were placed in Tyrode's buffer, dissected free of larger bronchioles and blood vessels, and then chopped with scissors into 25–75-mg fragments. The fragments were washed and then stored overnight in Tyrode's buffer at room temperature. Before use the next day, the tissue was again washed with buffer. Portions of lung tissue (about 400 mg) were placed in each of a series of vials containing buffer at 37 °C. After a 10-min incubation in Tyrode's buffer, test drug or vehicle was added, and 10 min later, the tissue was incubated with anti-IgE in a final dilution of 3:1000. After another 30-min incubation, samples of the supernatant were removed for assay. Histamine was assayed as described above in the guinea pig chopped lung test.

The Role of Position 4 in Angiotensin II Antagonism: A Structure–Activity Study†

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A number of [Sar^1,(pX)Phe^4]-ANG II and [Sar^1,(pX)Phe^4,Ile^8]-ANG II analogues were prepared. A good correlation between pX structure in [Sar^1,(pX)Phe^4]-ANG II and antagonist activity could not be found. However, the data suggest a general trend: Position 4 para substituents that are hydrophilic and capable of donating a hydrogen atom in a hydrogen bond promote agonist activity, while para substituents that are hydrophobic and incapable of donating a hydrogen atom promote antagonist activity. These properties were found to be optimal in the *p*-chloro substituent. The resulting analogue [Sar^1,(pCl)Phe^4]-ANG II is a potent ANG II antagonist in vivo. The pX substituents that promote antagonist activity in the [Sar^1,(pX)Phe^4]-ANG II series were unfavorable in [Sar^1,(pX)Phe^4,Ile^8]-ANG II analogues. ANG II analogues that are antagonists by virtue of an alteration in position 8 require a position 4 agonist side chain. Concurrent modifications of positions 4 and 8 do not give rise to potent antagonists with reduced partial agonist activity.

Among the first reported[1] antagonists of angiotensin II was the analogue [Phe^4,Tyr^8]-ANG II **3**[2,3] (Table I)[1,3-10] which contained an alteration of the tyrosine in position 4. Other weak antagonists were reported shortly after: [(pF)Phe^4-ANG II **4**[7] and [Phe^4]-ANG II **1**[4] (Table I). In that same period antagonists of much greater potency were discovered by alteration of the 8-position: [Ala^8]-ANG II **9**,[9] [Leu^8]-ANG II **11**,[11] [Ile^8]-ANG II **12**,[3] and [Cys^8]-ANG II **10**.[3]

Substitution of sarcosine for aspartic acid in position 1 was later shown to enhance antagonist action by blocking aminopeptidase action and increasing antagonist affinity[12] as shown in the potent antagonists [Sar^1,Ala^8]-ANG II **13**[9] and [Sar^1,Ile^8]-ANG II **14**.[12] Although the development of [Sar^1,X^8]-ANG II antagonists continued,[13] investigations of [Sar^1,X^4]-ANG II antagonists were not reported until recently. This may have been due to the lower potency of the [X^4]-ANG II antagonists compared to the [X^8]-ANG II antagonists (Table I).

[Sar^1,(SAcm)Phe^4]-ANG II **5** (Table I) was recently described to be a potent ANG II antagonist by Escher et al.[14] and [Sar^1,(OMe)Tyr^4]-ANG II **6**, also a potent antagonist, Table I, was recetly reported by Goghari et al.[6] In previous work[5,14] Escher has shown that hydrogen bonding and the inductive effects (electronegativity) of the para substituent are important for optimal agonist activity of position 4 analogues of [Sar^1]-ANG II. We have been pursuing the structure–antagonist activity relationship of [Sar^1,X^4]-ANG II antagonists. This paper describes our attempt to optimize the activity of position 4 antagonist analogues.

The partial agonist activity of [Sar^1,Ala^8]-ANG II **13** and other position 8 modified antagonists has precluded their use as antihypertensive agents[13] in humans. Structural modifications that could reduce such partial agonist activity could improve the therapeutic potential of ANG II antagonists. Since antagonist action may be obtained by modification of either position 4 or position 8, both residues must be responsible for receptor stimulation. Hence, modifications of both positions 4 and 8 in the same peptide may result in the creation of an ANG II antagonist devoid of agonist activity. This possibility was explored in the present study.

