF); TLC R_f (A) 0.59, R_f (B) 0.84, R_f (C) 0.82. Anal. ($C_{74}H_{97}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 1.12; Phe, 1.01; Val, 1.00; Asp, 0.95; Cys(Bzl), 1.02; Pro, 1.07; Arg, 1.05; Gly, 1.03; NH_3, 2.0.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Tyr(*n*-Pr)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (IV). The heptapeptidyl resin A (1.63 g, 0.62 mmol) was converted to the acyl octapeptidyl resin, and the product was worked up as for I to give IV (0.62 g, 67.3% based on initial Gly content of the resin): mp 213–215 °C; $[\alpha]^{25}_D$ −16.5° (c 1 DMF); TLC R_f (A) 0.59, R_f (B) 0.86, R_f (C) 0.84. Anal. ($C_{75}H_{99}N_{13}O_{13}S_3 \cdot 2H_2O$) C, H, N. Amino acid analysis: Tyr, 1.00; Phe, 0.94; Val, 1.00; Asp, 1.07; Cys(Bzl), 0.89; Pro, 1.04; Arg, 1.05; Gly, 1.03; NH_3, 2.11.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Tyr(*i*-Pr)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (V). The Boc heptapeptidyl resin A (1.2 g, 0.4 mmol) was converted to the acyl octapeptidyl resin and ammonolyzed, and the product was worked up as for I to give V (0.51 g, 85.8% based on initial Gly content of the resin): mp 216–218 °C; $[\alpha]^{25}_D$ −22.0° (c 0.8, DMF); TLC R_f (A) 0.57, R_f (B) 0.76, R_f (C) 0.76. Anal. ($C_{75}H_{99}N_{13}O_{13}S_3 \cdot 2H_2O$) C, H, N. Amino acid analysis: Tyr, 0.88; Phe, 0.84; Val, 1.00; Asp, 1.15; Cys(Bzl), 1.09; Pro, 1.04;, Arg, 1.06; Gly, 1.03; NH_3, 2.00.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (VI). The heptapeptidyl resin B (1.2 g, 0.4 mmol) was converted to the acyl octapeptidyl resin and ammonolyzed, and the product was worked up as for I to give VI (0.54 g, 86.6% based on initial Gly content of the resin: mp 212–214 °C; $[\alpha]^{25}_D$ −8.1° (c 1, DMF); TLC R_f (A) 0.68, R_f (B) 0.77, R_f (C) 0.85. Anal. ($C_{79}H_{99}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 1.04; Phe, 0.99; Val, 1.00; Asp, 0.83; Cys(Bzl), 1.00; Pro, 0.94; Arg, 0.96; Gly, 1.02; NH_3, 2.05.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Tyr(Me)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (VII). The Boc heptapeptidyl resin B (1.68 g, 0.55 mmol) was converted to the protected acyl octapeptidyl resin, ammonolyzed, and worked up as for I to give VII (0.50 g, 63.8% based on initial Gly content of the resin): mp 203–205 °C; $[\alpha]^{24}_D$ −14.8° (c 0.5, DMF); TLC R_f (A) 0.90, R_f (B) 0.98. Anal. ($C_{73}H_{95}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 0.86; Phe, 1.03; Val, 1.00; Asp, 0.88; Cys(Bzl), 1.00; Pro, 0.94; Arg, 0.96; Gly, 1.02; NH_3, 2.00.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (VIII). The heptapeptidyl resin B (1.2 g, 0.4 mmol) was converted to acyl octapeptidyl resin, ammonolyzed, and worked up as for I to give VIII (0.49 g, 82.5% based on initial Gly content of the resin): mp 212–214 °C; $[\alpha]^{25}_D$ −8.2° (c 0.9, DMF); TLC R_f (A) 0.70, R_f (B) 0.65, R_f (C) 0.85. Anal. ($C_{74}H_{97}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 0.99; Phe, 0.94; Val, 1.00; Asp, 0.99; Cys(Bzl), 0.90; Pro, 0.94; Arg, 1.02; Gly, 1.14; NH_3, 2.11.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Tyr(*n*-Pr)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (IX). The heptapeptidyl resin B (1.2 g, 0.4 mmol) was converted to acyl octapeptidyl resin, ammonolyzed, and worked up as for I to give IX (0.46 g, 74.7% based on initial Gly content of the resin): mp 212–214 °C; $[\alpha]^{25}_D$ −8.0° (c 0.5, DMF);

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TLC R_f (A) 0.64, R_f (B) 0.89. Anal. ($C_{75}H_{99}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 1.06; Phe, 1.09; Val, 1.00; Asp, 0.94; Cys(Bzl), 1.00; Pro, 0.87; Arg, 1.07; Gly, 1.12; NH_3, 2.1.

