Novel Gonadotropin-Releasing Hormone Antagonists: Peptides Incorporating Modified N^{ω}-Cyanoguanidino Moieties

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In order to minimize the deleterious effects of histamine release resulting from the administration to rats and humans of some potent gonadotropin-releasing hormone (GnRH) antagonists, various arginine residues were replaced with the less basic N^{ω}-cyano-$N^{\omega'}$-alkyl- or -arylhomoarginine, -arginine, or -*p*-aminophenylalanine and N^{ω}-triazolyllysine, -ornithine or -*p*-aminophenylalanine residues in active analogues. These novel analogues were synthesized on a solid-phase support via a two-step modification of the N^{ω}-NH_2 of lysine, ornithine, or *p*-aminophenylalanine residues in otherwise protected resin bound peptides. Most analogues were tested in the rat antiovulatory assay (AOA) and three in vitro assays: a pituitary cell culture assay, a binding assay to pituitary cell membranes, and a histamine release assay. Introduction of the cyanoguanidino and N^{ω}-triazolyl moieties into GnRH analogues yielded several water-soluble antagonists which showed a desirable therapeutic ratio (low histamine release activity to high in vivo potency). Among them, "Azaline" (**10**, [Ac-DNal[1],DCpa[2],DPal[3],Lys[5](atz),DLys[6](atz),ILys[8],DAla[10]]GnRH), inhibited ovulation in the rat by 90% at 2 μg/rat with an ED_{50} in the in vitro histamine release assay comparable to that of GnRH itself.

Introduction

Mammalian gonadotropin-releasing hormone (GnRH, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), first isolated and characterized by Matsuo et al.[1] and by Burgus et al.,[2] is well-known to exert regulatory control over reproductive functions. Since its discovery, thousands of GnRH analogues have been synthesized in a wide-ranging search for potent and long-acting agonists and antagonists for use as therapeutic agents for endocrine diseases and nonsteroidal contraception.[3]

In the agonist series, increasing the hydrophobic character of the peptide by introduction of D aromatic residues (such as DTrp and DNal) in position 6 improved potency in vitro and resulted in analogues with a prolonged duration of action.[4] Accordingly, the first generation of potent antagonists with modifications at positions 1, 2, and 3 contained such substitutions in position 6. Later, Nekola et al.[5] found that substitution of basic amino acids such as DArg in position 6 significantly increased potency of GnRH antagonists containing a hydrophobic aromatic N-terminus. This led to the development of analogues typified by the "Nal-Arg" analogue ([Ac-DNal[1],DPpa[2],DTrp[3],DArg[6]]GnRH), which gave 100% inhibition of ovulation in rats on the day of proesterus at a dose of 1.0 μg/rat.[6] However, subcutaneous (sc) injection of Nal-Arg and structurally related antagonists produced transient edema of the face and extremities in rats[7] and a systemic effect in humans. Subsequently the Nal-Arg antagonist was withdrawn from clinical investigation because of its histamine-related side effects. Further structure–activity relationship studies indicated that the combination of a hydrophobic N-terminal region and basic amino acid residue such as Arg or Lys in positions 6 and 8 resulted in potent antagonists, which caused the release of histamine.[8] On the other hand, the agonist [DTrp[6],Pro[9]-NHEt]GnRH or the antagonist [Ac-Δ^3Pro[1],DPpa[2],DTrp[3,6]]GnRH containing aromatic amino acids at position 6 did not elicit the edematous effect.[7]

Recent work in this field has focused on devising GnRH antagonists with minimal histamine-releasing activity and high inhibitory potency in vivo.[9,10] One recent approach

† National Institutes of Health.

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Table I. Characterization of GnRH Antagonists with Ac-DNal[1]-DCpa[2]-DPal[3] Substitutions and Modified Basic Residues in Positions 5, 6, and/or 8

	compounds	t_R (% CH_3CN)[a]	$[\alpha]_D$,[b] deg
1	[Har[5](mCN),DHar[6](mCN),ILys[8],DAla[10]]GnRH	4.00 (32.7)	-30
2	[Har[5](iCN),DHar[6](iCN),ILys[8],DAla[10]]GnRH	4.68 (35.4)	-28
3	[Har[5](bCN),DHar[6](bCN),ILys[8],DAla[10]]GnRH	4.11 (37.2)	-26
4	[Har[5](hCN),DHar[6](hCN),ILys[8],DAla[10]]GnRH	4.12 (46.8)	-26
5	[Har[5](chCN),DHar[6](chCN),ILys[8],DAla[10]]GnRH	5.02 (42.6)	-25
6	[Har[5](2mpCN),DHar[6](2mpCN),ILys[8],DAla[10]]GnRH	3.77 (27.6)	-25
7	[Arg[5](bCN),DArg[6](bCN),ILys[8],DAla[10]]GnRH	5.11 (39.0)	-38
8	[Arg[5](2mpCN),DArg[6](2mpCN),ILys[8],DAla[10]]GnRH	4.48 (27.0)	-34
9	[Aph[5](bCN),DAph[6](bCN),ILys[8],DAla[10]]GnRH	4.02 (43.2)	-23
10	[Lys[5](atz),DLys[6](atz),ILys[8],DAla[10]]GnRH	3.72 (27.0)	-34
11	[Orn[5](atz),DOrn[6](atz),ILys[8],DAla[10]]GnRH	4.93 (25.8)	-43
12	[Aph[5](atz),DAph[6](atz),ILys[8],DAla[10]]GnRH	4.48 (30.6)	-33
13	[Har[5](SbCN),DHar[6](SbCN),ILys[8],DAla[10]]GnRH	4.89 (45.0)	-27
14	[NicLys[5],DNicLys[6],HAr(bCN)[8],DAla[10]]GnRH	3.77 (36.0)	-33
15	[NicLys[5],DNicLys[6],Har(bur)[8],DAla[10]]GnRH	4.30 (33.0)	

[a] t_R = retention time in minutes, % CH_3CN. Peptides (10 μg/10 μL) dissolved in 0.1% TFA were applied to a Vydac C_{18} column (5 μm, 300-Å pore size; 4.5 × 250 mm) under isocratic conditions, 0.1% TFA/H_2O with % CH_3CN shown, at a flow rate of 2.0 mL/min. UV detection was 0.1 AUFS at 210 nm. [b] c = 1 (weight of lyophilized peptide in 50% HOAc/H_2O).