† The abbreviations for natural amino acids and nomenclature for peptide structures follow the recommendations for peptide structures of the IUPAC–IUB commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 977). Abbreviations for nonnative amino acids include Bph = *p*-(dihydroxyboryl)-phenylalanine, (OMe)Tyr = *O*-methyltyrosine, (pF)Phe = *p*-fluorophenylalanine, (SAcm)Phe = *p*-[(acetamidomethyl)thio]-phenylalanine. Other abbreviations in this paper include TEA = triethylamine, TFA = trifluoroacetic acid, DCC = *N,N'*-dicyclohexylcarbodiimide.

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Table I. Activities of [Y⁴]-ANG II Analogues in the Literature

	-X-Arg-Val-Y-Ile-His-Pro-Z- (1 2 3 4 5 6 7 8)			agonist		antagonist	
no.	X¹	Y⁴	Z⁸	in vitro[a] ANG-II-like[c]	in vivo[b] ANG-II-like[d]	in vitro[a] pA_2	in vivo[b] ID_{50}[e]
1[f]	Asp	Phe	Phe	11.0	20.0		(+)[f]
2[g]	Sar	Phe	Phe	22		7.9 (Ru)	
3[i]	Asp	Phe	Tyr			6.62 (RU)[h]	
4[i]	Asp	(pF)Phe	Phe			6.30 (RU)[h]	
5[j]	Sar	(SAcm)Phe	Phe	9.0		8.09	
6[g]	Sar	(OMe)Tyr	Phe	<0.1 (RU)		7.5 (RU)	
7[g]	Sar	(OMe)Tyr	Ile	<0.1 (RU)		6.6 (RU)	
8[g]	Sar	(OEt)Tyr	Phe	0.2 (RU)		<6.0 (RU)	
9[k]	Asp	Tyr	Ala			8.32	
10[i]	Asp	Tyr	Cys			8.22 (RU)[h]	
11[l]	Asp	Tyr	Leu	<0.1 (RS)[l]		8.78	
12[i]	Asp	Tyr	Ile			8.3 (RU)[h]	
13[m]	Sar	Tyr	Ala			8.61	
14[n]	Sar	Tyr	Ile			9.33	
15[g]	Sar	Phe	Ile	<0.1 (RU)		6.7 (RU)	

[a] Agonist, "ANG II-like" activity, and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[29] ANG II antagonists bearing sarcosine in position 1 are slowly reversible antagonists depressing both the slope and maximum of the ANG II dose–response curve in vitro at high doses but not at low doses.[29] The pA_2 values reported here for comparative purposes were determined at low doses in the range of competitive inhibition, and consequently may be overestimated.[8] [b] Residual "ANG II-like" activity and antagonist activity, ID_{50}, were measured in vivo, in the rat blood pressure assay described by Regoli et al.[12] [c] ANG II-like activity in vitro is expressed as percent activity relative to ANG II. [d] ANG II-like activity in vivo is expressed by the mmHg of blood pressure increase produced by a 1 μg bolus intravenous injection of compound. [e] ID_{50} in nanograms/rat per minute (using 250-g rats). [f] Reference 4, a slight antagonist effect was observed. [g] Reference 6. [h] For comparative purposes, these values were derived from the negative log of the K_1 reported in ref 3. (RU) = rat uterus. [i] Reference 2, 3. [j] Reference 5. [k] Reference 1, 7. [l] Reference 8. [m] Reference 9. [n] Reference 10.

