Design of Potent and Selective Linear Antagonists of Vasopressor (V_1-Receptor) Responses to Vasopressin†

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We report the solid-phase synthesis of 21 linear analogues of A and D, two nonselective antagonists of the vasopressor (V_1) and antidiuretic (V_2) responses to arginine vasopressin (AVP). A is Aaa-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH_2 (where Aaa = adamantylacetyl at position 1). D is the des-Arg^9 analogue of A. Nine new analogues of A (**1–9**) and 12 new analogues of D (**10–21**) were obtained. The following substitutions either alone or in combination were incorporated in A and/or in D: phenylacetic acid (Phaa) and *tert*-butylacetic acid (t-Baa) at position 1; D-Tyr^2, D-Tyr(Me)^2; Gln^4; Arg^6, Lys^6, Orn^6, MeAla^7. The nine new analogues of A are (**1**) [Arg^6], (**2**) [Lys^6], (**3**) [Orn^6], (**4**) [Phaa^1,Lys^6], (**5**) [Phaa^1,Orn^6], (**6**) [D-Tyr^2], (**7**) [D-Tyr^2,Arg^6], (**8**) [Phaa^1,D-Tyr^2], (**9**) [Phaa^1,D-Tyr^2,Arg^6]. The 12 new analogues of D are (**10**) [Arg^6], (**11**) [Lys^6], (**12**) [Orn^6], (**13**) [Phaa^1,Lys^6], (**14**) [Phaa^1,Gln^4,Lys^6], (**15**) [Phaa,D-Tyr(Me)^2,Lys^6], (**16**) [Phaa,D-Tyr(Me)^2,Gln^4,Lys^6], (**17**) [Phaa^1,D-Tyr^2,Gln^4,Lys^6], (**18**) [t-Baa^1,Lys^6], (**19**) [t-Baa^1,Gln^4,Lys^6], (**20**) [Arg^6,MeAla^7], (**21**) [t-Baa^1,Arg^6,MeAla^7]. All 21 peptides were examined for agonistic and antagonistic potencies in AVP V_2 and V_1 assays in rats. With the exception of **6**, the eight remaining new analogues of A are equipotent or more potent than A as V_1 antagonists. Peptides **2–9** are less potent than A as V_2 antagonists. Three, **4**, **5**, and **9**, exhibit significant gains in anti-V_1/anti-V_2 selectivities (selectivity ratio = 41, 14, and infinite, respectively), compared to A (anti-V_1, $pA_2 = 7.75 \pm 0.07$; selectivity ratio = 0.44). Peptide **9** is unique in both series. It is a highly potent V_1 antagonist (anti-V_1 $pA_2 = 8.62 \pm 0.11$ and is the first linear peptide to exhibit substantial antidiuretic agonism (4.1 ± 0.2 units/mg). With the exception of **12**, the remaining 11 analogues of D are 8–40 times more potent than D as V_1 antagonists. Eight of these peptides exhibit significant gains in anti-V_1/anti-V_2 selectivities compared to D (anti-V_1 $pA_2 = 7.43 \pm 0.06$; selectivity ratio = 1.6). Their corresponding anti-V_1 pA_2 values and selectivity ratios are (**13**) 8.81 ± 0.06; 26; (**14**) 9.05 ± 0.09; ~570; (**15**) 8.61 ± 0.03; ~35; (**16**) 8.93 ± 0.05; >290; (**17**) 8.91 ± 0.04, ~59; (**18**) 8.63 ± 0.10; ~41; (**19**) 8.40 ± 0.05; >1500; (**20**) 8.34 ± 0.06, ~53; the usefulness of a Gln^4/Val^4 interchange in enhancing anti-V_1/anti-V_2 selectivity is clearly demonstrated by the high selectivity ratios of **14**, **16**, and **19**. With anti-V_1 pA_2 values of 8.6 or better, seven of these linear molecules are as potent as any cyclic V_1 antagonists reported to date. Furthermore, peptides **14**, **16**, and **19** are as selective as or more selective than any cyclic V_1 antagonists reported to date. Peptide **14** is the first AVP antagonist cyclic or linear to possess a mean pA_2 value greater than 9. Many of these potent and selective linear AVP antagonists could serve as useful pharmacological tools for studies on (a) AVP receptor subtypes and (b) the putative physiological functions of AVP.

We recently reported that a ring structure is not a requirement for the binding of antagonists to the characteristic antidiuretic (V_2) receptors or vascular (V_1) receptors for arginine vasopressin (AVP).[1] Thus, linear analogues of the potent AVP V_2/V_1 antagonist [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-D-tyrosine,4-valine]arginine vasopressin [d$(CH_2)_5$D-Tyr(Et)VAVP][2] were found to be as effective as their parent in blocking the V_2-receptor responses to AVP in vivo.[1] Although a number of the early linear analogues also retained substantial anti-V_1 potency,[1] none of them were highly potent. Thus, for example, the linear V_2/V_1 antagonist Aaa-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH_2 (A, Table I), although twice as potent as d$(CH_2)_5$D-Tyr(Et)VAVP as a V_2 antagonist, retains only 40% of the V_1 antagonism of d$(CH_2)_5$D-Tyr(Et)VAVP.[1] Furthermore, none of the early linear antagonists exhibited greater V_1 antagonism than V_2 antagonism.[1] Since selective cyclic V_1 antagonists have been shown to be valuable pharmacological tools in numerous studies on the physiological roles of AVP,[3] potent and selective linear V_1 antagonists could likewise be very useful. We wondered whether it would be possible to design a potent and selective linear V_1 antagonist by simply making a linear analogue of a selective cyclic V_1 antagonist. An obvious choice for an initial attempt to design a selective linear V_1 antagonist was the highly potent and selective cyclic V_1 antagonist [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine vasopressin [d$(CH_2)_5$Tyr(Me)-

† 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 Aaa, 1-adamantaneacetic acid; t-Baa, *tert*-butylacetic acid; Phaa, phenylacetic acid: D-Tyr(Me), *O*-methyl-D-tyrosine; D-Tyr(Et), *O*-ethyl-D-tyrosine; AVP, arginine-vasopressin; d$(CH_2)_5$D-Tyr(Et)VAVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-D-tyrosine,4-valine]arginine vasopressin; d$(CH_2)_5$Tyr(Me)AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine vasopressin; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; TFA, trifluoroacetic acid; HOBt, *N*-hydroxybenzotriazole; ONp, *p*-nitrophenyl ester; Z, benzyloxycarbonyl; MeAla, *N*-methylalanine.