Table II. Characterization of GnRH Antagonists with Ac-DNal[1]-DCpa[2] and Modified Basic Residues in Positions 3, 6, and/or 8

	compounds	t_R (% CH_3CN)[a]	$[\alpha]_D$,[b] deg
16	[DPal[3],DHar[6](bCN),DAla[10]]GnRH	4.10 (36.6)	-21
17	[DHar(bCN)[3,6],ILys[8],DAla[10]]GnRH	4.15 (45.6)	-35
18	[DLys(atz)[3,6],ILys[8],DAla[10]]GnRH	4.60 (32.4)	-32
19	[DPal[3],DArg[6],Har(bCN)[8],DAla[10]]GnRH	4.70 (37.8)	-25
20	[DPal[3],DArg[6],Lys(atz)[8],DAla[10]]GnRH	4.13 (33.0)	-24
21	[DHar(bCN)[3],DArg[6],ILys[8],DAla[10]]GnRH	4.45 (40.8)	-28
22	[DLys(atz)[3],DArg[6],ILys[8],DAla[10]]GnRH	4.43 (33.0)	-31
23	[DPal[3,6],ILys[8],DAla[10]]GnRH	4.61 (27.0)	-23
24	[DHar(bCN)[3],DPal[6],ILys[8],DAla[10]]GnRH	3.80 (42.6)	-44
25	[DArg(bCN)[3],DPal[6],ILys[8],DAla[10]]GnRH	4.99 (43.2)	-38
26	[DAph(bCN)[3],DPal[6],Ilys[8],DAla[10]]GnRH	4.20 (40.2)	-42
27	[DAph(atz)[3],DPal[6],Ilys[8],DAla[10]]GnRH	4.63 (33.6)	-30
28	[DOrn(atz)[3],DPal[6],Ilys[8],DAla[10]]GnRH	4.46 (33.0)	-35

[a] t_R = retention time in minutes, % CH_3CN. Peptides (10 μg/10 μL) dissolved in 0.1% TFA were applied to a Vydac C_{18} column (5 μm, 300-Å pore size; 4.5 × 250 mm) under isocratic conditions, 0.1% TFA/H_2O with % CH_3CN shown, at a flow rate of 2.0 mL/min. UV detection was 0.1 AUFS at 210 nm. [b] c = 1 (weight of lyophilized peptide in 50% HOAc/H_2O).

toward reaching this goal in our laboratory was to attenuate the basicity of various arginine residues of active analogues by the introduction of an electronegative substituent (such as cyano) on the N^{ω}-guanidino function. This substituent stabilizes the guanidino functional group, resulting in a significant lowering of the pK_a of the guanidinium cation (the pK_a of the guanidinium cation in water is 14.46 while that of the cyanoguanidinium cation is -0.4[11]). This strategy of reducing guanidino basicity proved successful in the development of a new class of potent guanidino-containing histamine H_2 receptor antagonist.[12]

We report here on the synthesis of GnRH antagonists containing modified N^{ω}-cyano-$N^{\omega'}$-alkyl- or -arylguanidino moieties on homoarginine, arginine, or *p*-aminophenylalanine residues (cyanoguanidino peptides) as well as peptides incorporating an aminotriazolyl moiety on the distal amino function of lysine, ornithine, or *p*-aminophenylalanine.[13] The novel amino acid residues were synthesized on a partially protected resin-bound peptide by modifying lysine, ornithine, or *p*-aminophenylalanine residues that had been orthogonally protected in the prior steps of the synthesis. These new analogues, tested in bioassays including the rat antiovulatory assay (AOA)[14] and in vitro cell culture assay,[15] binding assay,[16] and histamine release assay,[8,17] are equipotent to some of the most potent antagonists prepared to date. Furthermore, several analogues were significantly less potent in releasing histamine from rat mast cells than previously prepared analogues.

Synthesis, Purification, and Characterization (See Tables I and II). Analogues were synthesized by the SPPS methodology either manually or on a Beckman 990 peptide synthesizer with use of previously described protocols on a methylbenzhydrylamine (MBHA) resin using the *tert*-

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Table III. Biological Characterization of GnRH Antagonists with Ac-DNal[1]-DCpa[2]-DPal[3] and Modified Basic Residues in Positions 5, 6, and/or 8

	compounds	in vitro relative Potencies	K_D, nM	AOA[a]	in vitro histamine release:[b] ED_{50} ± SEM, μg/mL
1	[Har[5](mCN),DHar[6](mCN),ILys[8],DAla[10]]GnRH		0.24 (0.18–0.32)	2.0 (2/10)	
2	[Har[5](iCN),DHar[6](iCN),ILys[8],DAla[10]]GnRH	0.3 (0.17–0.48)	0.32 (0.22–0.45)	0.5 (6/6), 1.0 (2/8)	
3	[Har[5](bCN),DHar[6](bCN),ILys[8],DAla[10]]GnRH		1.3 (0.9–1.9)	0.5 (11/11), 1.0 (4/14)	18 ± 0.25
4	[Har[5](hCN),DHar[6](hCN),ILys[8],DAla[10]]GnRH	0.14 (0.08–0.22)	3.3 (1.8–6.1)	1.0 (5/5), 2.5 (2/8)	
5	[Har[5](chCN),DHar[6](chCN),ILys[8],DAla[10]]GnRH	0.42 (0.27–0.67)	1.2 (0.7–1.7)	2.5 (7/9)	
6	[Har[5](2mpCN),DHar[6](2mpCN),ILys[8],DAla[10]]GnRH	1.3 (0.8–2.2)	0.27 (0.19–0.38)	1.0 (7/17)	43 ± 12
7	[Arg[5](bCN),DArg[6](bCN),ILys[8],DAla[10]]GnRH	1.0 (0.7–1.4)	2.1 (1.2–3.5)	1.0 (5/8), 2.5 (0/8)	
8	[Arg[5](2mpCN),DArg[6](2mpCN),ILys[8],DAla[10]]GnRH	0.9 (0.6–1.4)	0.41 (0.29–0.57)	2.5 (2/9)	
9	[Aph[5](bCN),DAph[6](bCN),ILys[8],DAla[10]]GnRH			5.0 (6/11), 2.5 (2/3)	
10	[Lys[5](atz),DLys[6](atz),ILys[8],DAla[10]]GnRH	0.23 (0.15–0.36)	0.48 (0.38–0.57)	2.0 (1/10)	139 ± 8.7
11	[Orn[5](atz),DOrn[6](atz),ILys[8],DAla[10]]GnRH	0.2 (0.1–0.3)	0.21 (0.14–0.32)	2.0 (1/10)	158 ± 10
12	[Aph[5](atz),DAph[6](atz),ILys[8],DAla[10]]GnRH	1.3 (0.8–2.0)		0.5 (8/18), 1.0 (1/17)	50 ± 6.7
13	[Har[5](SbCN),DHar[6](SbCN),ILys[8],DAla[10]]GnRH	1.3 (0.8–2.1)		25 (5/5), 50 (2/3)	
14	[NicLys[5],DNicLys[6],Har(bCN)[8],DAla[10]]GnRH	0.3 (0.2–0.5)	0.45 (0.29–0.67)	5.0 (14/20)	
15	[NicLys[5],DNicLys[6],Har(bur)[8],DAla[10]]GnRH		0.11 (0.07–0.16)	2.5 (7/7), 5.0 (0/7)	

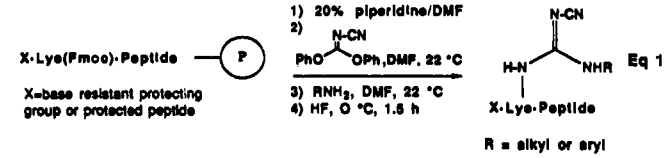
[a] AOA = antiovulatory assay: dosage in micrograms/rat (rats ovulating/total). [b] ED_{50} for [Ac-DNal[1],DFpa[2],DTrp[3],DArg[6]]GnRH was 0.17 ± 0.01 μg/mL and for [Ac-DNal[1],DCpa[2],DPal[3],NicLys[5],DNicLys[6],ILys[8],DAla[10]]GnRH was >300 μg/mL.