Table II. Position 4 Antagonist [Sar¹,(pX)Phe⁴,Phe⁸]-ANG II Analogues

		agonist		antagonist				
no.	X⁴	in vitro[a] ANG-II-like[c]	in vivo[b] ANG-II-like[d]	in vitro[a] pA_2	in vivo[b] ID_{50}[e]	σ_p[f]	π[g]	hydrogen[h] bonding
16	OH	100	50 ± 6.5	0		−0.37	−0.67	H^+
17	NH_3^+	15[i]				−0.66	−1.23	H^+
18	$B(OH)_2$	10		0		0.12	−0.55	H^+
19	SO_2NH_2	0.9[j]		0[j]		0.57	−1.82	H^+
20	SH	0.9[j]		≪6.0[j]		0.15	0.39	H^+
5	SAcm	9.0[j]		8.09[j]		∼0.21[k]	∼0[k]	H^+
21	SO_3^-	0[j]		≪6.0[j]		0.09	−4.76 (W)	H^-
22	NO_2	(+)[l]		(+)[l]		0.78	−0.28	H^-
6	OCH_3	0	20 ± 3.7	7.1	250 ± 52	−0.27	−0.02	H^-
		<0.1 (RU)[m]		7.5 (RU)[m]				
2	H	22[m]		7.5 (RU)[m]		0	0	H^-
23	F	0	22 ± 4.2	7.5	150 ± 45	0.06	0.14	H^-
8	OEt	0.1	2.5 ± 0.75	pa[n]	150 ± 42.5	−0.24	0.38	H^-
		<0.1 (RU)[m]		<6.0 (RU)[m]				
24	Cl	0	5.5 ± 2.1	7.5	30 ± 5.6	0.23	0.71	H^-
25	I	0	80.0 ± 18	6.6	pa[n]	0.18	1.12	H^-

[a] Agonist, "ANG II-like" activity, and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[29] ANG II antagonists bearing sarcosine in position 1 are slowly reversible antagonists depressing both the slope and maximum of the ANG II dose–response curve in vitro at high doses but not at low doses.[29] The pA_2 values reported here for comparative purposes were determined at low doses in the range of competitive inhibition, and consequently may be overestimated.[8] [b] Residual "ANG II-like" activity and antagonist activity, ID_{50}, were measured in vivo, in the rat blood pressure assay described by Regoli et al.[12] [c] ANG II-like activity in vitro is expressed as percent activity relative to ANG II. [d] ANG II-like activity in vivo is expressed by the mmHg of blood pressure increase produced by a 1 μg bolus intravenous injection of compound. [e] ID_{50} on nanograms/rat per minute (using 250-g rats). [f] σ_p is a measure of inductive effects[15] on phenyl using $\sigma_p^- = e^-$, $\sigma_p^+ = e^+$. [g] π is a measure of lipophilicity (π = +).[15] [h] Hydrogen bonding: H^+ = capable of donating H bond, H^- = incapable of donating H bond. [i] Reference 14. [j] Reference 5. [k] Estimated from values in ref 15. [l] Mixed agonist–antagonist effects seen above 100 ng/mL. [m] Reference 6, (RU) = rat uterus. [n] pa = partial agonist; quantitation not possible.

Results and Discussion

Table II contains a series of analogues of [Sar¹(pX)-Phe⁴]-ANG II, **2**, **5–7**, **16–25**, rank ordered with potent agonists at the top, potent antagonists at the bottom, and analogues with mixed agonist/antagonist activities in the middle. Several of the analogues were reported previously by Escher et al.[5,14] and Goghari et al.[6] The pX substituent inductive effects,[15] σ_p, lipophilicities,[15] π, and hydrogen bonding characteristics, H^+ and H^-, are also listed in Table II. Although Escher had observed a correlation between inductive effects on the phenyl ring with agonist activities,[14] the inductive effects on the phenyl ring do not correlate with antagonist activities. No good correlation could be found between pX structure and antagonist activity that was as tight as the agonist correlation. Instead, a general trend may be discerned from the table, which offers an explanation for the activities of some of the analogues. The [Sar¹,(pX)Phe⁴]-ANG II analogues **5** and **16–22** with agonist activity tend to contain para substituents that are hydrophilic (π = −) and capable of donating a hydrogen atom in a hydrogen bond (H^+). The [Sar¹,-(pX)Phe⁴]-ANG II analogues **6**, **7**, and **21–25** with antagonist activity tend to contain para substituents that are more lipophilic (π = +) and incapable of donating a hy-