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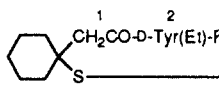
Table I. Structures and Antagonistic Properties of Potent and Selective Linear Nonapeptide V_1 Antagonistic Analogues of A

	antagonist	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)[d] ratio
	 1 2 3 4 5 6 7 8 9 CH_2CO-D-Tyr(Et)-Phe-Val-Asn-Cys-Pro-Arg-Gly-NH_2[e]	0.45 ± 0.11[c]	8.22 ± 0.12	1.1 ± 0.2	7.81 ± 0.07	2.4
A	Aaa-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH_2[f]	1.2 ± 0.2	7.75 ± 0.07	0.53 ± 0.07	8.11 ± 0.07	0.44
1	Aaa-D-Tyr(Et)-Phe-Val-Asn-Arg-Pro-Arg-Arg-NH_2	0.50 ± 0.05	8.13 ± 0.05	0.71 ± 0.07	7.98 ± 0.05	1.4
2	Aaa-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Arg-Arg-NH_2	0.50 ± 0.07	8.14 ± 0.06	1.4 ± 0.3	7.70 ± 0.09	2.8
3	Aaa-D-Tyr(Et)-Phe-Val-Asn-Orn-Pro-Arg-Arg-NH_2	1.6 ± 0.2	7.64 ± 0.06	~0.9[g]	~7.9[g]	~0.6
B	Phaa-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH_2[f]	0.26 ± 0.03	8.42 ± 0.05	0.69 ± 0.05	7.99 ± 0.03	2.7
4	Phaa-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Arg-Arg-NH_2	0.24 ± 0.05	8.47 ± 0.09	~9.8[h]	~6.85[h]	~41
5	Phaa-D-Tyr(Et)-Phe-Val-Asn-Orn-Pro-Arg-Arg-NH_2	0.29 ± 0.05	8.39 ± 0.07	~4.0[h]	~7.23[h]	~14
6	Aaa-D-Tyr(H)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH_2	3.0 ± 0.5	7.37 ± 0.07	2.1 ± 0.4	7.54 ± 0.07	0.7
7	Aaa-D-Tyr(H)-Phe-Val-Asn-Arg-Pro-Arg-Arg-NH_2	0.40 ± 0.04	8.22 ± 0.04	1.7 ± 0.5	7.66 ± 0.10	4.3
8	Phaa-D-Tyr(H)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH_2	2.2 ± 0.5	7.54 ± 0.10	~20[h]	~6.5[h]	~9
9	Phaa-D-Tyr(H)-Phe-Val-Asn-Arg-Pro-Arg-Arg-NH_2	0.16 ± 0.04	8.62 ± 0.11	agonist 4.1 ± 0.2 units/mg[i]		
C	t-Baa-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH_2[f]	0.71 ± 0.10	7.99 ± 0.06	0.83 ± 0.07	7.91 ± 0.04	1.2

[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 = antiantidiuretic ED/antivasopressor ED. [e] Cyclic V_2/V_1 antagonist parent, d$(CH_2)_5$D-Tyr(Et)VAVP[2]. [f] Data from Manning et al.[6] [g] This antagonist had a very slow onset and long duration of action which made more precise estimates of V_2-antagonistic potency impractical. [h] These peptides showed weak agonism as well as antagonism on these assays. Only rough estimates of their EDs and pA_2s are shown. [i] USP units. For comparison, AVP has about 400 units of antidiuretic activity per mg.

AVP].[4] However its linear analogue Aaa-Tyr(Me)-Phe-Gln-Asn-Abu-Pro-Arg-Gly-NH_2 was surprisingly found to retain only about 1% of the V_1 antagonism of its cyclic parent.[5] This rather disappointing finding further increased our doubts about the possibility of obtaining a linear analogue that would exhibit the same degree of V_1-antagonistic potency and receptor selectivity as do many cyclic AVP V_1 antagonists. However, findings from a parallel[6] and a subsequent preliminary study[5] on the effects of a variety of structural modifications of the aforementioned V_2/V_1 antagonist (A) at positions 1, 6, and 9 partially allayed these fears and also suggested an alternative approach. In these studies[5,6] we found that a number of modifications of A alone[6] and in combination[5] resulted in analogues that exhibited somewhat enhanced V_1 antagonism relative to V_2 antagonism.[5,6] The most promising modifications of A included the following: replacement of the Aaa[1] by either phenylacetic acid (Phaa[1])[6] or *tert*-butylacetic acid (tBaa[1]);[6] an Arg[6]/Abu[6] or a Lys[6]/Abu[6] interchange[5] and deletion of the C-terminal Gly (desGly[9])[6,7] or the C-terminal arginine (desArg[9]).[6,7] We wondered whether additional combinations of these or related modifications in the linear V_2/V_1 antagonist A would lead to more significant gains in anti-V_1/anti-V_2 potency and selectivity. When our initial results showed promise,[5] we expanded the study to include the following additional modifications in A: D-Tyr[2], D-Tyr(Me)[2], Gln[4], Orn[6], and MeAla[7] substitutions. Utilizing various combinations of all of these modifications, i.e., Phaa[1], t-Baa[1], D-Tyr(R)[2] (where R = H, Me), Gln[4], Arg[6], Lys[6], Orn[6], and desArg[9], gave the two series of analogues reported here: series 1, a nonapeptide series based on A; series 2, an octapeptide series based on its desArg[9] analogue D. A and D are referred to as a nonapeptide and an octapeptide, respectively, to correlate with the number of positions that can be substituted in each one.

Series 1. Analogues of Aaa[1]-D-Tyr(Et)[2]-Phe[3]-Val[4]-Asn[5]-Abu[6]-Pro[7]-Arg[8]-Arg[9]-NH_2 (A). We report here nine analogues (**1–9**) of the nonapeptide A. Analogues **1–9** possess the following single or multiple modifications at positions 1, 2, and 6: (**1**) Arg[6]; (**2**) Lys[6]; (**3**) Orn[6]; (**4**) Phaa[1], Lys[6]; (**5**) Phaa[1], Orn[6]; (**6**) D-Tyr[2]; (**7**) D-Tyr[2], Arg[6]; (**8**) Phaa[1], D-Tyr[2]; (**9**) Phaa[1], D-Tyr[2], Arg[6].

Series 2. Analogues of Aaa[1]-D-Tyr(Et)[2]-Phe[3]-Val[4]-Asn[5]-Abu[6]-Pro[7]-Arg[8]-NH_2 (D). We report here 12 analogues (**10–21**) of the octapeptide D. Analogues **10–21** possess the following single or multiple modifications at positions 1, 2, 4, 6, and 7; (**10**) Arg[6]; (**11**) Lys[6]; (**12**) Orn[6]; (**13**) Phaa[1], Lys[6]; (**14**) Phaa[1], Gln[4], Lys[6]; (**15**) Phaa[1], D-Tyr(Me)[2], Lys[6]; (**16**) Phaa[1], D-Tyr(Me)[2], Gln[4], Lys[6]; (**17**) Phaa[1], D-Tyr[2], Gln[4], Lys[6]; (**18**) t-Baa[1], Lys[6]; (**19**) t-Baa[1], Gln[4], Lys[6]; (**20**) Arg[6], MeAla[7]; (**21**) t-Baa[1], Arg[6], MeAla[7]. The structures of the 21 new nonapeptide and octapeptide antagonists are given in Tables I and II. Preliminary data on the properties of **13** and **18** have been reported.[3,5]

Peptide Synthesis. Starting from Boc-Arg(Tos)-resin we synthesized the protected precursors (I–XXI) of the free peptides (**1–21**) either entirely by the solid-phase method[8,9] (peptides III–XXI) or by a combination of solid-phase and solution methods of peptide synthesis (peptides I and II) by 8 + 1 couplings using previously described procedures.[6,10] Both approaches work equally