Table IV. Biological Characterization of GnRH Antagonists with Ac-DNal[1]–DCpa[2] and Modified Basic Residues in Positions 3, 6, and/or 8

	compounds	in vitro relative Potencies	K_D, nM	AOA[a]	in vitro histamine release:[c] ED_{50} ± SEM, μg/mL
16	[DPal[3],DHar[6](bCN),DAla[10]]GnRH		1.7 (1.1–2.6)	2.5 (4/17)	
17	[DHar(bCN)[3,6],ILys[8],DAla[10]]GnRH	1.4 (0.9–2.3)		2.5 (5/9)	ca. 50
18	[DLys(atz)[3,6],ILys[8],DAla[10]]GnRH	0.6 (0.3–1.1)		1.0 (6/6), 2.5 (1/8)	>150
19	[DPal[3],DArg[6],Har(bCN)[8],DAla[10]]GnRH	1.2 (0.7–2.0)	1.0 (0.7–1.5)	2.5 (5/6), 5.0 (1/6)	3.5 ± 1.4
20	[DPal[3],DArg[6],Lys(atz)[8],DAla[10]]GnRH			1.0 (5/10), 2.5 (0/7)	19 ± 1.0
21	[DHar(bCN)[3],DArg[6],ILys[8],DAla[10]]GnRH	1.4 (0.8–2.3)	1.2 (0.8–1.8)	0.5 (7/7), 1.0 (2/8)	6.0 ± 0.3
22	[DLys(atz)[3],DArg[6],ILys[8],DAla[10]]GnRH	0.3 (0.2–0.5)	0.27 (0.19–0.35)	5.0 (2/5), 10 (0/6)	9.5 ± 0.9
23	[DPal[3,6],ILys[8],DAla[10]]GnRH		0.08 (0.05–0.12)	1.0 (8/14), 2.0 (0/10)	32 ± 1.0
24	[DHar(bCN)[3],DPal[6],ILys[8],DAla[10]]GnRH	1.7 (1.0–2.8)		0.5 (8/8), 1.0 (2/10)	58 ± 8.9
25	[DArg(bCN)[3],DPal[6],ILys[8],DAla[10]]GnRH	1.6 (0.9–3.0)		1.0 (5/5), 2.5 (0/7)	
26	[DAph(bCN)[3],DPal[6],ILys[8],DAla[10]]GnRH	0.2 (0.1–0.3)		5.0 (9/9), 15 (4/6)	14 ± 2.1
27	[DAph(atz)[3],DPal[6],ILys[8],DAla[10]]GnRH	0.06 (0.04–0.09)		10 (4/4)	
28	[DOrn(atz)[3],DPal[6],ILys[8],DAla[10]]GnRH	0.2 (0.15–0.30)		1.0 (5/6), 2.5 (1/7)	45 ± 3.3

[a] AOA = antiovulatory assay: dosage in micrograms/rats (rats ovulating/total). [b] ED_{50} for [Ac-DNal[1],DFpa[2],DTrp[3],DArg[6]]GnRH was 0.17 ± 0.01 μg/mL and for [Ac-DNal[1],DCpa[2],DPal[3],NicLys[5],DNicLys[6],ILys[8],DAla[10]]GnRH was >300 μg/mL.

butyloxycarbonyl (Boc) group for N^{α}-amino protection.[9] The distal amino of the lysine, ornithine, or *p*-aminophenylalanine residues to be modified was protected as the 9*H*-fluorenylmethoxycarbonyl (Fmoc) derivative. The fully assembled peptide resin was treated with piperidine in DMF to remove the Fmoc group. Via the procedure



of Webb and Labaw[18] for the preparation of cyanoguanidines, diphenyl cyanocarbonimidate (PCI) in DMF was added, at room temperature, to the selectively deprotected peptide resin. After completion of the PCI coupling (negative ninhydrin test), an amine, RNH_2 (or other nucleophile), was reacted. The peptides were cleaved from the resin with HF and purified using two reverse-phase HPLC systems.[19] Retention times using isocratic conditions and specific rotations are given in Tables I and II. Amino acid analyses, including quantitation of Cpa and Nal, were consistent with expected results. The new amino acids were not characterized nor were they detected by standard amino acid analysis in our standard system. Calculated values for protonated molecular ions were in agreement with those obtained using FAB mass spectrometry (supplementary material). FT-IR of selected analogues were also consistent.[20]

Bioassays (See Tables III and IV). In vitro, the peptides were tested for their ability to inhibit GnRH-mediated LH secretion by cultured pituitary cells. Potencies of GnRH antagonists were expressed relative to a standard ([Ac-Δ^3Pro[1],DFpa[2],DTrp[3,6]]GnRH).[15] In binding studies, the K_D values for the potent agonist [DAla[6],NMeLeu[7],Pro[9]-NHEt]GnRH (taken as standard) was deter-

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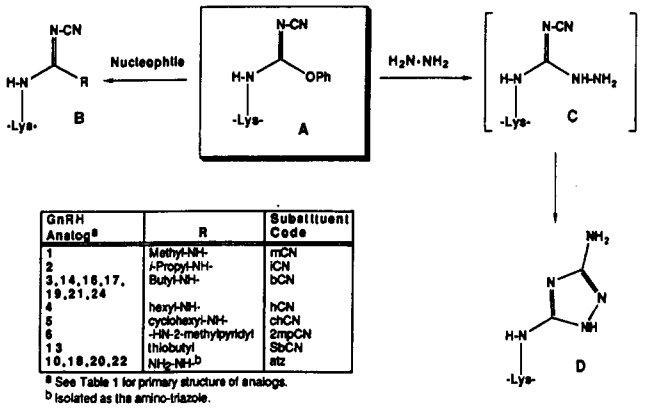
(20) The FTIR spectra of compounds 3 and 14 exhibited an absorbance at ca. 2165 cm^{-1} indicating the presence of a cyano group which was absent in compound 10.

Scheme I



GnRH Analog[a]	R	Substituent Code
1	Methyl-NH-	mCN
2	i-Propyl-NH-	iCN
3,14,16,17, 19,21,24	Butyl-NH-	bCN
4	hexyl-NH-	hCN
5	cyclohexyl-NH-	chCN
6	-HN-2-methylpyridyl	2mpCN
13	thiobutyl	SbCN
10,18,20,22	NH_2-NH-[b]	atz

[a] See Table I for primary structure of analogs. [b] Isolated as the amino-triazole.

mined from a Scatchard analysis to be approximately 0.3 nM. All the other K_D values were calculated from the potencies of the analogues (relative to the standard) determined from displacement data.[16] The assay for histamine release by rat mast cells has been reported previously.[8,17] The AOA was carried out as described by Corbin and Beattie;[14] cycling rats (250–300 g at the time of the assay) were injected subcutaneously with the peptides dissolved in saline or other appropriate solvents (200 μL) at noon on proestrus. Results are expressed in terms of the dosage in micrograms/rat (rat ovulating/total number of treated rats).