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Table III. Position 4 Antagonist [Sar[1],(pX)Phe[4],Ile[8]]-ANG II Analogues

		agonist		antagonist				
no.	X^4	in vitro[b] ANG-II-like[c]	in vivo[b] ANG-II-like[d]	in vitro[a] pA_2	in vivo[b] ID_{50}[e]	σ_p[f]	π[g]	hydrogen[h] bonding
14	OH	0	10.0 ± 1.1	9.1	10 ± 1.7	−0.37	−0.67	H^+
26	$B(OH)_2$	0	00.0	5.7	500 ± 85.3	0.12	−0.55	H^+
27	NO_2	0	5.0 ± 1.1	7.1		0.78	−0.28	H^-
15	H	0	2.7 ± 1.1	8.5	80 ± 9.8	0	0	H^-
		<0.1 (RU)[i]	6.7 (RU)[i]					
7	OCH_3	0	3.0 ± 1.1	7.1	100 ± 13.2	−0.27	−0.02	H^-
28	F	0	0.0	6.8	750 ± 230.5	0.06	0.14	H^-
29	Cl	0	2.0 ± 1.1	<6.0	500 ± 89	0.23	0.71	H^-
30	OEt	0	5.0 ± 1.2	<6.0	>1000	−0.24	0.38	H^-

[a] Agonist, "ANG II-like" activity, and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[29] ANG II antagonists bearing sarcosine in position 1 are slowly reversible antagonists depressing both the slope and maximum of the ANG II dose–response curve in vitro at high doses but not at low doses.[29] The pA_2 values reported here for comparative purposes were determined at low doses in the range of competitive inhibition, and consequently may be overestimated.[8] [b] Residual "ANG II-like" activity and antagonist activity, ID_{50}, were measured in vivo, in the rat blood pressure assay described by Regoli et al.[12] [c] ANG II-like activity in vitro is expressed as percent activity relative to ANG II. [d] ANG II-like activity in vivo is expressed by the mmHg of blood pressure increase produced by a 1 μg bolus intravenous injection of compound. [e] ID_{50} in nanograms/rat per minute (using 250-g rats). [f] σ_p is a measure of inductive effects[15] on phenyl with $\sigma_p^- = e^-$, $\sigma_p^+ = e^+$. [g] π is a measure of lipophilicity ($\pi = +$).[15] [h] Hydrogen bonding: H^+ = capable of donating H bond, H^- = incapable of donating H bond. [i] (RU) = rat uterus; ref 6.

drogen atom in a hydrogen bond (H^-).

The trend is most apparent in potent agonists and antagonists. For example, the hydroxyl group (−OH) is fairly hydrophilic and capable of donating a hydrogen atom, and the resulting analogue **16** is a potent agonist. The chloro group (−Cl) is quite lipophilic and lacks a hydrogen atom for a hydrogen bond. The corresponding analogue **24** (Table II) displays potent in vivo antagonist activity that falls within the same order of magnitude as that of [Sar[1],Ile[8]]-ANG II **14** (Table III).

The OEt, fluoro, hydrogen, and OMe groups are much less lipophilic than the chloro group, hence the corresponding analogues **7**, **23**, **2**, and **6** are weaker antagonists in vivo than the Cl analogue **24**, roughly decreasing in order of lipophilicity. Analogue **6** displays in vitro activity in the aorta (pA_2 = 7.1) that is comparable to the activity reported by Goghari et al.[6] in the rat uterus (pA_2 = 7.5, Table I).

Substituents with mixed properties give rise to [Sar[1],-(pX)Phe[4]]-ANG II analogues with mixed activities. The sulfonate group (SO_3^-) is very hydrophilic. Since it is ionic at neutral pH, it cannot donate a hydrogen atom, and the resulting analogue **21** is inactive as an agonist or antagonist. Although the nitro group is not as hydrophilic as the sulfonate group, it too cannot donate a hydrogen atom; hence the corresponding analogue **22** is a weak mixed agonist–antagonist. The (acetamidomethyl)thio group ($SCH_2NHCOCH_3$) is also paradoxical. This group is fairly lipophilic yet quite capable of donating a hydrogen atom in a hydrogen bond. Both properties contribute to analogue **5** with fairly high agonist and antagonist activities.[5]

