11·HCl, 110827-06-0; 12, 110827-01-5; 12·HCl, 54398-93-5; 13, 73728-55-9; 14, 95264-32-7; 15, 110827-02-6; 16, 55341-64-5; 17, 36272-09-0; 18 (isomer 1), 110827-04-8; 18 (isomer 2), 110827-05-9; 19, 110827-07-1; PNMT, 9037-68-7; Me_3SiCN, 7677-24-9; $SOCl_2$, 7719-09-7; MeMgI, 917-64-6; H_2NOH·HCl, 5470-11-1; 1-fluorenecarboxylic acid, 6276-03-5; 2-fluorenecarboxaldehyde, 30084-90-3; 2-(2-fluorenyl)-2-[(trimethylsilyl)oxy]acetonitrile, 110827-03-7; 2-acetylfluorene, 781-73-7.

Potent, Long-Acting Luteinizing Hormone-Releasing Hormone Antagonists Containing New Synthetic Amino Acids: *N*,*N*'-Dialkyl-D-homoarginines[1]

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Received May 26, 1987

A new series of unnatural amino acids has been prepared and incorporated into antagonistic analogues of luteinizing hormone-releasing hormone (LH-RH), on the basis of the hypothesis that stabilization of a proposed phospholipid membrane interaction might yield analogues with high potency and a prolonged duration of action. Thus a series of N^G,$N^{G'}$-dialkyl-D-homoarginine analogues [H-D-hArg(R_2)-OH; R = Me, Et, Pr, *i*-Pr, Bu, hexyl, cyclohexyl, (Et, Me_2NPr)] was conveniently prepared by semisynthesis from D-Lys using the appropriate dialkylcarbodiimide. A number of the analogues that were prepared by using these new amino acid analogues exhibited very high potency and a prolonged duration of action. One of the most potent members of the series, [N-Ac-D-Nal(2)[1],D-pCl-Phe[2],D-Trp[3],D-hArg(Et_2)[6],D-Ala[10]]LH-RH (detirelix), had an ED_{50} of 0.7 μg in the rat antiovulatory assay when administered at noon on proestrus and only 2.5 μg when administered 24 h earlier, at noon on diestrus II. This antagonist is undergoing detailed biological and clinical evaluation.

During the course of the numerous synthetic studies directed toward the understanding of the relationship between structure and biological activity of analogues of luteinizing hormone-releasing hormone (LH-RH; <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), the importance of hydrophobic substitutions for the Gly residue in position 6 was recognized.[2-4] Thus, substitution of the D form of natural amino acids yielded higher potencies as the hydrophobicity of the side chain increased[5,6] (e.g., [D-Trp[6],Pro[9]-NHEt]LH-RH;[5] 100 × LH-RH potency, rat estrus suppression assay[7]). Unnatural D-amino acids of substantially greater hydrophobicity[7-10] were then incorporated, and even higher potencies resulted (e.g., [D-Nal(2)[6]]LH-RH; nafarelin; 200 × LH-RH potency, rat estrus suppression assay[8]). Quantitative structure–activity relationships (QSAR) were derived[11,12] from earlier data which confirmed that the most potent monosubstituted LH-RH agonists should be those with substitutions substantially more hydrophobic than D-Trp. These potency increases were variously ascribed to increased receptor binding[13-15] and/or protection from proteolysis.[16-19] However, in the case of nafarelin, a significant role is played by this molecule's ability to bind to serum albumin[20] and phospholipid membranes (P. Felgner, unpublished). The efficient association with serum albumin (80% bound by equilibrium dialysis measurements), presumably with the hydrophobic binding site on serum albumin, is thought to play a role[21] in the prolonged biological half-life ($t_{1/2}$ = 2.4–3.3 h in women[22] for a dose of 5 μg, sc) of nafarelin. This complex may serve as a depot for the drug which protects it from proteolysis and clearance through the kidney. Thus, a hydrophobic depot effect may be important for highest potency in the LH-RH agonist series.