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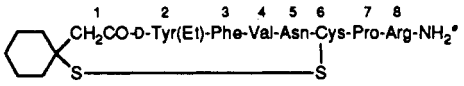
Table II. Structures and Antagonistic Properties of Potent and Selective Linear Octapeptide V_1 Antagonistic Analogues of D

	antagonist	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) ratio[d]
		0.45 ± 0.04[c]	8.17 ± 0.04	1.5 ± 0.3	7.69 ± 0.08	3.3
D	Aaa-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-NH_2[f]	2.6 ± 0.3	7.43 ± 0.06	4.2 ± 0.4	7.21 ± 0.04	1.6
10	Aaa-D-Tyr(Et)-Phe-Val-Asn-Arg-Pro-Arg-NH_2	0.66 ± 0.07	8.01 ± 0.05	1.4 ± 0.3	7.73 ± 0.08	2.1
11	Aaa-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Arg-NH_2	0.66 ± 0.09	8.02 ± 0.06	1.1 ± 0.2	7.81 ± 0.08	1.7
12	Aaa-D-Tyr(Et)-Phe-Val-Asn-Orn-Pro-Arg-NH_2	3.2 ± 0.5	7.33 ± 0.06	1.1 ± 0.3	7.81 ± 0.10	0.3
13	Phaa-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Arg-NH_2[g]	0.11 ± 0.01	8.81 ± 0.06	2.9 ± 0.2	7.36 ± 0.04	26
14	Phaa-D-Tyr(Et)-Phe-Gln-Asn-Lys-Pro-Arg-NH_2	0.070 ± 0.017	9.05 ± 0.09	~40[h]	~6.3[h]	~570
15	Phaa-D-Tyr(Me)-Phe-Val-Asn-Lys-Pro-Arg-NH_2	0.17 ± 0.01	8.61 ± 0.03	~6[h]	~7.0[h]	~35
16	Phaa-D-Tyr(Me)-Phe-Gln-Asn-Lys-Pro-Arg-NH_2	0.083 ± 0.008	8.93 ± 0.05	>24[h]	<6.5[h]	>290
17	Phaa-D-Tyr-(H)-Phe-Gln-Asn-Lys-Pro-Arg-NH_2	0.085 ± 0.007	8.91 ± 0.04	~5[h]	~7.2[h]	~59
18	t-Baa-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Arg-NH_2[g]	0.17 ± 0.03	8.63 ± 0.10	>7[h]	~7.0[h]	~41
19	t-Baa-D-Tyr(Et)-Phe-Gln-Asn-Lys-Pro-Arg-NH_2	0.27 ± 0.03	8.40 ± 0.05	>410[h]	~5.2[h]	>1500
20	Aaa-D-Tyr(Et)-Phe-Val-Asn-Arg-MeAla-Arg-NH_2	0.37 ± 0.06	8.28 ± 0.08	1.2 ± 0.2	7.77 ± 0.06	3.2
21	t-Baa-D-Tyr(Et)-Phe-Val-Asn-Arg-MeAla-Arg-NH_2	0.30 ± 0.05	8.34 ± 0.06	~16[h]	~6.60[h]	~53

[a-d] See corresponding footnotes to Table I. [e] Cyclic V_2/V_1 antagonist parent, desGly-d$(CH_2)_5$D-Tyr(Et)VAVP.[7,15] [f] Data from Manning et al.[6] [g] Preliminary data reported in Manning et al.[3] and Manning et al.[5] [h] These peptides showed weak agonism as well as antagonism on these assays. Only rough estimates of their EDs and pA_2s are shown.

well in our hands.[6] The 8 + 1 approach was utilized simply for convenience at the time peptides I and II were being synthesized. HCl (1 M)/AcOH was used in all the deprotection steps except those involving Boc-Gln in which TFA was employed.[11] Neutralizations were carried out with 10% Et_3N/CH_2Cl_2. In the solid-phase method of peptide synthesis we introduced the following modifications of the DCC[12]/HOBt[13] coupling procedure. The protected amino acids (except asparagine and glutamine) were coupled by the DCC/HOBt procedure in CH_2Cl_2/DMF (9:1, v/v) using a 3-fold excess of the Boc-amino acids, 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 reactions were completed in 3 h, and usually one coupling was sufficient. The couplings of Boc-Asn-ONp and Boc-Gln-ONp and all other procedures were performed by methods already described.[10,11,14] Cleavage from the resin was either by ammonolysis[11,14c] in methanol to give the protected peptide amides or by HBr/TFA to give the protected precursors Ia and IIa required[6,8b,15,16] for the 8 + 1 couplings in solution. Na in liquid NH_3[17] was used to deblock each protected precursor as previously described.[3,5,6] The free peptides were desalted and purified by gel filtration on Sephadex G-15 in a two-step procedure using 50% and 2 M AcOH as eluents, respectively, as previously described.[6,18] It should be noted that although many of these peptides contain sequences that have been reported to be sensitive to Na in liquid NH_3,[9] i.e., Arg–Pro and Lys–Pro bonds,[9] under the deblocking conditions used here and elsewhere,[6] we found no evidence for cleavage of any of these peptides.

Bioassay Methods. Assays for vasopressor V_1 antagonistic activities were performed by following blood pressure responses in rats anesthetized with urethane and pretreated with phenoxybenzamine.[19] Assays for antidiuretic V_2 agonism or antagonism were done by measuring changes in urine flow in water-loaded rats under ethanol anesthesia.[20] 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 one-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.[21] 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.[22] When standard errors are presented in the tables, the means reflect results from at least four independent assay groups. 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

The antivasopressor (anti-V_1) and anti-antidiuretic (anti-V_2) potencies and the anti-V_2/anti-V_1 effective dose (ED) ratios of analogues **1–9** of the nonapeptide A and of analogues **10–21** of the octapeptide D are given in Tables I and II, respectively.

A cursory examination of the properties of the nine new analogues in Table I and of the 12 new analogues in Table II shows that striking gains in both anti-V_1 potency and in anti-V_1/anti-V_2 selectivity have been achieved with

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respect to the two parent analogues, the nonapeptide A and the octapeptide D. Thus seven of the analogues in Table I have anti-V_1 pA_2 values greater than that of A (anti-V_1 pA_2 = 7.75). Eleven of the 12 analogues in Table II have anti-V_1 pA_2 values greater than that of D (anti-V_1 pA_2 = 7.43). Also, with anti-V_1 pA_2 values of 8.6 or better, seven of these new linear analogues are as potent as the most potent cyclic V_1 antagonists reported to date.[4] These are **9** (Table I) and **13–18** (Table II). Furthermore these modifications of A and D, besides resulting in enhancements in anti-V_1 potencies, have in many instances brought about drastic reductions in anti-V_2 potencies. Thus many of these new analogues, particularly those in Table II, exhibit striking selectivity for V_1 receptors versus V_2 receptors. Peptide **9** (Table I) exhibits surprising antidiuretic agonism and is in fact the most potent linear V_2 agonist reported to date. In the interest of clarity, a detailed discussion of the effects of the various modifications of the nonapeptide analogues of A (Table I) and those of the octapeptide analogues of D (Table II) will be presented separately below.

