4p: ^{1}H NMR (free base) (Me_2SO-d_6) δ 10.0 (br s, 1 H, ex, C8-O*H*), 8.91 (t, 1 H, ex, -N*H*CH_2-), 8.08 (d, 1 H, J = 9.1, C10-*H*), 7.80 (d, 1 H, J = 8.8, C3-*H*), 7.77 (d, 1 H, J = 3.0, C7-*H*), 7.32 (dd, 1 H, J = 9.1, J = 3.0, C9-*H*), 6.70 (d, 1 H, J = 8.8, C4-*H*), 3.38 (qu*, 2 H, -NHC$H_2$$CH_2$-), 3.00 (s, 3 H, C1-C$H_3$), 2.48 (m, 6 H, $CH_2CH_2N(CH_2CH_3)_2$), 1.76 (qt, 2 H, -$CH_2CH_2CH_2$-).

Biological Tests. In Vitro Cytotoxicity Evaluation. The mouse L1210 leukemia cells (RPMI) were grown in RPMI 1640 medium supplemented with 5% fetal calf serum and penicillin (10^6 units/L) plus streptomycin (100 mg/L) in controlled air-5% CO_2 humidified atmosphere at 37 °C. L1210 mouse leukemia cells were seeded at a density of 5×10^4 cells/mL. The test compound, dissolved in 50% ethanol, was added, at four different concentrations, to the cell suspension. The cytotoxic activity (IC_{50} value) of the test compound was defined as the concentration causing a 50% growth inhibition after 48 h, measured by cell protein content, and was determined from dose-response curves.

In Vivo Antileukemic Evaluation. BDF_1 mice were injected ip with 10^6 P388 lymphotic leukemia cells on day 0 and treated ip on days 1-5 in accordance with the protocols described by the National Cancer Institute.[13] The mean survival time (MST) for each treatment group (eight mice) was calculated and the percent of T/C was determined by using the following formula:

$$\% \text{ T/C} = [(\text{MST treated})/(\text{MST control})] \times 100$$

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Synthesis and Some Pharmacological Properties of Potent and Selective Antagonists of the Vasopressor (V_1-Receptor) Response to Arginine-Vasopressin†

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We report the solid-phase synthesis of eight position-9-modified analogues of the potent V_1-receptor antagonist of arginine-vasopressin, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin (d$(CH_2)_5$Tyr(Me)AVP) (**1-8**) and five position-9-modified analogues of the closely related β,β-dimethyl less potent V_1 antagonist, [1-deaminopenicillamine,2-*O*-methyltyrosine]arginine-vasopressin (dPTyr(Me)AVP) (**9-13**). In d$(CH_2)_5$Tyr(Me)AVP the C-terminal Gly-NH_2 was replaced by (**1**) ethylenediamine (Eda), (**2**) methylamine (NHMe), (**3**) Ala-NH_2, (**4**) Val-NH_2, (**5**) Arg-NH_2, (**6**) Thr-NH_2, (**7**) Gly-Eda, (**8**) Gly-*N*-butylamide (Gly-NH-Bu); in dPTyr(Me)AVP the C-terminal Gly-NH_2 was replaced by (**9**) Ala-NH_2, (**10**) Val-NH_2, (**11**) Thr-NH_2, (**12**) Arg-NH_2, and (**13**) Tyr-NH_2. All 13 analogues were tested for agonistic and antagonistic activities in in vivo rat vasopressor (V_1-receptor) and rat antidiuretic (V_2-receptor) assays. They exhibit no evident vasopressor agonism. All modifications in both antagonists were well-tolerated with excellent retention of V_1 antagonism and striking enhancements in anti-V_1/anti-V_2 selectivity. With anti-V_1 pA_2 values of 8.75, 8.73, 8.86, and 8.78, four of the analogues of d-$(CH_2)_5$Tyr(Me)AVP (**1-3** and **6**) are equipotent with d$(CH_2)_5$Tyr(Me)AVP (anti-V_1 pA_2 = 8.62) but retain virtually none of the V_2 agonism of d$(CH_2)_5$Tyr(Me)AVP. They are in fact weak V_2 antagonists and strong V_1 antagonists with greatly enhanced selectivity for V_1 receptors relative to that of d$(CH_2)_5$Tyr(Me)AVP. With anti-V_1 pA_2 values respectively of 8.16, 8.05, 8.04, 8.52, and 8.25, all five analogues (**9-13**) of dPTyr(Me)AVP are at least as potent V_1 antagonists as dPTyr(Me)AVP (pA_2 = 7.96) and three of these (**9**, **12**, **13**) actually show enhanced V_1 antagonism over that of dPTyr(Me)AVP. In fact, the Arg-NH_2[9] analogue (**12**) is almost equipotent with d$(CH_2)_5$Tyr(Me)AVP. These new V_1 antagonists are potentially useful as pharmacological tools for studies on the cardiovascular roles of AVP. Furthermore the analogues of dPTyr(Me)AVP may be useful in studies on the role(s) of AVP in the V_{1b}-receptor-mediated release of ACTH from corticotrophs.

Antagonists of the vasopressor (V_1-receptor) responses to arginine-vasopressin (AVP) originally reported from these laboratories have found widespread use as pharmacological tools in studies on the many putative physiological roles of AVP (for reviews see refs 1 and 2). Among the most widely used are [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin (d$(CH_2)_5$Tyr(Me)AVP)[3] and [1-deaminopenicillamine,2-*O*-methyltyrosine]arginine-vasopressin

† Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9). All amino acids are in the L configuration unless otherwise noted. Other abbreviations used are d$(CH_2)_5$Tyr(Me)AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin; dPTyr(Me)AVP, [1-deaminopenicillamine,2-*O*-methyltyrosine]arginine-vasopressin; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; BOP, benzotriazol-1-yltris(dimethylamino)phosphonium hexafluorophosphate; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; AcOH, acetic acid; TFA, trifluoroacetic acid; HOBt, *N*-hydroxybenzotriazole; ONp, *p*-nitrophenyl ester; Et_3N, triethylamine; Eda, ethylenediamine; NHMe, methylamine; NHBu, butylamide; Et_2O, diethyl ether.