Results and Discussion

Chemistry. A variety of peptides containing modified cyanoguanidino residues as well as the heterocyclic N-substituted lysine, ornithine, and *p*-aminophenylalanine derivatives have been synthesized using SPPS techniques (Tables I and II). As illustrated for the synthesis of modified homoarginine residues (eq 1), Fmoc-protected lysine substituted in positions to be modified on the resin-bound peptide, were deblocked. PCI was then added to afford intermediates as shown in Scheme I. These cyanoisourea intermediates A were not stable to HF cleavage under standard conditions and thus were not isolated or characterized. Monoamines such as *n*-butylamine and (2-methylpyridyl)amine reacted readily with A to give, after HF cleavage, peptides containing the desired N^{ω}-cyano-$N^{\omega'}$-alkyl- or -arylhomoarginine (**1–6, 14, 16, 17, 19, 21, and 24**), N^{ω}-cyano-$N^{\omega'}$-alkyl- or -arylarginine (**7, 8, 25**), or N^{ω}-cyano-$N^{\omega'}$-alkyl- or -aryl-*p*-aminophenylalanine (**9, 26**) residues. Hydrazine, a bifunctional nucleophile, upon reaction with A, afforded intermediate C, which cyclized under the reaction conditions[18] to give, after HF cleavage, peptides containing heterocyclic N^{ϵ}-substituted lysine (**10, 18, 20, 22**), ornithine (**11, 28**), or *p*-aminophenylalanine (**12, 27**) derivatives. In most cases the yields of these peptides were essentially the same as those obtained for the underivatized GnRH analogs suggesting that the two or three step procedure, **free amino function → A → B** or **free amino function → A → C → D**, was essentially quantitative. As shown by the HPLC trace (Figure 1a) of a crude peptide that is representative of this type of syntheses, a major component was present and isolated with a purity greater than 95% (Figure 1b) by preparative reverse-phase HPLC.[19] That this procedure should be applicable to modification of any suitably protected amino-containing residue was evidenced by the synthesis of analogues containing N^{ω}-cyano-$N^{\omega'}$-alkyl- or -arylarginine residues (**7, 8, and 25**) and N^{ϵ}-triazolylornithine derivatives (**11 and 28**). Similarly, *p*-(N^{ω}-cyano-$N^{\omega'}$-butylguanidyl)phenylalanine- and *p*-(*N*-triazolyl)phenylalanine-containing analogues (**9 and 26, 12 and 27**, respectively) were prepared. As illustrated by the synthesis of analogue **13** containing N^{ω}-cyano-*S*-butylisourea-modified residues, other reactive nucleophiles, for example mercaptides, can be used in the reaction with A to afford peptides containing new and unusual amino acids. Because of the mildness of the reaction conditions used to derivatize the distal free amino function, it is expected that chirality of the newly formed amino acid will be retained. We have no experimental results to the contrary.

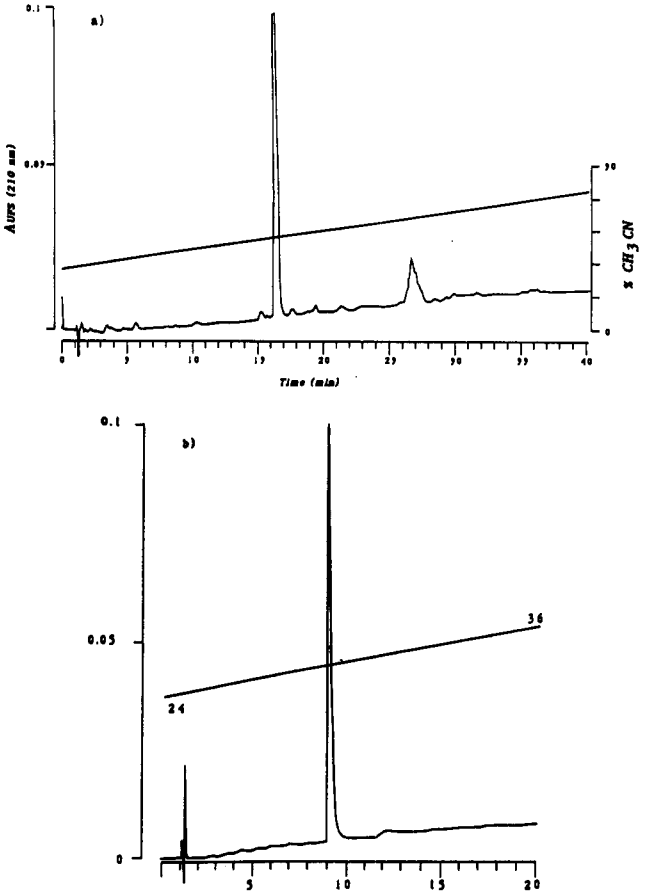
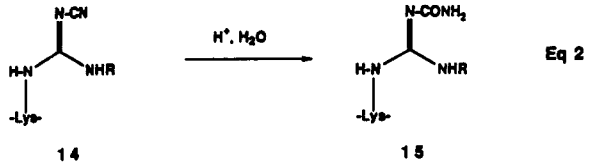


Figure 1. (a) Crude, lyophilized **10** (Azaline) from HF cleavage (5 μL, ca. 5 μg); Vydac 5 μm, C_{18}, 25 × 0.46 cm i.d. column; 0.1% TFA in water/acetonitrile; gradient: 18%–40′–42% CH_3CN at a flow rate of 2.0 mL/min. (b) Purified **10** (Azaline) (5 μL, 5 μg); 0.1% TFA in water/acetonitrile; gradient; 33%–10′–39% CH_3CN.

During the course of our studies we observed that the cyanoguanidino peptides [as the trifluoroacetate (TFA) salts such as for **14**] and stored as lyophilized powders at 4 °C, decomposed into a major hydrophilic impurity (from >95% to 50–70% purity by HPLC) over a 2–6-month period. Isolation of this decomposition product and characterization using mass spectroscopy showed that the mass of the decomposition product had increased by 18 at the residue that was modified with the alkylcyanoguanidino moiety. Further characterization by FTIR (the nitrile absorbance at 2166.8 cm^{-1} in the parent compound, **14**, disappeared in the decomposition product) combined with the MS data and literature precedence[21] suggested that, in the presence of water of hydration, hydrolysis of the nitrile had occurred under the acidic conditions induced by the TFA counterion,[22] yielding the corresponding

(21) Cimetidine, the cyanoguanidino containing H2 receptor antagonist was similarly hydrolyzed in 5 days in dilute HCl at room temperature.[11b]

guanylurea derivative 15 (eq 2).[13,23] Similarly, peptides



14 15 Eq 2

with two or more such substitutions also hydrolyzed to give the expected hydrolysis products. In order to assess the lability of a cyanoguanidino function in acid, the hydrolysis of 14 in 0.1% TFA was monitored by HPLC and was found to be complete within 4 days at room temperature (data not shown). Repurification of the cyanoguanidino-containing peptides and isolation as the acetate salts resulted in peptides stable to hydrolysis as powders at 4 °C.[22] Subsequent to this observation, all cyanoguanidino peptides were reconverted to, or isolated as, the acetate salts. It should be noted that the N^t-triazolyl derivatives (e.g., 10–12, 18, 22, 27, and 28) are stable as TFA salts.

Biology. Results obtained by Ljungqvist et al.[10a,24] indicated that substitution of position 5 and 6 with weakly basic acylated lysine residues and an isopropyllysine in position 8[25] and DAla in position 10[26] in combination with a hydrophobic N-terminus (Ac-DNal-DCpa-DPal-)[9] resulted in analogues with a desirable therapeutic ratio (high potency in the AOA and low histamine response). This led us to synthesize a similar series of 5,6-substituted cyanoguanidino and *N*-triazolyl-containing analogues. Their relative biological potencies in several assays are summarized in Table III. We first optimized the $N^{\omega'}$ group of the cyanoguanidino moiety in the homoarginine series (1–6) and found that the potent analogues in the AOA in this series contained an $N^{\omega'}$-isopropyl or an $N^{\omega'}$-butyl group (2 and 3, respectively). The bulkier secondary amino group cyclohexyl (5) was the least potent in both the binding assay and AOA, while substitution of an aromatic group (6) at the $N^{\omega'}$ position was well tolerated and resulted in a potent analogue in the AOA, in vitro assay and binding assay. Shortening the carbon tether connecting the guanidino group with the peptide backbone (7 and 8 compared with 3 and 6) appears to result in some decrease of the in vivo potency of the analogues in this series as well as of their affinity for the GnRH receptor in vitro. Substituting a butyl thiol for the $N^{\omega'}$-amine (13) significantly decreased potency in the AOA assay (0% inhibition at 25 μg/rat and 33% at 50 μg/rat) while retaining good potency in the pituitary cell culture assay. Incorporating a N^{ω}-cyano-$N^{\omega'}$-butyl moiety on a *p*-aminophenylalanine residue (9) in this series of analogues was not favorable for AOA activity.