Partial agonist activities were observed with analogues **7** and **25**, hindering quantitation. An explanation for these partial agonist activities is not available. Partial agonist activity for analogue **7** was not described in a previous report.[6] The OEt analogue **7** was more potent in vivo than the OMe analogue **6**, which would follow on the basis of lipophilicity. One might expect, therefore, that the iodo analogue **25** would be superior to the Cl analogue **24** on the basis of enhanced lipophilicity. It is clear, nevertheless, that the chlorine group is superior as a para substitution in position 4 antagonists. The decreased antagonist activity of **25** could be ascribed to greater steric volume, but the much larger SAcm analogue **5** is a stronger antagonist in vitro than **25**. Thus the invokation of size restriction as an explanation for the decreased activity of **25** does not appear to be valid. The optimization of activity with *p*-Cl may be influenced by other factors such as polarizability.

The surprisingly strong agonist activity reported[6] for analogue **2** does not fit the trend either. Goghari et al.[6] could not explain the activities of **2** by their models. It may be that a lipophilic substituent is necessary to suppress agonist activity. The abnormal behavior of analogues **2**, **7**, and **25** limit the universality of the proposed correlation. For this reason the correlation can only be termed a "trend".

An alternate hypothesis for the activities of the position 4 antagonists may relate to the ability of the para substituent to accept a hydrogen bond. On that basis the F analogue **23** should be a stronger antagonist than the Cl analogue **24**, since fluorine compounds are better hydrogen bond acceptors than chlorine compounds.[16] On the contrary, **24** is more potent than **23**. Analogue **2**, should also be devoid of antagonist activity, since it lacks a substituent for hydrogen bonding. But **2** is a fairly potent antagonist.[6] These results suggest that it is the lack of an ability to donate a hydrogen bond and not the ability to accept a hydrogen bond that contribute to antagonist activity.

The factors that tend to promote antagonist activity in the [Sar[1],(pX)Phe[4]]-ANG II analogues of Table II are deleterious to antagonist activity in the corresponding [Sar[1],(pX)Phe[4],Ile[8]]-ANG II analogues of Table III. Substituents that tend to be more lipophilic and incapable of donating a hydrogen atom in a hydrogen bond give rise to [Sar[1],(pX)Phe[4],Ile[8]]-ANG II analogues **7** and **28–30** with antagonist activities that are lower than the parent [Sar[1],Tyr[4],Ile[8]]-ANG II **14** (Table III). Thus, ANG II analogues that are antagonists by virtue of an alteration in position 8 require a position 4 agonist side chain.

It should be noted that analogue **15**, which was previously tested in the rat uterus,[6] gave different results in the rabbit aorta, suggesting a possible difference in receptors between the two species.

The in vivo partial agonist activities of some of the [Sar[1],(pX)Phe[4],Ile[8]]-ANG II analogues are also shown in Table III. Although the partial agonist activities are lower in the analogues **7** and **28–30**, the antagonist potencies of these analogues are correspondingly lower both in vitro and in vivo.

Potent antagonists to ANG II have also been obtained with D-amino acid substitutions in position 8.[17] Table IV

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Table IV. Activities of [Sar[1],Y[4],Z[8]]-ANG II Analogues

no.	Y[4]	Z[8]	agonist: in vitro[a] ANG-II-like[c]	agonist: in vivo[b] ANG-II-like[d]	antagonist: in vitro[a] pA_2	antagonist: in vivo[b] ID_{50}[e]
31	Tyr	D-Phe	0	10.0 ± 1.2	9.0	12.5 ± 1.4
32	Tyr	D-Tyr	0	15.0 ± 2.2	8.25	20.0 ± 2.3
33	Phe	Tyr	0	5.5 ± 2.0	7.5	50.0 ± 4.8
34	Phe	D-Tyr	0	5.0 ± 1.8	7.0	30.0 ± 4.0
35	(pF)Phe	D-Phe	0	10.0 ± 1.5	6.85	50.0 ± 5.5

[a] Agonist, "ANG II-like" activity, and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[29] ANG II antagonists bearing sarcosine in position 1 are slowly reversible antagonists depressing both the slope and maximum of the ANG II dose–response curve in vitro at high doses but not at low doses.[29] The pA_2 values reported here for comparative purposes were determined at low doses in the range of competitive inhibition, and consequently may be overestimated.[8] [b] Residual "ANG II-like" activity and antagonist activity, ID_{50}, were measured in vivo, in the rat blood pressure assay described by Regoli et al.[12] [c] ANG II-like activity in vitro is expressed as percent activity relative to ANG II. [d] ANG II-like activity in vivo is expressed by the mmHg of blood pressure increase produced by a 1 μg bolus intravenous injection of compound. [e] ID_{50} in nanograms/rat per minute (using 250-g rats).