Single Modifications of the Nonapeptide A (Table I). Effects of Basic Amino Acid Substitutions at Position 6. Replacement of the Abu[6] of A by Arg[6] and Lys[6] led to modest enhancements in both V_1 antagonism and in anti-V_1/anti-V_2 selectivity in the two resulting peptides, **1** and **2**. With anti-V_1 pA_2 values = 8.13 and 8.14, respectively, peptides **1** and **2** are over twice as potent as A which has an anti-V_1 pA_2 = 7.75. The Lys[6] substitution afforded a more significant gain in anti-V_1/anti-V_2 selectivity than the Arg[6] substitution as reflected by the effective dose (ED) ratios of **2** = 2.8 and of **1** = 1.4 (cf. ED ratio for A = 0.44). The data for peptide **3** clearly shows that the Orn[6]/Abu[6] interchange in A effected virtually no change in either anti-V_1 potency or in anti-V_1/anti-V_2 selectivity. Remarkably, both the Arg[6] and Lys[6] peptides are 3 times as potent as the Orn[6] peptide **3**, anti-V_1 pA_2 = 7.64) as V_1 antagonists. This clearly shows that both the size and the basicity of the position 6 residue are important for optimal interaction with V_1 receptors.

Effects of D-Tyr[2] Substitution. This substitution brought about losses of both V_1 and V_2 antagonism in peptide **6** compared to A. Thus at first sight the substitution of D-Tyr for D-Tyr(Et) appeared to be of little value for the design of more potent and selective linear V_1 antagonists. Yet, as will be noted below, in the discussion of peptide **9**, this initial assumption was quite misleading.

Effects of Phaa[1] and t-Baa[1] Substitutions. The properties of B and C, as previously reported,[6] show clearly that both the Phaa[1] and t-Baa[1] substitutions brought about modest enhancements in both V_1 antagonism and in anti-V_1/anti-V_2 selectivity, with the Phaa[1] substitution being superior in both instances.

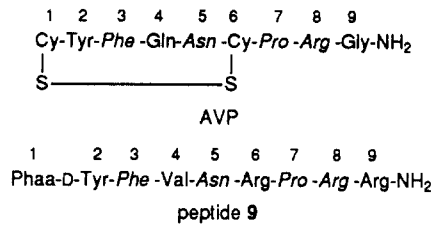
Relative Merits of Various Single Modifications in A. Comparison of the effects of these various single modifications at positions 1, 2, and 6 indicates that the Phaa[1], Lys[6], and Arg[6] single substitutions are more effective than the t-Baa[1], D-Tyr[2], and Orn[6] single substitutions in leading to both enhanced V_1 antagonism and enhanced anti-V_1/anti-V_2 selectivity.

Multiple Modifications of A. Effects of Phaa[1] + Lys[6] or Phaa[1] + Orn[6] Substitutions. Replacement of the Abu[6] residue of B, which has a Phaa[1] in place of Aaa[1], with either Lys[6] or Orn[6] led to retention of potent V_1 antagonism. Thus peptides B, **4**, and **5** have virtually identical anti-V_1 pA_2 values and are all almost twice as potent as A. Moreover peptides **4** and **5** exhibit significant reductions in anti-V_2 potencies relative to both A and B. Thus with ED ratios of ~41 and ~14, respectively, the Lys[6] and Orn[6] analogues of B are much more selective for V_1 receptors than for V_2 receptors than either A (ED ratio = 0.44) or B (ED ratio = 2.7). Thus, the combination of the Phaa[1] and Lys[6] substitutions in particular (peptide **4**), brought about a highly significant gain in anti-V_1/anti-V_2 selectivity compared to A (ED ratio = 0.44).

Effects of D-Tyr[2] + Arg[6] Substitutions. With an anti-V_1 pA_2 of 8.22 and an ED ratio of 4.3, peptide **7** is clearly more potent and selective than both A and peptide **6**. The latter has an anti-V_1 pA_2 of 7.37 and an ED ratio of 0.7. Peptides **7** and **6** differ only by an Arg[6]/Abu[6] interchange. These findings thus further illustrate the usefulness of the Arg[6]/Abu[6] interchange for enhancing anti-V_1 potency and selectivity.

Effects of Phaa[1] + D-Tyr[2] Substitutions. The combination of Phaa[1] + D-Tyr[2] to give peptide **8** (anti-V_1 pA_2 = 7.54: ED ratio = ~9) resulted in a slight reduction in anti-V_1 potency and a gain in anti-V_1/anti-V_2 selectivity compared to A (anti-V_1 pA_2 = 7.75; ED ratio = 0.44). However, as noted below, the replacement of the Abu[6] in **8** by Arg[6] resulted in a peptide, **9**, which has exceptional properties.

Linear V_2 Agonist/V_1 Antagonist. Peptide **9**, i.e., the Phaa[1], D-Tyr[2], Arg[6] analogue of A, exhibits unusual properties for a linear vasopressin molecule. It exhibits surprisingly high antidiuretic agonism. With a V_2 activity of 4.1 units/mg, it is clearly the most potent linear V_2 agonist reported to date. It is also the most potent and selective V_1 antagonist in Table I. With an anti-V_1 pA_2 = 8.62 it is equipotent with one of our most potent cyclic V_1 antagonists, d(CH_2)$_5$Tyr(Me)AVP (anti V_1 pA_2 = 8.61).[4] Comparison of the structures of AVP and of peptide **9** shows clearly how surprising these findings are. Thus AVP and peptide **9** have the following structures:



Thus peptide **9**, besides lacking the ring structure of AVP, differs from AVP at five positions (1, 2, 4, 6, and 9) and retains less than half of the original sequence of AVP, i.e., residues 3, 5, 7, and 8. Its structure bears only a tenuous relationship to that of AVP. The V_2 agonism of peptide **9** demonstrates very clearly that a ring structure is not a prerequisite for binding to or for activating V_2 receptors. This is in sharp contrast to the structural requirements for activation of V_1 receptors. Thus, while a ring structure has been shown not to be a requirement for binding to V_1 receptors, to date no linear molecule has been shown to exhibit V_1 agonism. These findings offer promising clues for the design of more potent linear V_2 agonists. These findings further illustrate the need for caution in evaluating the usefulness of any given modification on the basis of data from a single analogue. As noted above, the D-Tyr[2] substitution appeared to have little merit on the basis of the properties of the D-Tyr[2] analogue of A, i.e., peptide **6**. Yet when combined with the Phaa[1] and Arg[6] substitutions to give peptide **9**, it resulted in one of the most potent linear V_1 antagonists and the most potent linear V_2 agonist reported to date.

Relative Contributions of Different Modifications of the Nonapeptide A in Enhancing V_1 Antagonism and Selectivity. It is clear from the data presented in

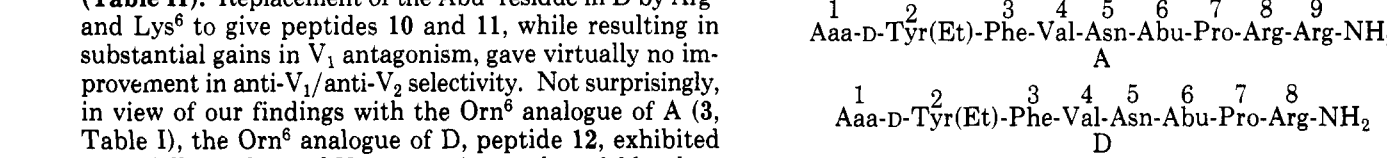
Table I that the basic substituents Lys or Arg are far superior to Abu at position 6 in leading to enhanced anti-V_1/anti-V_2 selectivity. A Lys[6] substituent appears to be more effective than an Arg[6] substituent in enhancing anti-V_1 selectivity. However, many more analogues need to be looked at before we can be certain of this. Both the Lys[6] and Arg[6] substitutions in A are clearly more effective than an Orn[6] substitution in enhancing V_1 antagonism and selectivity but Lys[6] and Orn[6] analogues of B, **4** and **5**, differed little in potency from B or each other. Also, peptides resulting from some combinations of two modifications of A, most notably peptide **4**, which contains the Phaa[1], Lys[6] modifications, resulted in a significant gain in anti-V_1/anti-V_2 selectivity compared to A. Remarkably, the combination of the three changes, Phaa[1], D-Tyr[2], Arg[6], to give peptide **9**, resulted in the most potent linear V_1 antagonist of the series. Peptide **9** is the first linear molecule to exhibit substantial V_2 agonism and consequently is a highly selective V_1 antagonist with an infinite ED ratio.