For the series of 5,6 substituted *N*-triazolyl analogs (10, 12), good GnRH antagonist potency was retained compared to that of the cyanoguanidino series. Interestingly, the most potent analogue 12 in the AOA was tethered to the peptide backbone via the aromatic linkage (i.e. *p*-aminophenylalanine). Replacement of the basic ILys in position 8 of Antide ([Ac-DNal[1],DCpa[2],DPal[3],NicLys[5],DNicLys[6],ILys[8],DAla[10]]GnRH; 100% inhibition at 1.0 μg/rat in AOA[10a]) with Har(bCN) gave an analogue 14 that was less potent than Antide in the AOA (30% inhibition at 5.0 μg/rat). Analogue 15 with a hydrolyzed cyanoguanidino moiety was slightly more potent than the parent compound 14. For this series of 5,6-substituted analogues and in many other instances, the potency of analogues in the binding assay and cell culture assay did not always correlate well with the observed in vivo potency measured in the AOA. Because the conditions in the three assays are quite different, this observation suggests that potencies may reflect different metabolism and pharmacokinetics such as different rate of release, at this low dose, from the injection site, or association to binding proteins in the circulation, or different mechanisms of action with different rates of association/dissociation from the receptors in vitro. Any one of these factors could be influenced by conformational and solubility parameters in vivo. Thus it should not be surprising that a good correlation between assays cannot be obtained in all cases (Tables III and IV).

Several of the analogs from this series were tested in the in vitro histamine release assay (Table III). Analogues 10 ("Azaline") and 11 (ED_{50} = 139 and 158 μg/mL, respectively), which both incorporated the *N*-triazolyl modification, were the least potent in eliciting histamine release. Analogues 3 and 6 were much more potent than 10 and 11 in the histamine release assay. Even the most potent analogues however were considerably less potent than Nal-Arg (ED_{50} = 0.17 μg/mL)[6] in the histamine release assay while in most cases retaining high GnRH antagonist potency. The most promising analogue identified so far, Azaline (10), had a histamine response similar to that of GnRH itself (ED_{50} = 139 μg/mL).

It has been well established that basic amino acids in positions 6 and/or 8 yield potent analogues that cause a high histamine action when combined with a hydrophobic N-terminal region (vide supra).[8] Thus we synthesized and tested in several assays a series of 3,6- and/or 8-substituted cyanoguanidino and *N*-triazolyl-modified analogues (Table IV) to investigate the possibility that substitution of the less basic cyanoguanidino and *N*-triazolyl residues could decrease the undesirable histamine response while retaining high GnRH antagonist potency. In the first series (16–21), the basic amino acids of the potent Nal-Arg antagonist ([Ac-DNal[1],DFpa[2],DTrp[3],DArg[6]]GnRH) were systematically replaced (at positions 3, 6, and 8) by the less basic Har(bCN) or Lys(atz) residues, while introducing the Ilys[8] and DAla[10] substitutions, first reported by Coy et al.[25] and Erchegyi et al.,[26] when appropriate. The most potent of this series in the AOA was 21 with a DHar(bCN) in position 3 and ILys in position 8 (75% inhibition at 1.0 μg/rat). Interestingly, 16 in which the DArg[6] has been substituted with DHar(bCN), although of lower potency in the pituitary cell culture assay than Nal-Arg, retained significant biological potency (76% inhibition at 2.5 μg/rat).

The series 24–28 was modeled after the potent antagonist [Ac-DNal[1],DCpa[2],DPal[3,6],ILys[8],DAla[10]]GnRH (23),[27]

(22) HPLC analysis indicated that these peptides contained as much as 17% TFA by weight (ca. 3 mequiv). After isolation as the acetate salts, the peptides contained ca. 0.5 mequiv of acetate.

(23) The absorbance at 2165 cm^{-1} in the cyanoguanidino peptide 14 was absent in the spectra of the decomposition product. It was not possible to assign additional bands due to the complexity of the regions from 2800 to 3500 cm^{-1}.

(24) Phillips, A.; Hahn, D. W.; McGuire, J. L.; Ritchie, D.; Capetola, R. J.; Bowers, C.; Folkers, K. Evaluation of anaphylactoid activity of a new LHRH antagonist. *Life Sci.* 1988, *43*, 883.

(25) Coy, D. H.; Nekola, M. V.; Erchegyi, J.; Coy, E. J.; Schally, A. V. Contraceptive effects of recent potent LHRH antagonist analogs. In *LHRH and Its Analogs–Contraceptive and Therapeutic Applications*; Zatuchni, G. I., Shelton, J. D., Sciarra, J. J., Eds.; Harper and Row: Philadelphia, 1981; p 37.

(26) Erchegyi, J.; Coy, D. H.; Nekola, M. V.; Pedroza, E.; Coy, E. J.; Mezo, I.; Schally, A. V. Luteinizing hormone-releasing hormone analogs with increased antiovulatory activity. *Biochem. Biophys. Res. Commun.* 1981, *100*, 915.

whereby a modified DHar, DArg, or DAph was introduced in position 3. Antagonist **24** with a DHar(bCN)[3] caused 80% inhibition of ovulation in the AOA assay at 1.0 μg/rat. When the carbon chain between the guanidino moiety and the backbone was decreased (**25**) by one methylene, the potency was reduced compared to that of the homoarginine derivative, while replacement of the Har(bCN) with Aph(bCN) (**26**) decreased potency further (0% inhibition at 5.0 μg/rat). Substitution with the *N*-triazolyl group in this series (**27–28**) did not seem to influence dramatically the potency of the analogues in the AOA and in vitro assay when compared to that of the corresponding analogues **26** and **25**.

Thus analogue **24** was the most potent of the series of DArg[6] and DPal[6] analogues (Table IV) (1.0 μg/rat, 80% inhibition). The overall potency in the AOA assay of these two series (**16–28**) were slightly less than for the Nal-Arg antagonist (1.0 μg/rat, 100% inhibition). Selected analogues from this series were also tested in the in vitro histamine release assay in order to establish whether these novel moderately basic cyanoguanidino and *N*-triazolyl residues reduced histamine activity compared to that of other potent analogues such as Nal-Arg (ED_{50} = 0.17 μg/mL). The ED_{50} values for analogues **17–22** were at least 20-fold higher than that for Nal-Arg. Interestingly, the biological results obtained for analogues **19–22** with an unmodified DArg[6] suggested that the deleterious effect of this strongly basic amino acid on histamine release could be attenuated by replacing residues 3 or 8 with a cyanoguanidino or *N*-triazolyl residue. However, the ILys[8] analogues elicited a weaker histamine response than Arg[6] analogues such as Nal-Arg. Therefore, it is more reasonable to compare the biological activities of **19** (ED_{50} = 3.5 μg/mL; 84% inhibition at 5.0 μg/rat) with that of the analogue[Ac-DNal[1],DCαMeCpa[2],DPal[3],DArg[6],ILys[8],D-Ala[10]]GnRH, which had an ED_{50} value of 4.0 μg/mL (100% inhibition at 1.0 μg/rat[8]). This comparison suggests that ILys is slightly more favorable in both the AOA and histamine assay than Har(bCN) in position 8 of this series. Replacement of the DArg[6] of **21** with a second DHar(bCN) in position 6 to afford **17** decreased histamine release potency from an ED_{50} of 6.2 μg/mL to one of ca. 50 μg/mL, respectively, although the GnRH antagonist potency was adversely affected with this substitution (80% inhibition at 1.0 μg/rat for **21** to 45% inhibition at 2.5 μg/rat for **17**). However, substitution of Lys(atz) in positions 6 and 8 yielded **18**, an even more potent analogue than **17** in the AOA (88% inhibition at 2.5 μg/rat) and less potent in the histamine release assay (ED_{50} > 150 μg/mL).