Table V. ANG II Analogue Data[a]

	amino acid analysis[b]								TLC R_f			HPLC		
no.	1	2	3	4	5	6	7	8	A	B	C	solvent (% CH_3CN)	K'	% purity
16	Sar	Arg	Val	Tyr	Ile	His	Pro	Phe	0.22	0.58	0.28	20	2.60	>98
	(+)	0.98	1.00	1.01	0.99	1.02	1.01	0.99						
18	Sar	Arg	Val	Bph*[c]	Ile	His	Pro	Phe*	0.38	0.58	0.57	24	2.1	97
	(+)	1.00	0.94	0.99	0.88	1.10	1.00	0.99						
22	Sar	Arg	Val	(NO_2)Phe	Ile	His	Pro	Phe	0.25	0.60	0.80	20	7.3	97
	(+)	1.00	1.00	(+)	0.96	1.01	1.04	1.00						
6	Sar	Arg	Val	(OMe)Phe	Ile	His	Pro	Phe	0.18	0.37	0.61	40	1.5	>95
	(+)	1.00	1.02	0.94	0.87	1.19	0.72	0.97						
23	Sar	Arg	Val	(pF)Phe	Ile	His	Pro	Phe	0.16	0.55	0.48	30	4.8	>98
	(+)	1.00	1.01	1.10	0.96	0.98	0.98	0.99						
24	Sar	Arg	Val	(pCl)Phe	Ile	His	Pro	Phe	0.23	0.42	0.64	25	3.2	>98
	(+)	1.07	1.05	1.02	0.92	0.96	0.93	1.06						
8	Sar	Arg	Val	(OEt)Phe	Ile	His	Pro	Phe	0.24	0.45	0.54	27	4.5	>98
	(+)	0.99	1.03	0.95	0.99	0.98	1.00	1.05						
25	Sar	Arg	Val	(pI)Phe	Ile	His	Pro	Phe	–	0.55	0.59	40	1.7	>98
	(+)	1.03	1.03	(+)	0.96	0.95	1.02	1.02						
14	Sar	Arg	Val	Tyr	Ile*	His	Pro	Ile*	0.19	0.67	0.75	20	2.80	>98
	(+)	0.99	0.97	0.98	1.01	1.07	0.99	1.01						
26	Sar	Arg	Val	Bph	Ile*	His	Pro	Ile*	0.27	0.59	0.57	25	3.49	97
	(+)	1.00	1.10	0.92	0.94	0.94	1.10	0.94						
27	Sar	Arg	Val	(NO_2)Phe	Ile*	His	Pro	Ile*	0.13	0.50	0.52	18	5.26	96
	(+)	0.99	1.01	(+)	0.97	1.02	1.03	0.97						
15	Sar	Arg	Val	Phe	Ile*	His	Pro	Ile*	0.12	0.46	0.46	21	2.1	>98
	(+)	1.03	1.00	1.03	0.99	0.99	0.96	0.99						
7	Sar	Arg	Val	(OMe)Phe	Ile*	His	Pro	Ile*	0.26	0.42	0.64	25	2.3	95
	(+)	1.04	1.03	0.91	0.98	1.01	1.05	0.98						
28	Sar	Arg	Val	(pF)Phe	Ile*	His	Pro	Ile*	0.19	0.67	0.59	30	2.3	95
	(+)	1.03	1.02	(+)	0.97	1.05	0.96	0.97						
29	Sar	Arg	Val	(pCl)Phe	Ile*	His	Pro	Ile*	0.25	0.54	0.74	25	2.0	>98
	1.18	1.00	0.91	0.86	1.00	1.08	0.93	1.00						
30	Sar	Arg	Val	(OEt)Phe	Ile*	His	Pro	Ile*	0.17	0.51	0.56	15	3.8	96
	(+)	1.03	1.02	0.88	0.95	1.15	1.03	0.95						
31	Sar	Arg	Val	Tyr	Ile	His	Pro	D-Phe	0.19	0.55	0.76	23	8.6	96
	(+)	1.01	1.01	1.02	0.96	1.02	0.99	1.02						
32	Sar	Arg	Val	Tyr	Ile	His	Pro	D-Tyr	0.14	0.44	0.48	15	8.0	>98
	(+)	1.03	1.02	0.99	0.98	1.01	0.99	0.99						
33	Sar	Arg	Val	Phe	Ile	His	Pro	Tyr	0.19	0.39	0.58	20	2.78	91
	(+)	1.05	1.03	0.92	0.95	0.97	1.05	1.03						
34	Sar	Arg	Val	Phe	Ile	His	Pro	D-Tyr	0.03	0.08	0.53	35	10.9	85
	(+)	1.05	1.03	1.03	0.95	0.98	1.04	0.92						
35	Sar	Arg	Val	(pF)Phe	Ile	His	Pro	D-Phe	0.29	0.57	0.66	27	2.99	98
	(+)	1.00	1.02	(+)	0.97	0.98	1.02	1.01						