Modifications of the Octapeptide D (Table II). Effects of Single Substitutions at Positions 6 and 7 (Table II). Replacement of the Abu[6] residue in D by Arg[6] and Lys[6] to give peptides **10** and **11**, while resulting in substantial gains in V_1 antagonism, gave virtually no improvement in anti-V_1/anti-V_2 selectivity. Not surprisingly, in view of our findings with the Orn[6] analogue of A (**3**, Table I), the Orn[6] analogue of D, peptide **12**, exhibited essentially unchanged V_1 antagonism and a 5-fold reduction in anti-V_1/anti-V_2 selectivity compared to D. Analogue **20**, which differs from peptide **10** in having a MeAla/Pro interchange at position 7 exhibits a modest gain in V_1 antagonism (pA_2 = 8.28) and in anti-V_1/anti-V_2 selectivity (ED ratio = 3.2) relative to peptide **10**. Thus it is clear that while the single modifications of D at positions 6 and 7 shown here lead to fairly effective V_1 linear antagonists, they effect only very modest improvements in selectivity for V_1 versus V_2 receptors.

Effects of Multiple Substitutions in D. When D was modified at more than one position with a variety of structural modifications, virtually all of the resulting analogues (**13–19**, **21**; Table II) exhibited striking enhancements of both V_1 antagonism and of anti-V_1/anti-V_2 selectivity. The combination of Phaa[1] or t-Baa[1] with Lys[6] resulting in peptides **13** and **18**, which have anti V_1 pA_2 values = 8.81 and 8.63, respectively. They also exhibit anti-V_1/anti-V_2 selectivities of 26 and ~41, respectively. Remarkably a Gln[4]/Val[4] interchange in peptides **13** and **18** to give peptides **14** and **19** resulted, on the one hand (peptide **14**), in a possible increase in anti-V_1 potency (pA_2 = 9.05) but, on the other (peptide **19**), in a significant decrease in anti-V_1 potency (pA_2 = 8.40). Nonetheless both peptides **14** and **19** exhibit strikingly enhanced anti-V_1/anti-V_2 selectivities of ~570 and >1500, respectively. Peptide **14** is thus the first V_1 antagonist, cyclic or linear, to have a mean V_1 pA_2 value greater than 9. It is thus the most potent and selective V_1 antagonist reported to date although its mean anti-V_1 potency is not significantly greater than those of peptides **13**, **16**, and **17**. Thus, notwithstanding the aforementioned failure[5] to obtain a linear V_1 antagonist by making a linear analogue of the Gln[4] containing potent cyclic V_1 antagonist, d$(CH_2)_5$Tyr(Me)[2]AVP[4], it is clear from the data presented in Table II that the Gln[4] substitution is very valuable for enhancing anti-V_1/anti-V_2 selectivity. Thus, the following pairs, peptides **13** and **14**, peptides **15** and **16**, and peptides **18** and **19**, differ from each other only by a Val[4]/Gln[4] interchange. Yet in each pair the Gln[4]-containing peptide is strikingly more selective for V_1 receptors than is its Val[4]-containing counterpart. It will also be noted that the combination of t-Baa[1] and MeAla[7] modifications led to a significant gain in anti-V_1/anti-V_2 selectivity of peptide **21** compared to either C or peptide **20**. Analogues **15–17**, which contain combinations of Phaa[1] with D-Tyr(Me)[2] or D-Tyr[2] with either Val[4] or Gln[4], are all highly potent and selective V_1 antagonists. They exhibit pA_2 values of 8.61, 8.93, and 8.91; and anti-V_1/anti-V_2 selectivities = ~35, >290, and ~59, respectively. Thus it is clear from the examination of the properties of the analogues in Table II that multiple substitutions in the octapeptide D have led to linear peptides that exhibit striking enhancements in V_1 antagonism and varying degrees of improved selectivity for V_1 versus V_2 receptors. Furthermore, these findings offer very promising clues to the design of even more potent and selective linear V_1 antagonists.

Conclusion

In this report we have shown that appropriate combinations of single modifications of the potent but nonselective linear V_2/V_1 antagonist A[1,6] and of its desArg[9] analogue D[6] have resulted in a number of highly potent



and highly selective antagonists of the vascular (V_1) responses to AVP. Thus the combination of the Phaa[1] and Lys[6] modifications of A to give peptide **4** (Table I) resulted in a 5-fold enhancement in V_1 antagonism and an 18-fold reduction in V_2 antagonism with respect to A. This represents a 90-fold gain in selectivity for V_1 receptors over V_2 receptors for peptide **4** compared to A. Remarkably, the combination of Phaa[1], D-Tyr[2], and Arg[6] in A to give peptide **9** resulted in a most surprising spectrum of activities. With an anti-V_1 pA_2 = 8.62, peptide **9** is clearly the most potent and selective of the nine new linear V_1/V_2 nonapeptide antagonists (Table I) and is in fact equipotent with one of our most potent cyclic V_1 antagonists, d-$(CH_2)_5$Tyr(Me)AVP.[4] With V_2 agonistic activity of 4 units/mg this trisubstituted analogue of A is also the most potent linear V_2 agonist reported to date. This later finding shows clearly that a ring structure is not required for the activation of V_2 receptors and offers very promising clues for the design of even more potent linear V_2 agonists. While multiple modifications of the nonapeptide A gave promising gains in both V_1 antagonism and in anti-V_1/anti-V_2 selectivity, it is clear from Table II that similar modifications in the octapeptide D, i.e., the desArg[9] analogue of A, resulted in much more promising gains in both anti-V_1 potency and in anti-V_1/anti-V_2 selectivity. Six of the peptides in Table II (peptides **13–18**) have anti-V_1 pA_2 values greater than 8.6. Thus they are all as potent as or more potent than d$(CH_2)_5$Tyr(Me)AVP.[4] In fact, one of these, peptide **14**, the [Phaa[1],Gln[4],Lys[6]] analogue of D, with an anti-V_1 pA_2 = 9.05, is the first AVP antagonist, cyclic or linear, to possess a mean pA_2 value greater than 9. Seven peptides (**13–19**) exhibit striking gains in anti-V_1/anti-V_2 selectivity compared to D. The studies reported here offer convincing evidence of the usefulness of the additivity approach for the design of potent and selective linear AVP V_1 antagonists and, in addition, offer promising clues for the design of even more potent and more selective linear V_1 antagonists. Finally, many of the linear V_1 antagonists reported here could be of value as pharmacological tools for studies on the role(s) of AVP