Replacement of the DArg[6] with DPal[6] as in **24** also lowered the histamine response (ED_{50} = 58 μg/mL), to the same degree as the substitution with DHar(bCN)[6] (**17**). However, in this latter case a concomitant increase of potency in the AOA was observed (80% inhibition at 1.0 μg/rat). When DOrn(atz) was substituted in position 3 (**28**) of this series of DPal[6] analogues, the histamine response was not influenced (ED_{50} = 45 μg/mL) as was the GnRH antagonist potency (17% inhibition at 1.0 μg/rat and 86% at 2.5 μg/rat). The histamine release potency of two members of the DPal[6] series, **24** and **28**, was less than that of [Ac-DNal[1],DCpa[2],DPal[3,6],DAla[10]]GnRH, which had an ED_{50} = 9.8 ± 1.5 μg/mL.[27b] The two DPal[6] analogues which were tested in the in vitro histamine release assay both had large ED_{50} values. Thus again, the effect of the ILys[8] substitution for the Arg[6] appears to enhance these values. The data in the last column of Table IV suggest that substitution of cyanoguanidino and *N*-triazolyl residues in both the DArg[6] and DPal[6] series (Table IV) had no adverse affect on histamine release, and in most cases actually appeared to lower histamine release.

In summary, we have successfully demonstrated the applicability of a general synthetic method for modifying basic amino-containing residues on a resin-bound peptide. Relatively high levels of GnRH antagonistic potency were retained and, as hypothesized, the histamine-releasing potency of some potent analogues in the AOA was reduced upon introduction of these less basic residues. Introduction of these pI altering cyanoguanidino- and *N*-aminotriazolyllysine residues can be used to begin to assess the role of the basic residues in most bioactive peptides. Furthermore, the modifications presented here may also confer enzymatic stability to arginine- and lysine-containing peptides. The flexibility of this method, combined with its procedural simplicity and good yields, enhances its applicability to peptide synthesis for the study of structure–activity relationships.

Experimental Section

Instruments. Amino acid analyses [after 4 M methanesulfonic acid hydrolysis at 110 °C for 24 h] were performed on a Perkin-Elmer (Norwalk, CT) high-pressure liquid chromatograph using *o*-phthalaldehyde postcolumn derivatization and fluorescence detection. Preparative HPLC were run using a Waters Assoc. (Milford, MA) Prep LC/System 500A and Model 450 variable-wavelength UV detector, Fisher (Lexington, MA) Recordall Model 5000 strip-chart recorder and a Waters Prep LC 500A preparative gradient generator. Analytical HPLC were run on a system using two Waters M-45 pumps, a Shimadzu Chromatopac EIA integrator, and a rheodyne Model 7125 injector. The peptide synthesizer used was Beckman Model 990. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter. All melting points are uncorrected.

Starting Materials. Amino acid derivatives Boc-D-Ala, Boc-Arg(Tos), Boc-Leu, Boc-Orn(Fmoc), Boc-D-Orn(Fmoc), Boc-Lys(Fmoc) and Boc-D-Lys(Fmoc), Boc-Pro, Boc-Ser(Bzl), Boc-D-Trp, and Boc-Tyr(2,6-Cl_2Bzl) were obtained from Bachem Inc. (Torrance, CA). Boc-D-Nal, Boc-D-Cpa, Boc-D-Pal, Boc-ILys, Boc-NicLys, and Boc-D-NicLys were synthesized at the Southwest Foundation for Biomedical Research (under Contract NO1-HD-6-2928 with NIH) and made available by the Contraceptive Development Branch, Center for Population Research, NICHD. Boc-L- and Boc-D-[4-*N*-(Fmoc)amino]phenylalanine (Aph(Fmoc)) were synthesized from Boc-L- or Boc-D-4-nitrophenylalanine obtained from Bachem Inc. (Torrance, CA) as described below for the L isomer. The methylbenzhydrylamine resin used for peptide synthesis was obtained according to published procedures.[9] Resins with substitutions varying from 0.4 to 0.9 mequiv/g were used with no observable differences. All solvents were reagent grade. Diphenyl cyanocarboimidate (PCI) was prepared via a two-step procedure from diphenyl carbonate as described below.

Peptide Synthesis. The resin-bound peptides incorporating the Fmoc-protected amino functions were synthesized by SPPS methodology on a Beckman 990 peptide synthesizer with use of previously described protocols on a methylbenzhydrylamine (MBHA) resin (1–2.5 g per peptide) using *tert*-butyloxycarbonyl groups for N^{α}-amino protection. TFA treatment was extended to two times 15 min. Coupling time was 90–120 min following by acetylation (excess acetic anhydride in CH_2Cl_2 for 15 min). A 3-fold-excess protected amino acid was used based on the original substitution of the methylbenzhydrylamine-resin. N-Terminal acetylation was performed using the same protocol as that used for capping.

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1,1-Dichloro-1,1-diphenoxymethane. According to the method of Webb and Labaw[14] for the preparation of diphenyl cyanocarboimidate, diphenyl carbonate (428 g, 2.0 mol) was melted in a three-necked flask equipped with a thermometer, overhead stirrer, and a Vigreux column at 140 °C. PCl_5 (458 g, 2.0 mol) was added in portions, and then the mixture was heated at 155 °C for 16 h. The product was distilled under vacuum to afford 417 g (78% yield) of 1,1-dichloro-1,1-diphenoxymethane [bp 162 °C (5 mmHg)].

Diphenyl Cyanocarboimidate. A solution of cyanamide (138 g, 3.3 mol) in ethyl acetate (500 mL) was added dropwise to a solution of 1,1-dichloro-1,1-diphenoxymethane (416 g, 1.5 mol) in ethyl acetate (500 mL) at a rate such that the temperature of the pot remained below 60 °C. After the addition was complete (3.5 h), the mixture was filtered and precipitate was collected and dried. The white precipitate was then stirred with 2 L of water, and Na_2CO_3 was added until pH 8 was reached. The precipitate was then filtered, washed with water, and dried to afford 181 g (51% yield) of diphenyl cyanocarboimidate (mp 156–158 °C; lit.[14] mp 156–158 °C) as a white fluffy solid.

N^{α}-Boc-4-nitro-L-phenylalanine. Commercially available 4-nitro-L-phenylalanine (25 g, 0.11 mol) was treated with di-*tert*-butyl dicarbonate (28.7 g, 0.132 mol) using conditions described previously[9] to give N^{α}-Boc-4-nitro-L-phenylalanine (29.1 g, 0.095 mol, 95%): mp 114–116 °C; $[\alpha]_D$ = +7.9° (c = 1, MeOH) [lit.[28] mp 110–112 °C; $[\alpha]_D$ = +8.0° (c = 1, MeOH)].