Since competitive antagonists of LH-RH must be present at the receptors continuously in order to compete with endogenous pulses of LH-RH, a prolonged biological $t_{1/2}$ is even more critical than for LH-RH agonists. Increased hydrophobicity at position 6 as well as high global hydrophobicity evolved as the formula for highest potency in the antagonist series also.[23-25] For example, [*N*-Ac-

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(1) Contribution No. 207 from the Institute of Bio-Organic Chemistry, Syntex Research. Some of these data were presented in a preliminary form at the 8th American Peptide Symposium, Tucson, AZ, May 1983. The abbreviations for natural amino acids and protecting groups followed the recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature [*Eur. J. Biochem.* 1984, *138*, 9). The N^G,$N^{G'}$-dialkylhomoarginines are abbreviated hArg(R_2) for the convenience of the readers, with the appropriate alkyl residue inserted. Other abbreviations used are the following: Nal(2), 3-(2-naphthyl)alanine; Pal(3), 3-(3-pyridyl)alanine; Pal(4), 3-(4-pyridyl)alanine; pX-Phe, *p*-halophenylalanine.
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Scheme I

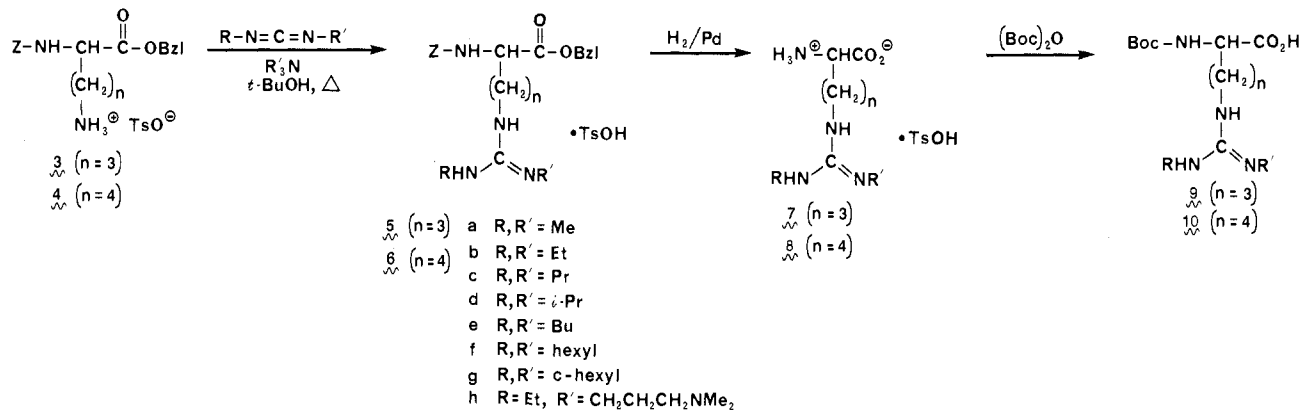

Table I. Amino Acid Intermediates[a]

no.	yield, %	$[\alpha]^{25}_D$ (c, MeOH), deg	formula	M_r[b]	anal.
6b	67	12.9 (0.1)	$C_{33}H_{44}N_4O_7S$	640.80	C, H, N[c]
6c	90	10.9 (0.4)	$C_{35}H_{48}N_4O_7S \cdot 0.5H_2O$	677.88	C, H, N
6d	72	4.7 (1.0)	$C_{35}H_{48}N_4O_7S$	668.87	C, H, N
6e	79	10.9 (0.6)	$C_{37}H_{54}N_4O_7S$	696.91	C, H, N
6f	72	10.1 (0.4)	$C_{41}H_{59}N_4O_7S$	752.01	C, H, N
6g	87	8.1 (0.9)	$C_{41}H_{55}N_4O_7S$	747.98	C, H, N
8b	100	−6.0 (0.1)	$C_{18}H_{32}N_4O_5S \cdot 1.5H_2O$	443.57	C, H, N[d]
8c	100	−7.1 (0.4)	$C_{20}H_{36}N_4O_5S \cdot H_2O$	462.61	C, H, N[e]
8d	100	−3.5 (0.3)	$C_{20}H_{36}N_4O_5S \cdot H_2O$	462.61	C, H, N[f]
8e	91	−6.3 (0.5)	$C_{22}H_{40}N_4O_5S \cdot 0.5H_2O$	478.61	C, H, N
8f	100	−8.3 (0.3)	$C_{26}H_{48}N_4O_5S \cdot 2H_2O$	564.79	C, H, N
8g	100	−7.6 (0.1)	$C_{26}H_{44}N_4O_5S \cdot H_2O$	542.74	C, H, N
9a	46	−15.6 (1)	$C_{13}H_{26}N_4O_4 \cdot H_2O$	320.40	C, H, N
10a	65	−19.5 (0.4)	$C_{14}H_{28}N_4O_4 \cdot 0.3H_2O$	321.81	C, H, N
10b	91	−7.7 (0.3)	$C_{16}H_{32}N_4O_4 \cdot 1.7HOAc$	444.54	C, H, N
10c	94	−11.3 (0.4)	$C_{18}H_{36}N_4O_4 \cdot HOAc$	432.57	C, H, N
10d	73	−1.2 (0.7)	$C_{18}H_{36}N_4O_4 \cdot HOAc \cdot 0.5H_2O$	441.58	C, H, N
10e	94	−11.1 (1.0)	$C_{20}H_{40}N_4O_4 \cdot HOAc$	460.62	C, H, N
10f	93	−6.8 (0.3)	$C_{24}H_{48}N_4O_4 \cdot 2HCl$	529.59	C, H, N[g]
10g	94	−0.7 (0.7)	$C_{81}H_{52}N_4O_7S \cdot H_2O$	642.86	C, H, N