Table III. Physicochemical Properties of the Protected Peptides I–XXI[a]

no.	formula	yield,[b] %	mp, °C	$[\alpha]^{25}_D$, deg (c = 1, DMF)	TLC, R_f A	B	D	E
I	$C_{87}H_{117}N_{19}O_{17}S_3$	59	178–181	−13.8		0.59	0.67	0.81
II	$C_{85}H_{117}N_{17}O_{17}S_3$	88	192–196	−11.8		0.65	0.73	0.96
III	$C_{84}H_{115}N_{15}O_{17}S_3$	51	204–207	−14.0	0.75	0.75	0.80	0.86
IV	$C_{81}H_{107}N_{17}O_{17}S_3$	21	211–215	−20.0	0.73	0.63		0.89
V	$C_{80}H_{105}N_{15}O_{17}S_3$	41	221–222	−18.5	0.72	0.70	0.79	0.82
VI	$C_{81}H_{108}N_{16}O_{15}S_2$	45	200–203	−18.6	0.62	0.58		0.91
VII	$C_{90}H_{119}N_{19}O_{17}S_3$	59	173–176	−15.5	0.29	0.27		0.65
VIII	$C_{77}H_{98}N_{16}O_{15}S_2$	72	233–234	−25.1	0.54	0.56		0.81
IX	$C_{86}H_{109}N_{19}O_{17}S_3$	25	206–209	−18.1	0.27	0.25		0.61
X	$C_{72}H_{99}N_{15}O_{14}S_2$	47	216–219	−15.5		0.56	0.70	0.65
XI	$C_{72}H_{99}N_{13}O_{14}S_2$	30	230–240	−19.8		0.57	0.70	0.71
XII	$C_{71}H_{97}N_{13}O_{14}S_2$	58	234–237	−19.3		0.55	0.71	0.74
XIII	$C_{68}H_{89}N_{13}O_{14}S_2$	54	227–231	−24.8		0.65	0.73	0.98
XIV	$C_{68}H_{88}N_{14}O_{15}S_2$	65	194–196	−30.9	0.63	0.64		0.54
XV	$C_{67}H_{87}N_{14}O_{14}S_2$	30	248–251	−24.6	0.62	0.60		0.78
XVI	$C_{67}H_{86}N_{14}O_{15}S_2$	64	204–206	−24.1	0.44	0.45		0.51
XVII	$C_{73}H_{90}N_{14}O_{15}S_2$	75	197–199	−25.3	0.57	0.59		0.53
XVIII	$C_{66}H_{93}N_{13}O_{14}S_2$	26	232–235	−24.4		0.64	0.73	0.92
XIX	$C_{66}H_{92}N_{14}O_{15}S_2$	42	175–177	−30.3	0.53	0.55		0.55
XX	$C_{71}H_{99}N_{15}O_{14}S_2$	50	194–196	−16.1		0.61	0.75	0.86
XXI	$C_{65}H_{93}N_{15}O_{14}S_2$	44	203–208	−19.0		0.54	0.69	0.72

[a] Structures of the protected peptides are given in the Experimental Section. [b] Yields were calculated on the basis of the arginine content of the starting resin for III–XXI and on the 8 + 1 coupling yield for I and II.

under physiological and pathophysiological conditions.

Experimental Section

Amino acid derivatives were purchased from Bachem Inc., 1-adamantaneacetic acid, phenylacetic acid, and *tert*-butylacetic acid were supplied by Aldrich Chemical Co., Inc. Dimethylformamide (DMF), anhydrous (99+%), Aldrich Chemical Co. Inc., was used for couplings. Other solvents and reagents were analytical grade. Boc-D-Tyr(Me) and Boc-D-Tyr(Et) were synthesized by previously published procedures.[23] The protected precursors required for the synthesis of 21 of the analogues reported here (compounds Ia, IIa below, and III–XXI, Table III) were synthesized by the manual solid-phase method[8,9] as described below using the following modification. The protected amino acids (except asparagine and glutamine) were coupled by the DCC/HOBt procedure[12,13] in CH_2Cl_2/DMF (9:1, v/v) using a 3-fold excess of the Boc-amino acids, 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 reactions were completed in 3 h as determined by the Kaiser test,[24] and usually one coupling was sufficient. The couplings of Boc-Asn-ONp and Boc-Gln-ONp, ammonolysis of the protected peptides (III–XXI) from the resin, and their purification were performed by methods already described.[6,10,11] The protected intermediates I and II were obtained by an 8 + 1 approach, using 1-adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Arg(Tos)-Pro-Arg(Tos)-OH (Ia) or 1-adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys(Tos)-Pro-Arg(Tos)-OH (IIa) and Arg(Tos)-NH_2, as previously described.[6,10] The syntheses of Ia and IIa is described below. The physicochemical properties of the 21 purified protected peptides (I–XXI) are given in Table III. All 21 protected precursors were converted to the required free linear peptides by deblocking with Na/liquid NH_3,[17] desalting, and purification in a two-step procedure, using gel filtration on Sephadex G-15 as previously described.[2,4,6,10,22] The physicochemical properties of the free peptides (1–21) are given in Table IV. Thin-layer chromatography (TLC) was performed on silica gel 60 precoated plates (0.25 mm, Merck). The following solvent systems were used: (A) butan-1-ol–acetic acid–water (4:1:5, v/v/v, upper phase), (B) butan-1-ol–acetic acid–water (4:1:1, v/v/v), (C) butan-1-ol–acetic acid–water (2:1:1, v/v/v), (D) butan-1-ol–acetic acid–water–pyridine (15:3:3:10, v/v/v/v), (E) chloroform–methanol (7:3, v/v), (F) butan-1-ol–acetic acid–water–ethyl acetate (1:1:1:1, v/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.[9] Optical rotations were measured with a Rudolph Autopol III polarimeter. For amino acid analysis,[25] peptides (approximately 0.5 mg) were hydrolyzed with constant boiling hydrochloric acid (500 μL) containing 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 = 1.00. All peptides gave the expected amino acid ratios ± 3%. Melting points of the protected peptides are uncorrected.

Table IV. Physicochemical Properties of the Free Peptides 1–21[a]

no.	yield, %[b,c]	$[\alpha]^{25}_D$, deg (c = 0.1, 50% ACOH)	TLC A	B	C	D	F
1	60	−60		0.10		0.20	
2	38	−53		0.04		0.24	
3	68	−69	0.15	0.05	0.60	0.25	0.62
4	48	−57	0.20	0.17		0.15	0.55
5	52	−70	0.13	0.04	0.54	0.22	0.60
6	55	−60	0.26	0.30	0.41		
7	58	−56			0.42	0.19	
8	57	−66	0.21	0.21	0.53		
9	69	−49			0.42	0.09	
10	55	−45		0.18		0.46	
11	52	−54		0.04		0.27	
12	85	−33		0.14		0.37	
13	43	−52		0.12		0.33	
14	47	−48	0.14	0.13	0.23		
15	68	−39	0.23	0.17	0.43		
16	50	−46	0.22	0.16	0.58		
17	70	−42	0.25	0.11	0.55		
18	58	−45		0.13		0.31	
19	39	−35	0.12	0.14	0.24		
20	62	−38		0.22		0.49	
21	15	−42		0.16		0.37	

[a] Structures of the free peptides are given in Tables I and II. [b] Yields are based on the amount of protected peptide used in the reduction step in each case and are uncorrected for acetic acid and water content. [c] all the free peptides gave the expected amino acid analysis ratios after hydrolysis ± 3%.