N^{α}-Boc-4-amino-L-phenylalanine. The Boc nitro amino acid (29.0 g, 0.095 mol) was reduced using 10% Pd/C (1 g) instead of Raney nickel following the method of Landis et al.[28] to give the desired amino acid (23.4 g, 0.0836 mol, 88%): mp 123–125 °C; $[\alpha]_D$ = +24.3° (c = 1, MeOH) [lit.[28] mp 130–132 °C; $[\alpha]_D$ = +23° (c = 1, MeOH).

N^{α}-Boc-4-[*N*-(9-fluorenylmethoxycarbonyl)amino]-L-phenylalanine. Boc-4-amino-L-phenylalanine (23.0 g, 0.0836 mol) in dioxane/water (1:1; 200 mL) was dissolved by addition of 1 M NaOH to pH 9. To this solution was added 9-fluorenylmethyl succinimidyl carbonate (33.8 g, 0.1 mol) in dioxane (200 mL) over a 40-min period. The solution pH was maintained at 9 using a pH-stat (delivering 1 M NaOH). After being stirred overnight, the dioxane was removed by rotary evaporation and the remaining aqueous solution extracted with ethyl acetate (2 × 300 mL). Acidification with $NaHSO_4$ to pH 2.5 followed by ethyl acetate extraction (2 × 300 mL), drying ($MgSO_4$), and solvent removal gave an oil. Crystallization in petroleum ether (bp 30–60 °C) gave the desired product (40 g, 0.79 mol, 94%: mp 109–113 °C; $[\alpha]_D$ = +23.7° (c = 1, EtOAc).

[Ac-DNal^1,DCpa^2,DPal^3,Har(bCN)^5,DHar(bCN)^6,ILys^8,DAla^{10}]GnRH (3). Ac-DNal-DCpa-DPal-(OBzl)Ser-(Fmoc)Lys-D(Fmoc)Lys-Leu-ILys-Pro-DAla-MBHA-resin (4 g, 0.52 mmol/g substitution) was treated with 10 mL of a solution of 20% piperidine (2 × 10 min followed by sequential washes with DMF, MeOH, 10% TEA/CH_2Cl_2 and CH_2Cl_2 washings (2 × 1 min each). Diphenyl cyanocarboimidate (PCI, 2.4 g, 10 mmol) was then added to the deblocked peptide resin with enough DMF to obtain a slurry. The mixture was agitated for 12 h and subjected to the following washing protocol (2 × 1 min for each solvent): MeOH, DMF, MeOH, DMF, MeOH, CH_2Cl_2, MeOH, CH_2Cl_2. After this step, the ninhydrin test was negative. The resin was then treated with 4 mL (excess) of butylamine and DMF was added to form a slurry. This reaction mixture was agitated for 24 h and then washed with MeOH and CH_2Cl_2 (2 × 1 min). After vacuum drying, the peptide was cleaved from the resin by treatment with anhydrous HF (100 mL) and anisole (4 mL) for 1.5 h at 0 °C. The HF and anisole were removed by use of aspirator and/or high vacuum. The peptide residue was treated with ether and filtered. Extraction of the peptide with water followed by lyophilization yielded a fluffy, white solid (0.4 g). Analogues **7**, **9**, **14**, **16**, **17**, **19**, **21**, and **24–26** were prepared via the procedure just described using appropriately substituted resins. Analogue **1** was prepared using a similar procedure except that methylamine (2 mL) was added to the product from the PCI reaction and the mixture was stirred at 0 °C for 48 h. Analogues **2**, **4**, and **5** were prepared by the procedure just described for **3** except that the product from the PCI reaction was reacted with isopropylamine, hexylamine, and cyclohexylamine, respectively.

[Ac-DNal^1,DCpa^2,DPal^3,Har(2mpCN)^5,DHar(2mpCN)^6,ILys^8,DAla^{10}]GnRH (6). The procedure as outlined above for the preparation of **3** was followed except that the resin obtained after treatment with PCI was treated with 2 mL (excess) of 2-(aminomethyl)pyridine/DMF and after 48 h of agitation, the mixture was washed (2 × 1 min DMF) and another portion of the amine (2 mL) was added followed by agitation for another 48 h to insure complete reaction of the more hindered and less reactive amine. The peptide was then washed and cleaved from the resin as described previously to afford 0.4 g of a fluffy, white solid. Analogue **8** was prepared using the procedure just described.

[Ac-DNal^1,DCpa^2,DPal^3,Lys(atz)^5,DLys(atz)^6,ILys^8,DAla^{10}]GnRH (10). The procedure as outlined above for the preparation of **3** was followed except that the resin obtained after treatment with PCI was treated with 2 mL (excess) of hydrazine and then agitated for 8–12 h. The reaction mixture was then washed, cleaved with HF, and lyophilized as previously described to yield 0.4 g of a white fluffy solid. Analogues **11**, **12**, **18**, **20**, **22**, **27**, and **28** were similarly synthesized.

[Ac-DNal^1,DCpa^2,DPal^3,Har(SbCN)^5,D(SbCN)^6,ILys^8,DAla^{10}]GnRH (13). The procedure as outlined above for the preparation of **3** was followed except that the resin obtained after treatment with PCI was treated with 1 mL of 1-butanethiol (13 mmol) and 1.4 mL (20 mmol) of triethylamine and then agitated for 24 h. The reaction mixture was then washed, cleaved with HF, and lyophilized as previously described to yield 0.4 g of a white fluffy solid.

Purification. The lyophilized, crude peptides were dissolved in 0.25 M triethylammonium phosphate (200 mL), pH 2.25 (TEAP 2.25), and loaded onto a 5 × 30 cm preparative reversed-phase HPLC catridge packed in our laboratory using a Vydac C_{18} silica (330-Å pore size, 15–20-μm particle size). The peptide was eluted with use of a flow rate of 100 mL/min on a Waters Prep 500 System with a mixture of A (TEAP 2.25) and B (60% CH_3CH, 40% A) with an appropriate gradient (90 min) such that retention time was ~45 min. The collected fractions were screened by use of analytical reversed-phase HPLC under isocratic conditions, 0.1% TFA/H_2O at a flow rate of 2.0 mL/min (Vydac C_{18} column, 5 μm, 300-Å pore size; 4.5 × 250 mm). Appropriate fractions were then combined and converted to the acetate salt by loading after dilution (1/1) in water on a preparative reversed-phase HPLC cartridge as described above and eluted with the use of a mixture of solvents A (0.5% AcOH) and B (60% CH_3CN, 40% A) and the following gradient: 20%B (10′) followed by a 20-min gradient to 90%B.