β-(*S*-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-D-Tyr(*i*-Pr)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg-(Tos)-Gly-NH_2 (X). The Boc heptapeptidyl resin B (1.2 g, 0.4 mmol) was converted to protected acyl octapeptidyl resin, ammonolyzed, and worked up as for I to give X (0.47 g, 79.2% based on initial Gly content of the resin): mp 208–211 °C; $[\alpha]^{25}_D$ −11.4° (*c* 0.5, DMF); TLC R_f (A) 0.61, R_f (B) 0.70, R_f (C) 0.90. Anal. ($C_{75}H_{99}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 0.91; Phe, 0.96; Val, 1.00; Asp, 1.01; Cys(Bzl), 1.04; Pro, 0.88; Arg, 1.01; Gly, 1.12; NH_3, 2.0.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-D-tyrosine,4-valine]arginine-vasopressin [d(CH_2)$_5$-D-TyrVAVP] (XI). A solution of the protected acyl octapeptide amide I (118 mg, 0.077 mmol) in sodium-dried and redistilled ammonia (500 mL) was treated at the boiling point and with stirring with sodium[16] from a stock of the metal contained in a small-bore glass tube until a light-blue color persisted in the solution for 30 s.[2,9,10] 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 solution. An excess of a solution of potassium ferricyanide[17] (0.01 M, 14 mL) was added gradually with stirring. The yellow solution was stirred for a further 20 min and 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 (1.7 g) was desalted on a Sephadex G-15 column (110 × 2.7 cm) eluting with aqueous acetic acid (50%)[18] with a flow rate of 5 mL/h. The eluate was fractionated and monitored for absorbance at 280 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 280 nm). Lyophilization of the pertinent fractions yielded the vasopressin analogue XI as a white powder (49 mg, 57%): TLC R_f (A) 0.20, R_f (C) 0.26; $[\alpha]_D$ −110.0° (*c* 0.25, 1 M AcOH). Amino acid analysis: Tyr, 1.12; Phe, 1.03; Val, 1.00; Asp, 0.86; Cys, 0.98; Pro, 1.04; Arg, 0.95; Gly, 0.92; NH_3, 2.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid,2-(*O*-methyl)-D-tyrosine,4-valine]arginine-vasopressin [d(CH_2)$_5$-D-Tyr(Me)VAVP] (XII). The analogue XII was prepared from intermediate II (140 mg, 0.096 mmol) in the manner detailed above for XI: yield 56 mg (53.0%); $[\alpha]^{23}_D$ −92.5° (*c* 1.0, 50% AcOH); TLC R_f (A) 0.26, R_f (C) 0.57. Amino acid analysis: Tyr, 0.99; Phe, 1.02; Val, 1.00; Asp, 1.01; Cys, 0.99; Pro, 1.02; Arg, 0.96; Gly, 1.00; NH_3, 1.95.