[a] All products were obtained as noncrystalline foams. [b] Calculated for the unsolvated amino acid derivatives. [c] Calcd: C, 61.85; H, 6.92; N, 8.75. Found: C, 61.36; H, 7.35; N, 9.24. [d] Calcd: C, 48.74; H, 7.95; N, 12.63. Found: C, 48.90; H, 7.24; N, 10.93. [e] Calcd: C, 51.92; H, 8.28; N, 12.11. Found: C, 52.19; H, 7.87; N, 11.76. [f] Calcd: C, 51.92; H, 8.28; N, 12.11. Found: C, 52.13; H, 7.82; N, 12.15. [g] Calcd: C, 54.43; H, 9.52; N, 10.58. Found: C, 54.44; H, 9.20; N, 10.06.

Pro[1],D-pF-Phe[2],D-Nal(2)[3,6]]LH-RH[9] (1) and similar, very hydrophobic structures[23] at one time were the most potent LH-RH antagonists, but had to be administered in corn oil (co) or propylene glycol/saline mixtures (pg) due to their low water solubility. A study designed to provide more water-soluble structures[26] led to [*N*-Ac-D-pCl-Phe[1],D-pCl-Phe[2],D-Trp[3],D-Arg[6],D-Ala[10]]LH-RH (**2**), in which a D-Arg residue replaced the usual hydrophobic, aromatic residue in position 6. This compound was surprisingly potent (78% block of ovulation at a 3-μg dose, pg, antiovulatory assay[26]) when administered to rats at noon on proestrus, but was more unusual in its longevity of action.[24,25] Thus, while the ED_{50} for **2** was 1.7 μg for dosing at noon on proestrus (PE), the ED_{50} for administration 24 h earlier, at noon on diestrus II (D_{II}), was only 3-fold higher.[25] For compound **1**, in contrast, the ED_{50} on D_{II} was ~40 times the PE ED_{50} dose.

As one explanation for the favorable effects of the D-Arg[6] substitution, we considered the possibility that a critical, negatively charged site[27] in the LH-RH receptor might interact with the positively charged side chain of this residue. However, since longevity of action was a major attribute of the D-Arg[6] substitution, we felt that it was more likely to be due to a hydrophilic type of depoting. This hydrophilic depot effect was hypothesized to be due to an electrostatic interaction between the positively charged guanidine function of the D-Arg residue and the

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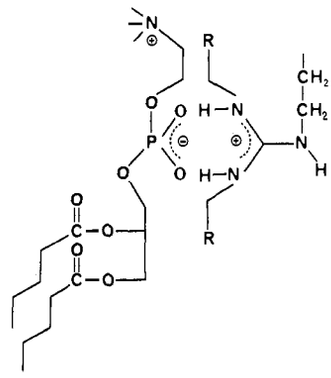