[a] See text for details of analytical procedures. [b] Amino acid analysis expressed in molar ratios of the D,L amino acids in the peptides. (+) = amino acid present in roughly 1 molar equiv (in cases where quantitation is difficult). * = amino acid present in two positions; value expressed is half the experimental value. [c] Bph is converted to Phe in 6 N HCl.[21] The other amino acids do not appear to suffer side-chain degradation.

contains a series of [Sar[1],(pX)Phe[4],D-AA[8]]-ANG II analogues, some of which were described previously.[16] The (pF)Phe[4] modification, which increases in vitro and in vivo antagonist activity in [Sar[1],(pF)Phe[4],Phe[8]]-ANG II **23** (Table II) but reduces in vitro and in vivo antagonist activity of the corresponding Ile[8] analogue **28** (Table III), also reduces in vitro and in vivo antagonist activity in the corresponding D-Phe[8] analogue **35** (Table IV). It appears, therefore, that concurrent antagonist modifications of positions 4 and 8 do not give rise to potent ANG II antagonists with reduced partial agonist activity.

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The enhancement of antagonist activity by increasing lipophilicity and removal of hydrogen bonding capacity at tyrosine was also observed with vasopressin antagonists.[18] It would be interesting to see if this effect can be observed in other peptide hormones.

It should be mentioned that halogen substituents on the ortho position of Tyr^4 were previously found to diminish the activities of ANG II. [Asn^1,(3-I)Tyr^4]-ANG II and [Asn^1,(3,5-I_2)Tyr^4]-ANG II displayed 33% and 13% of the pressor activity of [Asn^1]-ANG II, respectively.[19] Antagonist activities were not reported for these analogues.

The apparently different receptor requirements for position 4 agonist and antagonist analogues suggests that a position 4 agonist side chain (pX^h, where h = hydrophilic) interacts with receptor functionality that is different from that which interacts with a position 4 antagonist side chain (pX^l, where l = lipophilic). In the present study, the octapeptide sequence [Sar^1,(pX^l)Phe^4]-ANG II appears to reach maximal antagonist activity when X = Cl. It remains to be seen whether alternate sequences bearing a chlorophenylalanine group might provide ANG II antagonist activity with greater potency.

Experimental Section

tert-Butyloxycarbonyl amino acids and peptide reagents were obtained from Bachem Fine Chemicals, Inc., Protein Research Foundation, or Chemical Dynamics Corp., and were used without further purification. Boc-(pF)Phe[20] was prepared by standard procedures from commercially available (pF)Phe (Bachem). Thin-layer chromatography (TLC) was performed on Brinkman precoated silica gel plates (SIL-G-25).