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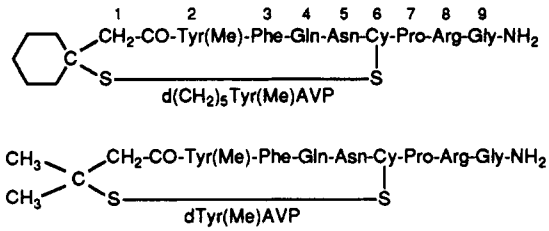
(dPTyr(Me)AVP).[4] Their structures are as follows:



These two molecules differ only in the nature of the alkyl substituents on the β-carbon at position 1. d(CH$_2$)$_5$Tyr(Me)AVP is a highly potent antagonist of the vasopressor (V$_1$-receptor) response to AVP. Its anti-V$_1$ pA_2 = 8.62. It is also a weak antidiuretic (V$_2$-receptor) agonist (0.31 units/mg).[3] dPTyr(Me)AVP is a less potent V$_1$ antagonist. Its anti-V$_1$ pA_2 = 7.98.[4] It also exhibits substantially more V$_2$ agonism, 3.5 units/mg.[4] However, it appears to be the most potent antagonist of the ACTH-releasing effects of AVP (V$_{1b}$-receptor) in vitro that has been tested to date.[5] Furthermore it has also been shown to be a V$_{1b}$ antagonist in vivo.[6] In attempting to enhance the V$_1$-antagonistic potencies and reduce the residual V$_2$ agonism of these two peptides, we have focused on modifications, deletions, and substitutions at the C-terminal Gly-NH$_2$ position. We have previously shown that deletion of the C-terminal Gly or Gly-NH$_2$ from d(CH$_2$)$_5$Tyr(Me)AVP results in good retention of V$_1$ antagonism and virtual elimination of V$_2$ agonism.[7,8] We have also reported that the C-terminal Gly-NH$_2$ of d(CH$_2$)$_5$Tyr(Me)AVP could be replaced by Tyr-NH$_2$ with good retention of V$_1$ antagonism. The ^{125}I derivative of the resulting compound, d(CH$_2$)$_5$[Tyr(Me)^{2}Tyr-NH$_2$^{9}]AVP exhibits high affinity for V$_{1a}$ receptors[9] and is thus a useful probe for radioautographic localization of V$_{1a}$ receptors. In selecting additional modification for the C-terminal position of d(CH$_2$)$_5$Tyr(Me)AVP and dPTyr(Me)AVP, we were guided by our previously reported findings on four series of C-terminally modified selective V$_2$ and nonselective V$_2$/V$_1$ AVP antagonists.[10] Thus we had shown that the selective AVP V$_2$ antagonist, d(CH$_2$)$_5$[D-Ile^2,Ile^4]AVP,[11] and the nonselective AVP V$_2$/V$_1$ antagonists d(CH$_2$)$_5$[D-Phe^2,Ile^4]AVP,[12] d(CH$_2$)$_5$[D-Tyr(Et)^2,Val^4]AVP,[13] and d(CH$_2$)$_5$[D-Tyr(Et)^2,Ile^4]AVP could have the C-terminal Gly-NH$_2$ replaced in each case by a wide variety of amino acid and non-amino acid substituents with excellent retention of V$_2$ antagonism and in some instances good enhancements of both V$_2$ antagonism and anti-V$_2$/V$_1$ selectivities.[10] We now report the synthesis and some pharmacological properties of eight position-9-modified analogues of d(CH$_2$)$_5$Tyr(Me)AVP and five position-9-modified analogues of dPTyr(Me)AVP. The eight analogues of d(CH$_2$)$_5$Tyr(Me)AVP have the C-terminal Gly-NH$_2$ replaced by (1) ethylenediamine (Eda); (2) methylamine (NHMe); (3) Ala-NH$_2$; (4) Val-NH$_2$; (5) Thr-NH$_2$; (6) Arg-NH$_2$; (7) Gly-Eda; and (8) Gly-*N*-butylamide (Gly-NH-Bu). The five analogues of dPTyr(Me)AVP (**9–13**) have the C-terminal Gly-NH$_2$ replaced by (**9**) Ala-NH$_2$; (**10**) Val-NH$_2$; (**11**) Thr-NH$_2$; (**12**) Arg-NH$_2$, and (**13**) Tyr-NH$_2$. Preliminary presentations on the pharmacological properties of analogues **1–3** and **6** have been presented elsewhere.[1,14-16]

Peptide Synthesis

The protected precursors (I–II, VII–IX, XI–XIII) (Tables III and IV) of the free peptides (**1–2, 7–9, 11–13**) (Table V) were synthesized entirely by the solid-phase method[17,19] using the standard Merrifield resin starting from the appropriate Boc-aminoacyl-resin. The precursors (III–VI) (Table III) and X (Table IV) of the free peptides (**3–6** and **10**) (Table V) were prepared by a combination of solid-phase and solution methods involving 8 + 1 couplings using the BOP-reagent.[20] Both approaches work

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Table I. Some Pharmacological Properties of Selective AVP Antagonists (1–8) Based on Modifications of d(CH_2)$_5$[Tyr(Me)^2]AVP at Position 9