[1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-ethyl)-D-tyrosine,4-valine]vasopressin [d-(CH_2)$_5$-D-Tyr(Et)VAVP] (XIII). The protected acyl octapeptide amide III (167 mg, 0.115 mmol) was reduced by sodium in liquid NH_3, reoxidized, deionized, and purified as for XI to give XIII: yield 29 mg (22%); $[\alpha]^{25}_D$ −63.2° (*c* 0.2, 1 M AcOH); TLC: R_f (A) 0.32; R_f (C) 0.49. Amino acid analysis: Tyr, 1.04; Phe, 1.00; Val, 1.00; Asp, 0.99; Cys, 1.09; Pro, 1.04; Arg, 1.01; Gly, 0.99; NH_3, 1.98.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-*n*-propyl)-D-tyrosine,4-valine]vasopressin [d-(CH_2)$_5$-D-Tyr(*n*-Pr)VAVP] (XIV). The analogue XIV was prepared from the intermediate IV (166 mg, 0.114 mmol) in the manner detailed above for XI: yield 21 mg (16%); $[\alpha]^{25}_D$ −64.7° (*c* 0.3, 50% AcOH); TLC R_f (A) 0.40, R_f (C) 0.55. Amino acid analysis: Tyr, 1.03; Phe, 0.94; Val, 1.00; Asp, 1.00; Cys, 1.12; Pro, 1.04; Arg, 0.95; Gly, 0.99; NH_3, 2.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-isopropyl)-D-tyrosine,4-valine]vasopressin [d-(CH_2)$_5$-D-Tyr(*i*-Pr)VAVP] (XV). The analogue XV was prepared from the intermediate V (124 mg, 0.083 mmol) in the manner detailed above for XI: yield 22 mg (23%); $[\alpha]^{25}_D$ −75.9° (*c* 0.3, 50% AcOH); TLC R_f (A) 0.22; R_f (C) 0.42. Amino acid analysis: Tyr, 0.88; Phe, 0.93; Val, 1.00; Asp, 0.94; Cys, 1.07; Pro, 1.04; Arg, 0.90; Gly, 0.85; NH_3, 2.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-D-tyrosine,4-valine,8-D-arginine]vasopressin [d-(CH_2)$_5$-D-TyrVDAVP] (XVI). The protected acyl octapeptide amide VI (121 mg, 0.079 mmol) was reduced by sodium in liquid NH_3, reoxidized, deionized, and purified as for XI to give 54.6 mg (61.8%): $[\alpha]^{25}_D$ −70.0° (*c* 0.3, 1 M AcOH); TLC R_f (A) 0.13, R_f (C) 0.36. Amino acid analysis: Tyr, 1.06; Phe, 0.94; Val, 1.00; Asp, 1.07; Cys, 1.00; Pro, 0.94; Arg, 0.98; Gly, 1.05; NH_3, 2.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid],2-(*O*-methyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin [d(CH_2)$_5$-D-Tyr(Me)VDAVP] (XVII). The analogue XVII was prepared from the intermediate VII (124 mg, 0.086 mmol) in the manner detailed above for XI: yield 35.5 mg (37%); R_f (A) 0.29, R_f (C) 0.44; $[\alpha]^{25}_D$ −94.8° (*c* 0.25, 1 M AcOH). Amino acid analysis: Tyr, 1.02; Phe, 0.96; Val, 1.00; Asp, 1.06; Cys, 1.08; Pro, 0.94; Arg, 0.96; NH_3, 2.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-ethyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin [d(CH_2)$_5$-D-Tyr(Et)VDAVP] (XVIII). The analogue XVIII was prepared from the intermediate VIII (124 mg, 0.084 mmol) in the manner detailed above for XI: yield 14.4 mg (15%); $[\alpha]^{25}_D$ −81.7° (*c* 0.2, 1 M AcOH); TLC R_f (A) 0.25, R_f (C) 0.44. Amino acid analysis: Tyr, 0.93; Phe, 0.98; Val, 1.00; Asp, 0.97; Cys, 0.93; Pro, 0.87; Arg, 0.93; Gly, 0.97; NH_3, 2.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-*n*-propyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin [d(CH_2)$_5$-D-Tyr(*n*-Pr)VDAVP] (XIX). The protected acyl octapeptide amide IX (115 mg, 0.078 mmol) was reduced by sodium in liquid NH_3, reoxidized, deionized, and purified as for XI to give XIX: yield 16.6 mg (18%); $[\alpha]^{25}_D$ −56.4° (*c* 0.25, 50% AcOH); TLC R_f (A) 0.10, R_f (B) 0.12. Amino acid analysis: Tyr, 1.10; Phe, 0.89; Val, 1.00; Asp, 1.03; Cys, 0.88; Pro, 0.94; Arg, 0.99; Gly, 1.03; NH_3, 2.2.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid,2-(*O*-isopropyl)-D-tyrosine,4-valine,8-D-arginine]vasopressin [d(CH_2)$_5$-D-Tyr(*i*-Pr)VDAVP] (XX). The analogue XX was prepared from intermediate X (123 mg, 0.082 mmol) in the manner detailed above for XI: yield 11.9 mg (13%); $[\alpha]_D$ −54.4° (*c* 0.25, 50% AcOH); TLC R_f (A) 0.20, R_f (C) 0.19. Amino acid analysis: Tyr, 1.12; Phe, 1.05; Val, 1.00; Asp, 1.09; Cys, 1.11; Pro, 0.94; Gly, 1.03; NH_3, 2.00

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