Figure 1. Schematic representation of possible interactions (electrostatic, H-bonding, hydrophobic) between the hArg(R_2) moiety and components of the phospholipid cell membrane. Reprinted with permission from ref 60.

negatively charged phosphate head group of the phospholipid bilayers of cells in the animal.[10]

In order to further stabilize the hypothesized interaction with phospholipid membranes, we designed a series of residues (Scheme I) that have both a potential for electrostatic interaction and the possibility of additional stabilization by hydrophobic interaction with the bilayer (Figure 1). In addition, previous work showed that hydrophobicity in the side chain at position 6 led to highly potent LH-RH agonists and antagonists. Amino acids combining a positively charged side chain and hydrophobic substitutions might therefore be expected to lead to higher potency by a mechanism other than the specific membrane interaction hypothesized above. These $N^G,N^{G'}$-dialkyl-D-arginine or $N^G,N^{G'}$-dialkyl-D-homoarginine residues were incorporated into LH-RH antagonist structures, and the potencies of many of the resulting analogues were determined at two time points in order to assess the effect of these substitutions on the duration of biological effect.

Chemistry. The desired amino acids could be conveniently prepared in their protected form (**9** or **10**) by reaction of a protected form of D-ornithine (**3**) or D-lysine (**4**) with the appropriate dialkylcarbodiimide (Scheme I). Although both $N^G,N^{G'}$-dialkyl-D-arginine (**9**) and $N^G,N^{G'}$-dialkyl-D-homoarginine (**10**) analogues were prepared (Table I), the lower cost of D-lysine caused us to focus primarily on structure **10**. D-Arg(Me_2) was prepared from D-Orn as described[28] and was converted to **9a** with $(Boc)_2O$.[29]

All peptides were synthesized by the Merrifield solid-phase method[30] using (benzhydrylamino)polystyrene-1% divinylbenzene resin.[31] N^{α}-*tert*-Butoxycarbonyl[32] (Boc) protection was used on all amino acids. The side-chain protection of amino acids was as follows: Arg(Tos), Tyr-(2,6-Cl_2Bzl),[33] Ser(Bzl). The side chains of the N,N'-dialkyl-D-arginine (**9**) and -D-homoarginine (**10**) residues were unprotected. Anhydrous liquid HF was used for the final deprotection and cleavage from the resin.[34] The crude peptides were purified by preparative high-performance liquid chromatography (prep-HPLC).[8] Boc-D-Nal(2), Boc-D-pCl-Phe, and Boc-D-pF-Phe were prepared from diethyl acetamidomalonate by a modification[35] of the Sorensen[36] procedure and resolved by using subtilisin Carlsberg as described.[8,37]

During the final deprotection with HF, we noted that analogues containing a secondary carbon attached to the guanidine function underwent partial fragmentation, with loss of the alkyl group. Thus, hArg(c-hexyl$_2$)-containing analogues rather cleanly gave hArg analogues (e.g., **12**), while hArg(iPr_2) analogues were dealkylated to a mixture of hArg(iPr_2)-, hArg(iPr)-, and hArg-containing products (e.g., **34**). This fragmentation was not observed with hArg analogues wherein a primary carbon atom is attached to the guanidine function (e.g., hArg(Me_2), hArg(Et_2), et al.), suggesting the involvement of a carbonium ion in the dealkylation reaction.

Bioassay. The analogues were tested in a standard rat antiovulatory assay[38] using either corn oil (co) or 40% propylene glycol/0.9% saline (pg) as vehicle. Most analogues were administered to the rats at two different times. The ED_{50}'s from administration at noon on PE serve as a measure of acute or short-term potency of the analogues (blockade of the preovulatory LH-RH surge two hours later), while those derived from dosing 24 h earlier, at noon on D_{II}, give a measure of the duration of action of the analogues.

Although the more acute assay has become the literature standard for comparison, the relative potencies of LH-RH antagonists in maintaining efficacy for 24 h may be a more important measure of suitability for therapeutic applications. The potency of many of these analogues was also determined in the PE assay using co as a vehicle for comparative purposes, since studies using this vehicle have been reported in the literature. The use of co may extend the duration of action of some analogues where the partition coefficient favors slow transfer from oil to aqueous phase.