no.	peptide	antivasopressor (anti-V_1) effective dose[a] (nmol/kg)	antivasopressor (anti-V_1) pA_2[b]	antiantidiuretic (anti-V_2) effective dose[a] (nmol/kg)	antiantidiuretic (anti-V_2) pA_2[b]	effective dose (ED)[a] ratio
	d(CH_2)$_5$[Tyr(Me)^2]AVP[e,f]	0.16 ± 0.01[c]	8.62 ± 0.03	agonist 0.31 units/mg	-	-
1	d(CH_2)$_5$[Tyr(Me)^2,Eda^9]AVP[g]	0.12 ± 0.02	8.75 ± 0.02	mixed ~33	~6.3	~275
2	d(CH_2)$_5$[Tyr(Me)^2,NHMe^9]AVP[g]	0.12 ± 0.01	8.73 ± 0.02	mixed ~84	~5.9	~700
3	d(CH_2)$_5$[Tyr(Me)^2,Ala-NH_2^9]AVP[g]	0.13 ± 0.03	8.75 ± 0.08	mixed ~77	~6.0	~590
4	d(CH_2)$_5$[Tyr(Me)^2,Val-NH_2^9]AVP	0.20 ± 0.04	8.57 ± 0.09	mixed ~115	~5.8	~575
5	d(CH_2)$_5$[Tyr(Me)^2,Thr-NH_2^9]AVP	0.22 ± 0.02	8.49 ± 0.04	mixed ~45	~6.2	~205
6	d(CH_2)$_5$[Tyr(Me)^2,Arg-NH_2^9]AVP	0.12 ± 0.02	8.78 ± 0.07	mixed ~18	~6.6	~150
	d(CH_2)$_5$[Tyr(Me)^2,Tyr-NH_2^9]AVP[h]	0.29 ± 0.02	8.47 ± 0.03	mixed ~63	~6.0	~217
7	d(CH_2)$_5$[Tyr(Me)^2,Gly-Eda^9]AVP	0.27 ± 0.03	8.39 ± 0.05	mixed ~16	~6.6	~60
8	d(CH_2)$_5$[Tyr(Me)^2,Gly-NH-Bu^9]AVP	0.25 ± 0.04	8.44 ± 0.07	agonist 0.027 ± 0.003 units/mg[e]	-	-

[a] The effective dose is defined as the dose (in nanomoles/kilogram) that reduces the response seen with $2x$ units of agonist to equal the response seen with x units of agonist administered in the absence of the antagonist. [b] Estimated in vivo pA_2 values represent the negative logarithms of the "effective doses" divided by the estimated volume of distribution (67 mL/kg). [c] Means ± SE. [d] ED ratio = anti-V_2 ED/anti-V_1 ED. [e] Data from Kruszynski et al. [f] Analogues 1–8 have the C-terminal Gly-NH_2 replaced by (1) ethylenediamine (NH(CH_2)$_2$-NH_2); (2) methylamide ($NHCH_3$); (3) Ala-NH_2; (4) Val-NH_2; (5) Thr-NH_2; (6) Arg-NH_2; (7) Gly-Eda; (8) Gly-*N*-butylamide (Gly-NH-(CH_2)$_3CH_3$). [g] Preliminary data reported in refs 1, 14–16. [h] Data from Elands et al.[9]

equally well. The 8 + 1 approach was utilized simply for convenience at the time the peptides **3–6** and **10** were being synthesized; 1 M HCl/AcOH was used in all the deprotection steps except those involving Boc-Gln in which TFA was employed.[21] Neutralizations were carried out with 10% Et_3N/CH_2Cl_2. Peptide and/or amide bond formation was carried out as follows: the protected amino acids (except asparagine and glutamine) and β-(benzylthio)-β,β-pentamethylenepropionic acid[22] and β-(benzylthio)-β,β-dimethylpropionic acid[23] were coupled by the DCC[24]/HOBt[25] procedure in CH_2Cl_2/DMF (9:1, v/v) using a 3-fold excess of the Boc-amino acid, DCC, and HOBt in relation to the resin. Thirty minutes after the beginning of the coupling, a 2-fold excess of Et_3N was added. The reaction was completed in 3 h, and usually one coupling was sufficient. The coupling of the active esters[26] Boc-Asn-ONp and Boc-Gln-ONp and all other procedures were performed by methods already described.[21] Cleavage from the resin was either by ammonolysis[21,27] in methanol to give the protected peptide amides or by aminolysis with methylamine, ethylenediamine, or *n*-butylamine in methanol essentially as described for the synthesis of 1-deamino[9-ethylenediamine]lysine-vasopressin[28] to give the appropriate substituted protected peptide amides. The precursors required for the 8 + 1 couplings in solution: β-(benzylthio)-β,β-pentamethylenepropionyl-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos) and β-(benzylthio)-β,β-dimethylpropionyl-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos) were cleaved from their respective resins by the HBr/TFA procedure.[8,18,29] Na in liquid NH_3[30] was used to deblock each protected precursors (I–XIII) as previously described,[3,4,11–13] and the resulting disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$.[31] The free peptides were desalted and purified by gel filtration on

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Table II. Some Pharmacological Properties of Selective AVP Antagonists (9–13) Based on Modifications of dP[Tyr(Me)^2]AVP at Position 9

no.	peptide	antivasopressor (anti-V_1) effective dose[a] (nmol/kg)	antivasopressor (anti-V_1) pA_2[b]	antidiuretic activity (units/mg)
	dP[Tyr(Me)^2]AVP[d]	0.80 ± 0.07[c]	7.96 ± 0.05[c]	3.5 ± 0.5
9	dP[Tyr(Me)^2,Ala-NH$_2$^{9}]AVP	0.47 ± 0.03	8.16 ● 0.03	0.040 ± 0.005
10	dP[Tyr(Me)^2,Val-NH$_2$^{9}]AVP	0.60 ± 0.01	8.05 ± 0.01	0.039 ± 0.002
11	dP[Tyr(Me)^2,Thr-NH$_2$^{9}]AVP	0.63 ± 0.08	8.04 ± 0.06	0.032 ± 0.002
12	dP[Tyr(Me)^2,Arg-NH$_2$^{9}]AVP	0.21 ± 0.02	8.52 ± 0.04	0.083 ± 0.006
13	dP[Tyr(Me)^2,Tyr-NH$_2$^{9}]AVP	0.38 ± 0.02	8.25 ± 0.02	0.029 ± 0.004

[a–c] See corresponding footnotes to Table I. [d] Data from Bankowski et al.[4]

Sephadex G-15 in a two-step procedure using 50% and 0.2 M AcOH as eluents, respectively, as previously described.[32] The purity of the free peptides was checked by TLC and HPLC.