Solid-Phase Synthesis. Chloromethylated resin (Chemical Dynamics Co., 1% cross-linked S-DVB, 200–400 mesh, 0.7–1.00 mmol/g) was esterified with Boc-Arg(Tos) to an incorporation

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of approximately 0.5 mmol/g by the cesium salt method.[26] Eight (or seven) separate cycles of deprotection, neutralization, and coupling were carried out for the synthesis of the peptidyl resins. Ammonolysis[11,14c] in MeOH or acidolytic cleavage (HBr/TFA) in CH_2Cl_2[6,8-10,15,16] 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.

1-Adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Arg(Tos)-Pro-Arg(Tos)-OH (Ia). Boc-Arg(Tos)-resin (6.6 g, 1.8 mmol) was subjected to seven cycles of deprotection, neutralization, and coupling (modified as noted above) to yield the protected *N*-acylheptapeptidyl-resin (7.9 g, weight gain 1.37 g, 87.8%). The protected peptidyl-resin (7 g, 1.5 mmol) was suspended in TFA (40 mL) containing anisole (10 mL) and hydrogen bromide was bubbled through the suspension for 30 min.[6,7] The filtrate was collected and the resin was resuspended in CH_2Cl_2 (20 mL), TFA (20 mL), and anisole (5 mL). HBr bubbling was resumed for a further 20 min, whereupon the filtrate was collected and the resin was washed with CH_2Cl_2–TFA (1:1, v/v, 30 mL × 2). The filtrates and the washings were combined and evaporated on a rotary evaporator. Addition of Et_2O (ca. 250 mL) to the residual anisole solution gave a precipitate, which following 3 h at 4 °C was collected, washed with Et_2O, and dried in vacuo over P_2O_5; 1.81 g. This material was dissolved in hot (ca. 70 °C) DMF (15 mL), reprecipitated with water, collected, and dried in vacuo over P_2O_5 to give the protected acylheptapeptide Ia: yield 1.22 g (45.1%); mp 182–185 °C; $[\alpha]^{25}_D$ = −10.1° (*c* = 1, DMF); TLC 0.56 (B), 0.69 (D), 0.20 (E).

1-Adamanylacetyl-D-Tyr(Et)-Phe-Val-Asn-Arg(Tos)-Pro-Arg(Tos)-Arg(Tos)-NH_2 (I, Table III) (8 + 1 Coupling). The protected *N*-acylheptapeptide Ia (161 mg, 0.11 mmol), HOBt (33.8 mg, 0.25 mmol), and HCl × Arg(Tos)-NH_2 (181.5 mg, 0.5 mmol) were dissolved in DMF (1 mL), and Et_3N was added gradually to give a solution of pH 7–8 to moist pH paper. The mixture was cooled to −10 °C and then DCC (22.7 mg, 0.11 mmol) in 0.1 mL of DMF was added. After 3 days of stirring at room temperature, the reaction was completed (TLC monitoring). The mixture was filtered, and 5 drops of water, 5 drops of EtOH, and 2 drops of 1 N HCl were added to the filtrate, and the peptide precipitated. The solid was collected, dried over P_2O_5, reprecipitated from DMF with EtOH and Et_2O, collected, and dried in vacuo over P_2O_5 to give the required acyloctapeptide amide (116 mg, 59%) (I, Table III).

1-Adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys(Tos)-Pro-Arg(Tos)-OH (IIa). Starting from Boc-Arg(Tos)-resin (5 g, 2.25 mmol), after seven cycles of deprotection, neutralization, and couplings, 6.5 g (weight gain 1.57 g, 86%) of the protected *N*-acylheptapeptidyl-resin was prepared. *N*-Acylheptapeptidyl-resin (5.4 g, 1.8 mmol) was treated with HBr/TFA and the resulting protected peptide purified by precipitations as above for Ia to give 0.66 g of IIa (yield 25.5%): mp 198–201 °C; $[\alpha]^{25}_D$ = −13.2° (*c* = 1, DMF); TLC 0.52 (B), 0.72 (D), 0.37 (E).

1-Adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys(Tos)-Pro-Arg(Tos)-Arg(Tos)-NH_2 (II, Table III) (8 + 1 Coupling). The interaction of *N*-acylheptapeptide (IIa) (213 mg, 0.15 mmol), HOBt (40.5 mg, 0.30 mmol), HCl × Arg(Tos)-NH_2 (181.5 mg, 0.5 mmol), and DCC (31 mg, 0.15 mmol) in DMF as described above for I gave the acyloctapeptide amide (153 mg, 88%) (II, Table III).

1-Adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Orn(Tos)-Pro-Arg(Tos)-Arg(Tos)-NH_2 (III, Table III). Boc-Arg(Tos)-resin (1.33 g, 0.6 mmol) was converted to protected acyloctapeptidyl-resin by manual method of solid-phase-synthesis methodology, i.e., eight cycles of deprotection, neutralization with 10% Et_3N in CH_2Cl_2, and coupling (mediated by DCC/HOBt modified as noted above or active esters) were used to incorporate successively Boc-Arg(Tos), Boc-Pro, Boc-Orn(Tos), Boc-Asn-ONp, Boc-Val, Boc-Phe, Boc-D-Tyr(Et), and 1-adamantaneacetic acid. The deprotections were performed with 1 M HCl/AcOH in all cases. The resulting protected peptidyl resin, 1-adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Orn(Tos)-Pro-Arg(Tos)-Arg(Tos)-resin (2.95 g, 0.6 mmol), was cleaved by ammonolysis. The protected peptide was extracted with hot (ca. 70 °C) DMF (ca. 30 mL) and the product precipitated by the addition of hot (ca. 70 °C) water (ca. 500 mL). Following overnight storage at 4 °C, the product was collected, dried in vacuo over P_2O_5, precipitated 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 (III, Table III). The same procedure was used for the synthesis of peptides (IV–XXI) except that for the protected acylheptapeptides (X–XXI) the Boc-Arg(Tos) coupling cycle was omitted. Pertinent physicochemical data for all protected acylhepta- and acyloctapeptide amides are given in Table III. Their structures are as follows: (I) 1-adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Arg(Tos)-Pro-Arg(Tos)-Arg(Tos)-NH_2; (II) 1-adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys(Tos)-Pro-Arg-(Tos)-Arg(Tos)-NH_2; (III) 1-adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Orn(Tos)-Pro-Arg(Tos)-Arg(Tos)-NH_2; (IV) 1-phenylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys(Tos)-Pro-Arg(Tos)-Arg-(Tos)-NH_2; (V) 1-phenylacetyl-D-Tyr(Et)-Phe-Val-Asn-Orn-(Tos)-Pro-Arg(Tos)-Arg(Tos)-NH_2; (VI) 1-adamantylacetyl-D-Tyr-Phe-Val-Asn-Abu-Pro-Arg(Tos)-Arg(Tos)-NH_2; (VII) 1-adamantylacetyl-D-Tyr-Phe-Val-Asn-Arg(Tos)-Pro-Arg(Tos)-Arg(Tos)-NH_2; (VIII) 1-phenylacetyl-D-Tyr-Phe-Val-Asn-Abu-Pro-Arg(Tos)-Arg(Tos)-NH_2; (IX) 1-phenylacetyl-D-Tyr-Phe-Val-Asn-Arg(Tos)-Pro-Arg(Tos)-Arg(Tos)-NH_2; (X) 1-adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Arg(Tos)-Pro-Arg-(Tos)-NH_2; (XI) 1-adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys(Tos)-Pro-Arg(Tos)-NH_2; (XII) 1-adamantylacetyl-D-Tyr-(Et)-Phe-Val-Asn-Orn(Tos)-Pro-Arg(Tos)-NH_2; (XIII) 1-phenylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys(Tos)-Pro-Arg(Tos)-NH_2; (XIV) 1-phenylacetyl-D-Tyr(Et)-Phe-Gln-Asn-Lys(Tos)-Pro-Arg(Tos)-NH_2; (XV) 1-phenylacetyl-D-Tyr(Me)-Phe-Val-Asn-Lys(Tos)-Pro-Arg(Tos)-NH_2; (XVI) 1-phenylacetyl-D-Tyr-(Me)-Phe-Gln-Asn-Lys(Tos)-Pro-Arg(Tos)-NH_2; (XVII) 1-phenylacetyl-D-Tyr-Phe-Gln-Asn-Lys(Tos)-Pro-Arg(Tos)-NH_2; (XVIII) 1-*tert*-butylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys(Tos)-Pro-Arg(Tos)-NH_2; (XIX) 1-*tert*-butylacetyl-D-Tyr(Et)-Phe-Gln-Asn-Lys(Tos)-Pro-Arg(Tos)-NH_2; (XX) 1-adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Arg(Tos)-MeAla-Arg(Tos)-NH_2; (XXI) 1-*tert*-butylacetyl-D-Tyr(Et)-Phe-Val-Asn-Arg(Tos)-MeAla-Arg(Tos)-NH_2.