Boc-*p*-(dihydroxyboryl)phenylalanine (Boc-Bph). Boc-Bph was prepared from Bph-OEt[21] according to the method of preparation for Boc-D-Bph[17] $[\alpha]^{25}_{289}$ −15.23° (*c* 0.65, MeOH) as an amorphous powder: TLC ($CHCl_3$–MeOH–AcOH, 85:10:5), R_f 0.57; ^1H NMR (CD_3OD) δ 7.44 (dd, 4 H), 4.38 (m, 1 H), 3.05 (m, 2 H), 1.38 (s, 9 H); $[\alpha]^{25}_{289}$ 16.67° (*c* 0.79, MeOH). The product was used without further purification.

Peptide Synthesis and Purification. All peptides were prepared by the solid-phase method on Beckman 990-B peptide synthesizers.[22,23] The C-terminal residue was esterified to a chloromethylated copolymer of polystyrene and 2% divinylbenzene (Bio-Rad) via a cesium salt procedure.[24] The degree of substitution was determined by amino acid analysis of a hydrolysate obtained by treating the amino acid-resin with HCl–PrOH (1:1) at 120 °C for 3 h.[25] Routine deprotection of Boc-amino protecting groups was accomplished with 30% TFA in CH_2Cl_2 and neutralization with 10% TEA in CH_2Cl_2. Coupling of each amino acid was performed with a 2.5 molar excess of *tert*-butyloxycarbonyl amino acid and DCC in CH_2Cl_2 with completeness of reaction monitored by the ninhydrin test.[26] Side chain protecting groups were as follows: Arg, tosyl; Tyr, α-Br-Z or 2,6-Cl_2Bzl; His, tosyl.

In most cases coupling was complete after 2 h. If the ninhydrin test remained positive, a recoupling cycle was performed. After the last coupling and deprotection the peptide was cleaved from resin by treatment with anhydrous HF containing 50% (v/v) anisole at 0 °C for 60 min. After vacuum evaporation of HF, the resin was rinsed with Et_2O to remove anisole and then rinsed with glacial HOAc and filtered. The filtrate was diluted with water and lyophilized to a powder of crude peptide material.

The crude peptides were purified to homogeneity either by (a) partitioning through 200 transfers of countercurrent distribution in *n*BuOH–HOAc–H_2O (4:1:5), (b) by partition chromatography[27] on Sephadex G-15 in nBuOH-HOAc-H_2O (4:1:5), or (c) by reversed-phase semipreparative HPLC[28] on a Whatman C^{18} column using the appropriate solvent mixture of CH_3CN–0.1 N NH_4OAc, pH 4. The volumes of chromatographic fractions containing pure peptide by TLC were reduced by partial rotary evaporation and dried to powders by lyophilization to constant weight.

Homogeneity of each peptide was determined by the following methods: (a) Amino acid analysis of 72 h acid hydrolysis (6 N HCl, 110 °C) performed on a Beckman Model 120C analyzer. (b) Analytical TLC on silica gel plates with solvent systems (A) *n*BuOH–AcOH–H_2O (4:1:5), (B) *n*BuOH–AcOH–H_2O–EtOAc (1:1:1:1), (C) nBuOH–AcOH–H_2O–pyridine (15:3:12:10), visualizing spots with Pauly reagent.[26] (c) Analytical reversed-phase HPLC on C_{18} silica gel column using the appropriate CH_3CN–0.1 N NH_4OAc (pH 4) mixture.

Analytical data for all peptides are listed in Table V.

Registry No. **6**, 88874-29-7; **7**, 92780-94-4; **8**, 101713-01-3; **14**, 37827-06-8; **15**, 98641-01-1; **16**, 51833-69-3; **18**, 119771-24-3; **22**, 119771-25-4; **23**, 119771-26-5; **24**, 119771-27-6; **25**, 119771-28-7; **26**, 119771-29-8; **27**, 119787-54-1; **28**, 119771-30-1; **29**, 119787-55-2; **30**, 119771-31-2; **31**, 111821-39-7; **32**, 111771-44-9; **33**, 111821-48-8; **34**, 111771-50-7; **35**, 119817-71-9; BOC-Bph, 119771-23-2.

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