Bioassay Methods

Assays for vasopressin V_1-antagonistic activities were performed by following blood pressure responses in rats anesthetized with urethane and pretreated with phenoxybenzamine.[33] Assays for antidiuretic V_2 agonism or antagonism were done by measuring changes in urine flow in water-loaded rats under ethanol anesthesia.[34] The USP posterior pituitary reference standard was used as a standard in agonistic assays and as an agonist in assays for antagonism. All injections of agonists and antagonists were given intravenously. In assays for antagonistic activities an "effective dose" (ED) of the antagonist was estimated. The ED is the dose of an antagonist that reduces the response to a subsequent dose of agonist to equal the response to half that dose of agonist given in the absence of antagonist. In practice, this is estimated by finding doses of antagonist above and below the ED and interpolating on a log scale.[35] An in vivo pA_2 can be estimated from the ED by dividing the ED by an arbitrarily assumed volume of distribution of 67 mL/kg.[36] When standard errors are presented in the tables, the means reflect results from at least four independent assay groups. None of these peptides exhibited any evident vasopressor agonism. Some peptides showed both agonistic and antagonistic activities on antidiuretic assays. In these instances the presence of agonism precluded reliable measurement of antagonistic potencies. EDs and pA_2s could only be roughly estimated, and means and standard errors are not indicated in the tables.

Results and Discussion

The antivasopressor (anti-V_1), antidiuretic agonistic activities, antiantidiuretic (anti-V_2) potencies, and the anti-V_2/anti-V_1 effective dose (ED) ratios of the position-9-modified analogues (1–8) of d(CH$_2$)$_5$Tyr(Me)AVP are given in Table I. The corresponding pharmacological data for the position-9-modified analogues (9–13) of dPTyr(Me)AVP are given in Table II. None of the peptides in Tables I and II exhibit any evident vasopressor agonism.

Examination of the properties of the peptides in both tables provides clear evidence that all substituents are well-tolerated in both peptides with excellent retention of V_1 antagonism in five of the eight analogues of d-(CH$_2$)$_5$Tyr(Me)AVP and two of the five analogues of dPTyr(Me)AVP. The remaining three analogues of dPTyr(Me)AVP exhibit a surprising enhancement of V_1 antagonism. Furthermore these position-9 substitutions brought about a highly significant reduction in V_2 agonism in both series of analogues. Thus all of the new peptides exhibit significant enhancements in selectivity for V_1 receptors versus V_2 receptors with respect to those of the respective parents, d(CH$_2$)$_5$Tyr(Me)AVP and dPTyr-(Me)AVP.

d(CH$_2$)$_5$Tyr(Me)AVP: Effects of Position-9 Modifications on Anti-V_1 Potency (Table I). With anti-V_1 pA_2 values in the range 8.73–8.80, four of the position-9-modified analogues of d(CH$_2$)$_5$Tyr(Me)AVP (1–3 and 6) are equipotent with d(CH$_2$)$_5$Tyr(Me)AVP (anti-V_1 pA_2 = 8.62).[3] Thus the C-terminal Gly-NH$_2$ in d(CH$_2$)$_5$Tyr-(Me)AVP can be replaced by the amino acid amides Ala-NH$_2$ and Arg-NH$_2$ and by the non-amino acids, ethylenediamine and methylamine, with full retention of V_1 antagonism. With anti-V_1 pA_2s = 8.57 and 8.49, respectively, the Val-NH$_2$^{9} and Thr-NH$_2$^{9} substituted analogues, although somewhat less potent than d(CH$_2$)$_5$Tyr(Me)-AVP, nonetheless further illustrate that position 9 can tolerate a wide variety of structural modification with good retention of V_1 antagonism. This point is clearly demonstrated by peptides 7 and 8 (Table I). Thus extension of the C-terminal glycine by ethylenediamine or butylamine results in excellent retention of anti-V_1 potencies exhibited by the resulting peptides 7 and 8, which have anti-V_1 pA_2s of 8.39 and 8.44, respectively. The data on peptides 1–8 together with earlier published data for the desGly^9 and desGly-NH$_2$^{9} analogues of d(CH$_2$)$_5$Tyr(Me)AVP[7,8] thus provide convincing evidence that the C-terminal Gly-NH$_2$ can be deleted or replaced by a wide variety of substituents with excellent retention of V_1-antagonistic potency.

d(CH$_2$)$_5$Tyr(Me)AVP: Effects of Position-9 Modifications on Anti-V_1 Selectivity (Table I). d-(CH$_2$)$_5$Tyr(Me)AVP, although a highly potent and selective V_1 antagonist, nonetheless retains some antidiuretic agonism (0.31 units/mg). Replacement of the C-terminal Gly-NH$_2$ in d(CH$_2$)$_5$Tyr(Me)AVP by all of the position-9 substituents reported here has led to a drastic reduction of V_2 agonism in each case. Thus peptide 8, having a Gly-N-Bu substituent at position 9, exhibits only 0.027 units/mg of antidiuretic activity. All of the remaining peptides 1–7 are mixed V_2 agonists/V_2 antagonists with anti-V_2 pA_2 values in the range ~5.8–6.64. Peptides 1–6 exhibit effective dose (ED) ratios in the range of 150–700. All of these peptides are thus much more selective for V_1

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receptors with respect to V_2 receptors than is d-$(CH_2)_5$Tyr(Me)AVP. It is clear therefore that replacement of the C-terminal Gly-NH_2 in d$(CH_2)_5$Tyr(Me)AVP by the amino acid and non-amino acid substituents reported here has resulted in a series of potent V_1 antagonists which are much more selective for AVP V_1 receptors than is d-$(CH_2)_5$Tyr(Me)AVP. All of these position-9-modified antagonists of d$(CH_2)_5$Tyr(Me)AVP, but especially the Eda^9-, the N-Me^9-, the Ala-$NH_2{}^9$-, and the Arg-$NH_2{}^9$-substituted analogues are potentially useful tools for studies on the role(s) of AVP in cardiovascular regulation.