1-Adamantylacetyl-D-Tyr(Et)-Phe-Val-Asn-Arg-Pro-Arg-Arg-NH_2 (1, Table IV). A stirred solution of the protected acylheptapeptide amide I (Table III) (70 mg, 0.04 mmol) in sodium-dried ammonia (ca. 300 mL) was treated at the boiling point with sodium 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. NH_4Cl (ca. 0.1 g) was added to discharge the color. The ammonia was evaporated and air was passed through the flask. After 5 min, the residue was dissolved in 50% acetic acid (ca. 10 mL) and the resulting solution 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 eluate was fractioned and monitored for absorbance at 254 nm. The fractions comprising the major peak were checked by TLC (A, B), 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 (2 N) with a flow rate of 4 mL/h. The peptide was eluted in a single peak (absorbance at 254 nm). Lyophilization of the pertinent fractions gave the linear analogue 1 (31.2 mg) (Table IV). With minor modifications this procedure was utilized to give all the remaining free peptides (2–21). The physicochemical properties of all 21 free peptides are given in Table IV.

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Registry No. 1, 129520-56-5; 2, 129520-57-6; 3, 129520-58-7; 4, 129520-59-8; 5, 129520-60-1; 6, 129493-15-8; 7, 129520-61-2; 8, 129520-62-3; 9, 129520-63-4; 10, 121199-52-8; 11, 121199-51-7; 12, 121199-50-6; 13, 129520-64-5; 14, 129520-65-6; 15, 129520-66-7; 16, 129520-67-8; 17, 129520-68-9; 18, 129520-69-0; 19, 129520-70-3;

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Synthesis and Structure–Activity Relationships of New 9-*N*-Alkyl Derivatives of 9(*S*)-Erythromycylamine[1]

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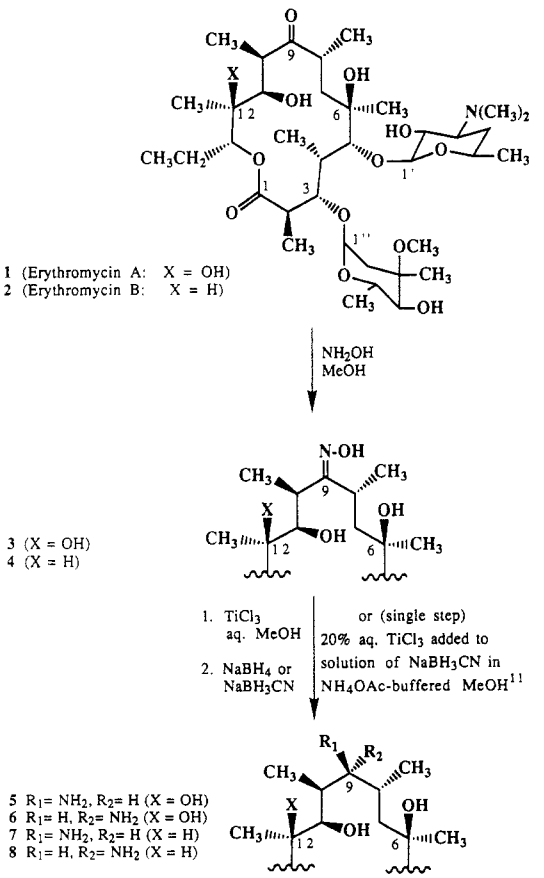
A series of new 9-*N*-alkyl derivatives of 9(*S*)-erythromycylamine has been synthesized by reductive alkylation of erythromycylamine with aliphatic aldehydes and sodium cyanoborohydride. Alternative syntheses employing hydrogenation methods have also been developed. These new 9-*N*-alkyl derivatives possess excellent antimicrobial activity in vitro and in vivo, especially when administered orally to treat experimental infections in mice. From structure–activity studies, 9-*N*-(1-propyl)erythromycylamine (LY281389) was selected as the most efficacious derivative. These methods have also been extended to the synthesis of some 9-*N*,*N*-dialkyl derivatives of erythromycylamine.

Introduction

9(*S*)-Erythromycylamine is a well-known semisynthetic derivative of erythromycin; it possesses excellent antimicrobial activity but is poorly absorbed after oral administration to humans.[2,3] Several approaches to improving the oral bioavailability of erythromycylamine have been investigated. Ketones and aromatic aldehydes have been condensed with erythromycylamine, yielding Schiff bases which can hydrolyze back to erythromycylamine;[3,4] however, these derivatives failed to increase serum concentrations of antibiotic after oral administration to humans despite good oral absorption in dogs.[3] In contrast to ketones and aromatic aldehydes, aliphatic aldehydes have been condensed with erythromycylamine to produce 9-*N*,11-*O*-oxazine derivatives.[3] Dirithromycin is a relatively new member of this oxazine class;[5] it produces high concentrations of antibiotic in tissues after oral administration and is currently being evaluated in clinical trials.[6,7]

We have recently reported that reductive amination of certain tylosin-related macrolides with dialkylamines produced a new series of antibiotics possessing an expanded spectrum of antimicrobial activity and good oral bioavailability.[8,9] In order to explore a similar approach

Scheme I. Synthesis of 9(*S*)-Erythromycylamine (5) and Related Compounds



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