Biological Testing. Histamine release in vitro, binding assay, and AOA were carried out as described in the respective references.[6,14-17]

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(29) **Abbreviations.** IUPAC rules are used for nomenclature except for the following: Ac = acetate; Nal = 3-(2-naphthyl)-alanine; Cpa = 4-chlorophenylalanine; DFpa = D-4-fluorophenylalanine; Pal = 3-(3-pyridyl)alanine; NicLys = nicotinyllysine; ILys = N^{ϵ}-isopropyllysine; aph = 4-aminophenylalanine; mCN = N^{ω}-cyano-$N^{\omega'}$-methyl; iCN = N^{ω}-cyano-$N^{\omega'}$-isopropyl; bCN = N^{ω}-cyano-$N^{\omega'}$-butyl; hCN = N^{ω}-cyano-$N^{\omega'}$-hexyl; chCN = N^{ω}-cyano-$N^{\omega'}$-cyclohexyl; 2mpCN = N^{ω}-cyano-$N^{\omega'}$-2-methylpyridyl; Lys(atz) = N^{ϵ}-[5′-(3′-amino-1′*H*-1′,2′,4′-triazolyl)]lysine; Orn(atz) = N^{δ}-[5′-(3′-amino-1′*H*-1′,2′,4′-triazolyl)]ornithine; Aph(atz) = 4-*N*-[5′-(3′-amino-1′*H*-1′,2′,4′-triazolyl)]phenylalanine; bur = butylisoureido.

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Registry No. 1 (free base), 130883-26-0; 1·xHOAc, 134485-04-4; 2 (free base), 134457-18-4; 2·xHOAc, 134485-05-5; 3 (free base), 134457-19-5; 3·xHOAc, 134485-06-6; 4 (free base), 134457-20-8; 4·xHOAc, 134485-07-7; 5 (free base), 134457-21-9; 5·xHOAc, 134457-40-2; 6 (free base), 134457-22-0; 6·xHOAc, 134457-42-4; 7 (free base), 134457-23-1; 7·xHOAc, 134457-43-5; 8 (free base), 134457-24-2; 8·xHOAc, 134485-08-8; 9 (free base), 134457-25-3; 9·xHOAc, 134457-44-6; 10 (free base), 134457-26-4; 10·xHOAc, 134485-09-9; 11 (free base), 134457-27-5; 11·xHOAc, 134457-45-7; 12 (free base), 134457-28-6; 12·xHOAc, 134485-10-2; 13 (free base), 134457-29-7; 13·xHOAc, 134457-46-8; 14 (free base), 134457-30-0; 14·xHOAc, 134457-47-9; 14·xTFA, 134457-64-0; 15 (free base), 134457-48-0; 15·xHOAc, 134457-49-1; 16 (free base), 134457-31-1; 16·xHOAc, 134457-50-4; 17 (free base), 134457-32-2; 17·xHOAc, 134457-51-5; 18 (free base), 134457-33-3; 18·xHOAc, 134457-52-6; 19 (free base), 134457-34-4; 19·xHOAc, 134485-11-3; 20 (free base), 134457-35-5; 20·xHOAc, 134457-53-7; 21 (free base), 134457-54-8; 21·xHOAc, 134457-55-9; 22 (free base), 134457-36-6; 22·xHOAc, 134485-12-4; 23 (free base), 134457-56-0; 23·xHOAc, 134457-57-1; 24 (free base), 134457-58-2; 24·xHOAc, 134457-59-3; 25 (free base), 134485-03-3; 25·xHOAc, 134485-13-5; 26 (free base), 134457-37-7; 26·xHOAc, 134457-61-7; 27 (free base), 134457-38-8; 27·xHOAc, 134457-62-8; 28 (free base), 134457-39-9; 28·xHOAc, 134457-63-9; GnRH, 33515-09-2; BOC-Phe(4-NO_2)-OH, 33305-77-0; BOC-Phe(4-NH_2)-OH, 55533-24-9; BOC-Phe(4-NHFmoc)-OH, 114346-31-5; $(PhO)_2C{=}NCN$, 79463-77-7; $BuNH_2$, 109-73-9; $MeNH_2$, 74-89-5; *i*-$PrNH_2$, 75-31-0; $Me(CH_2)_5NH_2$, 111-26-2; c-$C_8H_{11}NH_2$, 108-91-8; BuSH, 109-79-5; 2-pyridinemethanamine, 3731-51-9; histamine, 51-45-6.

Supplementary Material Available: Table of MS characterization data of GnRH antagonists (1 page). Ordering information is given on any current masthead page.

Synthesis and Biological Activity of Angiotensin II Analogues Containing a Val-His Replacement, Valψ[CH(CONH$_2$)NH]His

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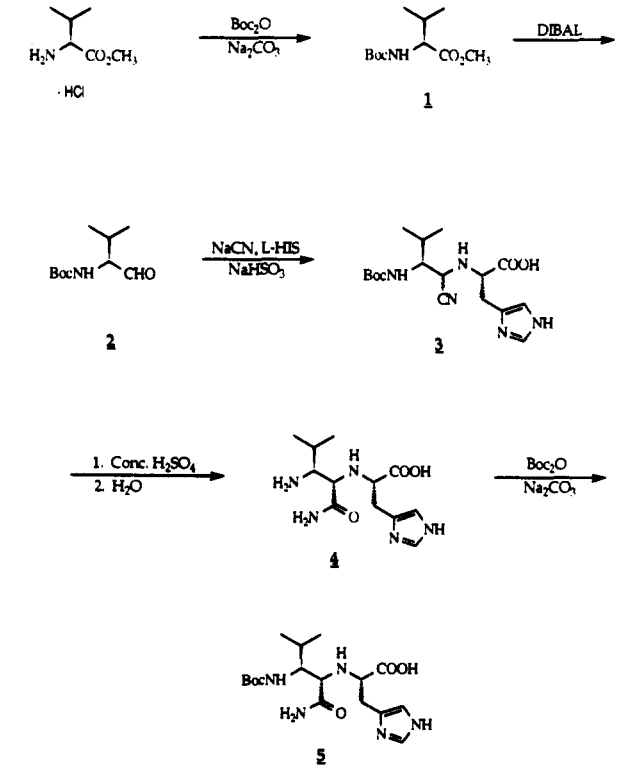
The dipeptide mimic Valψ[CH(CONH$_2$)NH]His (**4**) was incorporated into angiotensin II (AII) analogues to provide an octapeptide saralasin derivative (**29**) as well as tetrapeptide analogue **19**. Three C-terminal tetrapeptides (**21**, **25**, and **28**) were also prepared. All compounds were tested for their ability to displace ^{3}H-AII from rabbit adrenal gland homogenate and as antagonists of AII and AI on guinea pig ileum. The octapeptide analogue **29** was 700 times less active than the parent peptide **30**. All the C-terminal fragments **19**, **21**, **25**, and **28** have no measurable AII antagonist activity. Of the four tetrapeptide fragments, only **21** showed any appreciable binding activity.

Introduction

The octapeptide angiotensin II (AII) (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) is a potent pressor agent that acts on smooth muscle to elicit its actions.[1] Over 400 analogues of angiotensin II have been synthesized to define the role of various amino acid substituents in the biological response of the parent hormone.[2] [Sar^1,Val^5,Ile^8]-AII (**30**) and [Sar^1,Val^5,Ala^8]-AII (saralasin)[3] (**32**) are two potent antagonists that have resulted from the replacement of the C-terminal Phe by aliphatic amino acids. These compounds, however, still retain transient agonist activity and short in vivo half-lives. Modification of the peptide bonds[4] to make them less susceptible toward hydrolysis while retaining the antagonist activity has not been very successful.[5] One such amide bond replacement has been the synthesis of methyleneamino peptide analogues.[6] One or two replacements of the amide bonds at the N-terminal of **32** by the methyleneamino group does not appreciably alter the activity of the hormone but the activity rapidly decreases as these replacements are extended toward the C-terminal.

In extending this strategy we have utilized a single amide bond replacement at a sterically sensitive region of an-

Scheme I



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