dPTyr(Me)AVP: Effects of Position-9 Modifications on V_1 Antagonism (Table II). With anti-V_1 pA_2 values in the range 8.04–8.52, all of the position-9-modified antagonists of dPTyr(Me)AVP are at least as potent as dPTyr(Me)AVP (anti-V_1 pA_2 = 7.96). Peptides **9**, **12**, and **13** are actually more potent than dPTyr(Me)AVP. Thus, surprisingly, replacement of the C-terminal Gly-NH_2 by this series of amino acid amides has resulted in V_1 antagonists which are as much as four times more potent than dPTyr(Me)AVP. With an anti V_1 pA_2 = 8.52, the Arg-$NH_2{}^9$ analogue is clearly the most potent of the series and appears to be as potent as d$(CH_2)_5$Tyr(Me)AVP.

dPTyr(Me)AVP: Effects of Position-9 Modifications on Anti-V_1 Selectivity (Table II). Replacement of the C-terminal Gly-NH_2 in dPTyr(Me)AVP by Ala-NH_2, Val-NH_2, Thr-NH_2, Arg-NH_2, and Tyr-NH_2 resulted in all cases in a drastic loss of V_2 agonism. Thus dPTyr(Me)-AVP exhibits 3.5 units/mg of antidiuretic activity. In the five new analogues this has been reduced to 0.029–0.083 units/mg. This represents a 40–120-fold reduction in antidiuretic activity by the five new position-9-substituted analogues of dPTyr(Me)AVP. These reductions in V_2 agonism coupled with the significant enhancements of V_1 antagonism clearly demonstrate substantial gains in anti-V_1 selectivity. Thus all five peptides (**9–13**) are as potent or more potent and are more selective V_1 antagonists than dPTyr(Me)AVP. Some of them, the Arg-$NH_2{}^9$ analogue (**12**) for example, may have merit as potential tools for studies on the cardiovascular role(s) of AVP. With their increased anti-V_1 potencies and greatly reduced V_2 agonism, all of these peptides may also have value as antagonists of the ACTH releasing effects of AVP, i.e. as V_{1b}-receptor antagonists. In this regard, the Tyr-$NH_2{}^9$ analogue (**13**) is a potentially useful radioiodinated ligand for studies on V_{1b}-receptor localization and characterization.

Relative Contributions of the Amino Acid Substitutions in d$(CH_2)_5$Tyr(Me)AVP in dPTyr(Me)AVP in Enhancing V_1 Antagonism and Selectivity. It is clear from the data in Tables I and II that the same series of amino acid substitutions at position-9 in these two V_1 antagonists resulted in two quite distinct patterns. Thus on the one hand, in the case of the 9-substituted analogues of d$(CH_2)_5$Tyr(Me)AVP there was excellent retention of V_1 antagonism, but in no instance was there an enhancement of V_1 antagonism relative to that of d$(CH_2)_5$Tyr-(Me)AVP. On the other hand, three of the position-9-modified analogues of dPTyr(Me)AVP exhibit enhanced V_1 antagonism relative to that of dPTyr(Me)AVP. Thus the effects of these identical amino acid substitutions in these two antagonists have had quite distinct effects on V_1-antagonistic potencies. With respect to anti-V_1 selectivity their effects in both peptides appear to have been very similar. Thus for both series, anti-V_1 selectivity was greatly enhanced. The rank order of effectiveness of the position-9 substituents in retaining or enhancing V_1 antagonism also reveals other contrasts. For the 9-substituted analogues of d$(CH_2)_5$Tyr(Me)AVP, the rank order of effectiveness in decreasing order is Arg-NH_2, Ala-NH_2, Val-NH_2, Tyr-NH_2 and Thr-NH_2, whereas for dPTyr-(Me)AVP the corresponding rank order is Arg-NH_2, Tyr-NH_2, Ala-NH_2, Val-NH_2, Thr-NH_2. Thus in both series the Arg-$NH_2{}^9$ substitution ranks at the top in leading, respectively, to retention and enhancement of V_1 antagonism. However, it is clear that the rank order number of effectiveness in the two series do not fully correlate. The number two ranking of the Tyr-$NH_2{}^9$ analogue of dPTyr(Me)AVP relative to the number five ranking of the corresponding Tyr-$NH_2{}^9$ analogue of d-$(CH_2)_5$Tyr(Me)AVP illustrates this point very clearly. These findings on the nonequivalent effects of the same substitutions in two very closely related peptides show clearly the pitfalls inherent in predicting the effects of any given amino acid substitution based on its effects in a single peptide. However, this caveat aside, the effects of the Arg-$NH_2{}^9$ and Ala-$NH_2{}^9$ substitutions in enhancing both V_1 antagonism and selectivity in d$(CH_2)_5$Tyr(Me)-AVP and in dPTyr(Me)AVP correlate rather well with their effects in enhancing anti-V_2 potency, and in the case of the Ala-$NH_2{}^9$ analogue, anti-V_2/anti-V_1 selectivity, in the potent and selective V_2 antagonist, d$(CH_2)_5$[D-Ile^2,Ile^4]AVP.[10,16] Furthermore, the enhancement of V_1 antagonism brought about by the Arg-$NH_2{}^9$ substitution in the nonselective V_2/V_1 antagonist, d$(CH_2)_5$[D-Phe^2,Ile^4]AVP[10] correlates very well with the data presented here for its effectiveness in retaining and enhancing V_1 antagonism, respectively, in d$(CH_2)_5$Tyr(Me)AVP and in dPTyr(Me)AVP.

Conclusion

The potent vasopressin V_1 antagonist d$(CH_2)_5$Tyr-(Me)AVP (anti-V_1 pA_2 = 8.62)[3] and the less potent V_1 antagonist dPTyr(Me)AVP (anti-V_1 pA_2 = 7.96)[4] were modified at position 9 by replacing the C-terminal Gly-NH_2 with the same five amino acid amides: Arg-NH_2, Ala-NH_2, Thr-NH_2, Val-NH_2, and Tyr-NH_2. The C-terminal Gly-NH_2 of d$(CH_2)_5$Tyr(Me)AVP was also substituted by ethylenediamine and methylamine and extended by ethylenediamine and by butylamine. We have shown that these modifications in both molecules are very well tolerated with excellent retention of V_1-antagonistic potencies. Four of the position-9 substituents in d-$(CH_2)_5$Tyr(Me)AVP (Eda, N-Me, Ala-NH_2, and Arg-NH_2) resulted in molecules which are equipotent with d-$(CH_2)_5$Tyr(Me)AVP. Three of the position-9-modified analogues of dPTyr(Me)AVP exhibit enhanced V_1 antagonism relative to that of dPTyr(Me)AVP. All eight analogues of d$(CH_2)_5$Tyr(Me)AVP and all five analogues of dPTyr(Me)AVP exhibit substantial gains in anti-V_1 selectivity relative to that of d$(CH_2)_5$Tyr(Me)AVP and dPTyr(Me) AVP, respectively. Thus some of the analogues of d$(CH_2)_5$Tyr(Me)AVP, e.g., **1–3** and **6**, may have advantages over d$(CH_2)_5$Tyr(Me)AVP as potent and selective V_1 antagonists. All of the analogues reported here are potentially useful as pharmacological tools for studies on the cardiovascular actions of AVP. Furthermore the analogues of dPTyr(Me)AVP may have value as probes for studies on the ACTH-releasing effects (V_{1b}-receptor) of AVP. In this regard also, the Tyr-NH_2 analogue (**13**) when radiolabeled with ^{125}I may be of value as a probe for V_{1b}-receptor localization and characterization.

Experimental Section

Amino acid derivatives were purchased from Bachem Inc. or from Chemalog Inc. Dimethylformamide (DMF), anhydrous (99+%), Aldrich Chemical Co., Inc., was used for couplings. Other solvents and reagents were analytical grade. Boc-Tyr(Me)[37],

Table III. Physicochemical Properties of Protected Peptides I–VIII: β-(Benzylthio)-β,β-pentamethylenepropionyl-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-X

no.	X	formula	yield[a], %	mp, °C	$[\alpha]^{25}_D$, deg (c = 1, DMF)	TLC, R_f A	B	C
I	NH-$(CH_2)_2$-NH_2	$C_{73}H_{96}N_{14}O_{13}S_3$	76.2	185–186 dec	−37.4	0.45	0.67	0.08
II	NH-CH_3	$C_{72}H_{93}N_{13}O_{13}S_3$	78.4	195–198	−39.7	0.67	0.86	0.78
III	Ala-NH_2	$C_{74}H_{96}N_{14}O_{14}S_3$	84.2	185–189 dec	−31.7	0.54	0.80	0.46
IV	Val-NH_2	$C_{76}H_{99}N_{14}O_{14}S_3$	82.5	232–233	−36.3	0.72	0.79	0.82
V	Thr(Bzl)-NH_2	$C_{82}H_{104}N_{14}O_{15}S_3$	81.0	217–218	−26.6	0.82	0.81	0.88
VI	Arg(Tos)-NH_2	$C_{84}H_{110}N_{17}O_{16}S_4$	64.6	170–171	−28.6	0.71	0.78	0.82
VII	Gly-NH-$(CH_2)_2$-NH_2	$C_{75}H_{99}N_{15}O_{14}S_3$	29.0	162–165	−32.0	0.36	0.82	0.01
VIII	Gly-NH-$(CH_2)_2$-CH_3	$C_{77}H_{101}N_{14}O_{14}S_3$	49.0	189–190	−34.0	0.76	0.88	0.73

[a] Yields were calculated on the basis of arginine content of the starting resin for I, II, VII, and VIII and on the 8 + 1 coupling yield for III–VI.

Table IV. Physicochemical Properties of Protected Peptides IX–XIII: β-(Benzylthio)-β,β-dimethylpropionyl-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-X

no.	X	formula	yield,[a] %	mp, °C	$[\alpha]^{25}_D$, deg (c = 1, DMF)	TLC, R_f A	C	D
IX	Ala-NH_2	$C_{71}H_{92}N_{14}O_{14}S_3$	31.6	213–216	−33.6	0.50	0.67	0.50
X	Val-NH_2	$C_{73}H_{96}N_{14}O_{14}S_3$	92.2	197–210	−40.5	0.66	0.78	0.70
XI	Thr(Bzl)-NH_2	$C_{79}H_{100}N_{14}O_{15}S_3$	59.9	192–194	−38.8	0.64	0.90	0.63
XII	Arg(Tos)-NH_2	$C_{81}H_{105}N_{17}O_{16}S_4$	51.0	171–173	−32.9	0.50	0.58	0.51
XIII	Tyr(Bzl)-NH_2	$C_{84}H_{102}N_{14}O_{15}S_3$	66.4	201–203	−41.5	0.56	0.77	0.63

[a] Yields were calculated on the basis of C-terminal amino acid content of the starting resin, except X where the yield of 8 + 1 coupling is given.

Table V. Physicochemical Properties of the Free Peptides 1–13 Given in Tables I and II

no.	yield,[a,b] %	$[\alpha]^{25}_D$, deg (c = 0.3, 1 M AcOH)	TLC, R_f A	B	D	E	HPLC t_R[d,e]
1	46.8	−57.6	0.24	0.26	0.10	–	4.88
2	50.9	−73.2	0.32	0.51	0.28	–	5.16
3	42.1	−58.0	0.20	0.31	0.19	–	5.19
4	54.7	−65.0[c]	0.17	–	0.20	–	5.19
5	51.3	−64.0[c]	0.21	0.43	0.19	–	5.16
6	38.8	−59.1[c]	0.13	0.25	0.10	–	5.04
7	35.0	−53.4	0.26	0.27	0.10	–	5.22
8	48.3	−43.3	0.53	0.84	0.62	–	5.19
9	43.7	−39.0	0.20	–	0.21	0.35	5.08
10	32.7	−47.0	0.28	–	0.30	0.59	5.07
11	11.0	−30.0	0.16	–	0.15	0.58	5.15
12	28.5	−49.1	–	0.20	0.06	0.38	4.93
13	38.2	−41.0	0.28	–	0.30	0.35	5.03

[a] Yields are based on the amount of protected peptide used in the reduction–reoxidation step in each case and are uncorrected for acetic acid and water content. [b] All peptides gave the expected amino acid analysis ratios after hydrolysis ±3%. [c] c = 0.1, 50% AcOH. [d] t_R = retention time in minutes. Peptides were dissolved in HPLC-grade methanol and applied to a Waters μBondapak C_{18} column (15–20 μM, 3.9 mm × 15 cm); conditions: (a) H_2O (0.1% TFA), (B) CH_3CN–H_2O = 9:1 (0.1% TFA); flow rate 1.5 mL/min; developing 0 to 100% B for 15 min; detection was 0.5 AUFs at 210 nm. [e] All peptides were at least 96–99% pure as shown by the HPLC pattern.

β-(benzylthio)-β,β-pentamethylenepropionic acid,[22] and β-(benzylthio)-β,β-dimethylpropionic acid[23] were synthesized by previously published procedures. The protected precursors I–II, VII–IX, XI–XIII (Tables III and IV) and protected acylheptapeptides β-(benzylthio)-β,β-pentamethylenepropionyl-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)Pro-Arg(Tos) (IV) and β-(benzylthio)-β,β-dimethylpropionyl-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos) (V) required for the 8 + 1 couplings in solution were synthesized by the manual solid-phase method[8,17,18,21] as described below using the following modification. The Boc-amino acids (except asparagine and glutamine) and the β,β-dialkyl derivatives of S-Bzl-β-mercaptopropionic acid were coupled by the DCC/HOBt procedure[24,25] in CH_2Cl_2/DMF (9:1, v/v) using a 3-fold excess of protected acyl component, DCC, and HOBt in relation to the resin. Thirty minutes after the beginning of the coupling, a 2-fold excess of Et_3N was added. The reaction was completed in 3 h as determined by the Kaiser test,[38] and usually one coupling was sufficient. The couplings of Boc-Asn-ONp and Boc-Gln-ONp, ammonolysis or aminolysis of the protected peptides from the resin (I–II, VII–IX, XI–XIII) and their purification were performed by methods already described.[10,21,28,39] The protected precursors III–VI and X were obtained by the 8 + 1 approach using protected acylheptapeptide acids cleaved from the resin by the HBr/TFA procedure and Ala-NH_2, Val-NH_2, Thr(Bzl)NH_2, Arg(Tos)NH_2, respectively, in the presence of the BOP coupling reagent.[20] The physicochemical properties of the 13 purified protected peptides (I–XIII) are given in Tables III and IV. All protected precursors were converted to the required free cyclic peptides by deblocking with Na/liquid NH_3,[3,4,11–13,30] oxidiative cyclization with $K_3[Fe(CN)]$,[31] desalting, and purification in a two-step procedure using gel filtration on Sephadex G-15 as previously described.[32] The physicochemical properties of the free peptides (1–13) are given in Table V. Thin-layer chromatography (TLC) was performed on silica gel precoated plates (0.25 mm, Merck). The following solvent systems were used: (A)

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butan-1-ol–acetic acid–water (4:1:5, v/v/v, upper phase), (B) butan-1-ol–acetic acid–water–pyridine (15:3:3:10 v/v/v/v), (C) chloroform–methanol (7:3, v/v), (D) butan-1-ol–acetic acid–water (4:1:1, v/v/v), and (E) butan-1-ol–acetic acid–water (2:1:1, v/v/v). Loads of 10–50 μg were applied, and chromatograms were a minimum length of 10 cm. The chlorine gas procedure for the KI-starch reagent was used for detection.[19] Analytical reversed-phase high-performance liquid chromatography (HPLC) was performed on a Waters 810 instrument with a μBondapak C_{18} column (15–20 μL, 3.9 mm × 15 cm) and UV detection (see Table V). All peptides were at least 96–99% pure. Optical rotations were measured with a Rudolph Autopol III polarimeter. For amino acid analysis,[40] peptides (approximately 0.5 mg) were hydrolyzed with constant-boiling hydrochloric acid (500 μL) containing a 1% solution of phenol in water (10 μL) in evacuated and sealed ampules for 24 h at 110 °C. The analyses were performed on a Beckman System 6300 amino acid analyzer. Molar ratios were referred to Phe or Gly = 100. All peptides gave the expected amino acid ratios ±3%. Melting points of the protected peptides are uncorrected.

Solid-Phase Synthesis. Chloromethylated resin (Chemalog 1% cross-linked S-DVB, 200–400 mesh, 0.7–1.00 mmol/g) was esterified with either Boc-Gly, Boc-Ala, Boc-Thr(Bzl), Boc-Tyr(Bzl), or Boc-Arg(Tos) to an incorporation of approximately 0.5 mmol/g by the cesium salt method.[41] Eight (or seven) cycles of deprotection, neutralization, and coupling were carried out for the synthesis. Ammonolysis[21,27] and/or aminolysis[28] in MeOH or acidolytic cleavage with HBr/TFA[8,18,29] were used to split the protected peptides from the resin. All of the protected precursors were purified by the same general method: extraction with hot DMF followed by reprecipitations with H_2O and EtOH/Et_2O until adjudged pure by TLC.

[β-(Benzylthio)-β,β-dimethylpropionyl]-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Ala-NH_2 (IX, Table IV). (i) Ammonolytic Cleavage. Boc-Ala-resin (2.0 g, 0.72 mmol) was subjected to eight cycles of deprotection, neutralization, and coupling with Boc-Arg(Tos), Boc-Pro, Boc-Cys(Bzl)-Boc-Asn-ONp, Boc-Gln-ONp, Boc-Phe, Boc-Tyr(Me), and β-(benzylthio)-β,β-dimethylpropionic acid. The resulting peptidyl resin (2.83 g, 84.3%) was cleaved by ammonolysis. The protected peptide was extracted with hot (ca. 70 °C) DMF (ca. 30 mL), and the product was precipitated by the addition of hot (ca. 70 °C) water (ca. 500 mL). After cooling, the product was collected, dried in vacuo over P_2O_5, reprecipitated from hot DMF (5 mL) with EtOH (100 mL) and Et_2O (300 mL), collected, and dried in vacuo over P_2O_5 to give the required acyloctapeptide amide (IX, Table IV). The same procedure was used for the synthesis and purification of the protected of acylpeptide amides XI–XIII (Table IV).

[(β-Benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH-$(CH_2)_3$-CH_3 (VIII, Table III). (ii) Aminolytic Cleavage. Boc-Gly-resin (1.7 g, 1.2 mmol) was converted to protected acyloctapeptidyl resin in eight cycles of deprotection, neutralization, and coupling (mediated by DCC/HOBt modified as noted above or active esters) with Boc-Arg(Tos), Boc-Pro, Boc-Cys(Bzl), Boc-Asn-ONp, Boc-Gln-ONp, Boc-Phe, Boc-Tyr(Me), and β-(benzylthio)-β,β-pentamethylenepropionic acid, respectively. The resulting protected peptidyl resin (2.0 g, 1.2 mmol) was suspended in dry methanol (10 mL) (freshly distilled from Na), and *n*-butylamine (20 mL) was added; the suspension was stirred at room temperature for 4 days. The solvents were evaporated, and the protected peptide was extracted with hot DMF (ca. 30 mL) and reprecipitated by the addition of hot water (ca. 500 mL). Following overnight storage at 4 °C, the product was collected, dried in vacuo over P_2O_5, and reprecipitated from hot DMF (5 mL) with EtOH (50 mL) and Et_2O (ca. 300 mL). The solid was collected and dried in vacuo over P_2O_5 to give the required acyloctapeptide *n*-butylamide (VIII, Table III). Similar procedures were used for the synthesis and purification of the related C-terminally substituted amides of protected peptides (I, II, and VII, Table III). The appropriate protected acylpeptidyl resin (ca. 1 mmol) was reacted with a solution of ethylenediamine (35 mL) in anhydrous methanol (150 mL) or methylamine (20 mL) in methanol (150 mL) to give the substituted amides I, VII, and II (Table III), respectively.

[(β-Benzylthio)-β,β-pentamethylenepropionyl]-Tyr-(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos) (XIV). (iii) Acidolytic Cleavage.[8,18,29] This compound was resynthesized as previously described:[8] yield 45%; mp 184–185 °C (lit.[8] mp 182–186 °C); $[\alpha]^{25}_D$ −21.6° (*c* = 0.5 DMF) (lit[8] $[\alpha]^{25}_D$ −21.5° (*c* = 0.5 DMF)); TLC 0.73 (A), 0.73 (B), 0.22 (C).

[(β-(Benzylthiol-β,β-dimethylpropionyl]-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos) (XV). This protected precursor was split from the resin by acidolytic cleavage:[8,18,29] yield 85.2%; mp 186–189 °C; $[\alpha]^{25}_D$ −40.9° (*c* = 1, DMF).

[β-(Benzylthiol)-β,β-pentamethylenepropionyl]-Tyr-(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Ala-NH_2 (III, Table III), (8 + 1 Coupling with BOP Reagent). To a solution of the protected peptidyl acid (XIV) (348.0 mg, 0.26 mmol) in DMF (5 mL) were added (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP-reagent)[20] (123.0 mg, 0.28 mmol) and *N*,*N*-diisopropylethylamine (0.19 mL, 1.05 mmol). The mixture was stirred at room temperature for 5 min, and HCl-Ala-NH_2 (65.0 mg, 0.52 mmol) was added. Stirring was continued for 6 h (the progress of the coupling reaction was monitored by TLC). The reaction mixture was poured into 5% $KHCO_3$ solution (ca. 400 mL). The precipitate was filtered, washed with water, and dried over P_2O_5. The crude product was finally reprecipitated from hot DMF (5 mL) and ethanol (ca. 50 mL) by addition of ether (ca. 300 mL) to give the desired acyloctapeptide amide (III, Table III). The protected acyloctapeptide amides (IV–VI, Table III) were obtained in the same manner using Val-NH_2, Thr-NH_2, and Arg-NH_2, respectively; compound (X, Table IV) was synthesized from acid (XV) and Val-NH_2 according to the same procedure.

[1-(β-Mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,9-alaninamide]vasopressin, d$(CH_2)_5$[Tyr-(Me)^2,AlaNH$_2^9$]AVP (3, Table V). A solution of the protected acyloctapeptide amide (III, Table III) (150 mg) in sodium-dried ammonia (ca. 500 mL) was treated at the boiling point and with stirring with sodium from a stick of metal contained in a small-bore glass tube until a light-blue color persisted in the solution for ca. 30 s. NH_4Cl 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 (50%, 80 mL) and quickly poured into ice-cold water (ca. 1000 mL). The pH was adjusted to approximately 7.0 with concentrated ammonium hydroxide. Following neutralization, an excess of a solution of potassium ferricyanide (0.01 M, 14 mL) was added gradually with stirring. The yellow solution was stirred for an additional 20 min, and, after acidification with 0.2 N HOAc to pH 4, for 10 min with anion-exchange resin (Bio-Rad AG-3, Cl^- form, 10 g-damp weight). The suspension was slowly filtered through a bed of resin (10 g damp weight), and the bed was washed with 0.2 M HOAc (3 × 100 mL); the combined filtrate and washings were lyophilized. The resulting powder (1.5 g) was desalted on a Sephadex G-15 column (110 × 2.7 cm) eluting with aqueous acetic acid (50%) with a flow rate of 5 mL/h. The elute was fractionated and monitored for absorbance at 254 nm. The fractions comprising the major peak were checked by TLC, pooled, and lyophilized. The residue (102 mg) was further subjected to gel filtration on Sephadex G-15 column (100 × 1.5 cm), eluting with aqueous acetic acid (0.2 M) with a flow rate of 4 mL/h. The peptide was eluted in a single peak (absorbance at 254 nm). Lyophilization of pertinent fractions gave the desired vasopressin analog (3, Table V). With minor modifications this procedure was utilized to give the remaining 12 free peptides in Table V.

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