Biological Results and Discussion

As discussed above, members of the class of LH-RH antagonists containing a D hydrophobic amino acid in position 6 (e.g., standard **1**, Table II) had high acute potency, but the analogues with a D-Arg[6] substitution (standards **2** and **11**) had a prolonged duration of action (compare D_{II} ED_{50}'s). Our hypothesis was that this was due to an interaction that was not sterically demanding, i.e., a hydrophilic depot effect due to interaction with phospholipid rather than a specific electrostatic interaction within the receptor. This hypothesis received its first test with analogue **12**, which was obtained inadvertently by dealkylation of the corresponding D-hArg(c-hexyl$_2$) analogue, as described above. This homologue was slightly more potent acutely (PE/pg) and had a roughly equal duration of action compared to the standard **2**, suggesting that significant structural variation in this position was possible.

Although the fragmentation reaction prevented the preparation of the D-hArg(c-hexyl$_2$) analogues by this route, D-hArg(iPr_2), another sterically hindered, hydrophobic analogue, was sufficiently stable to allow purification and evaluation of **34**. This analogue was substantially less potent than **12**, suggesting that the guanidine function in

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Table II. LH-RH Antagonists

no.	LH-RH analogue[a]	$[\alpha]^{25}_D$ (c, HOAc), deg	HPLC:[b] k'	TLC[c] BAW	TLC[c] BEAW	rat antiovulatory assay: ED_{50},[d] μg/inj PE[e] pg[f]	PE[e] co[f]	D_{II}[e]/ co[f]
1	[*N*-Ac-Pro^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH[g]	−50.0 (0.8)	1.72	0.49	0.71	2.2	3.3	140
2	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-Arg^6,D-Ala^{10}]LH-RH[h]		0.82	0.36	0.65	1.7	1.2	5
11	[*N*-Ac-D-Nal(2)^1,D-pF-Phe^2,D-Trp^3,D-Arg^6]LH-RH[g,i]	−31.1 (0.8)	0.58	0.39	0.63	2.4	1.1	9
12	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-hArg^6,D-Ala^{10}]LH-RH	−18.0 (0.5)	0.77	0.36	0.64	1.3	2.0	<8
13	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Me$_2$)^6,D-Ala^{10}]LH-RH	−12.7 (0.3)	1.01	0.29	0.60	1.6	1.0	5.6
14	[*N*-Ac-D-Nal(2)^1,D-pF-Phe^2,D-Trp^3,D-hArg(Me$_2$)^6]LH-RH	−26.5 (0.3)	0.74	0.26	0.53	1.7	0.8	5.0
15	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6,D-Ala^{10}]LH-RH	−18.4 (0.3)	1.47	0.32	0.61	1.7	1.6	3.3
16	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6]LH-RH	−26.0 (0.4)	1.12	0.29	0.57	2.0	2.4	16
17	[*N*-Ac-D-Nal(2)^1,D-pF-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6]LH-RH	−32.6 (0.3)	1.07	0.29	0.60	1.6	1.4	3.5
18	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6]LH-RH	−29.3 (0.4)	1.36	0.28	0.59	2.0	1.0	2.5
19	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6,D-Ala^{10}]LH-RH (detirelix[j])	−21.6 (0.3)	1.67	0.32	0.61	0.7	0.5	2.5
20	[*N*-Ac-D-Nal(2)^1,D-pF-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6,D-Ala^{10}]LH-RH	−26.0 (1.0)	1.28	0.30	0.62	0.4		
21	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Nal(2)^3,D-hArg(Et$_2$)^6,D-Ala^{10}]LH-RH	−26.0 (0.1)	3.09	0.30	0.61		1.7	
22	[*N*-Ac-D-Trp^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6]LH-RH	−18.2 (0.3)	0.80	0.25	0.59		3.4	
23	[*N*-Ac-D-Trp^1,D-pCl-Phe^2,D-Nal(2)^3,D-hArg(Et$_2$)^6,D-Ala^{10}]LH-RH	−19.7 (0.1)	1.82	0.27	0.61	5.5		
24	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6,Pro^9-NHEt]LH-RH	−26.6 (0.2)	2.66	0.28	0.62	2.4		>16
25	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6,D-Ala^{10}]LH-RH	−16.0 (0.3)	1.66	0.26	0.59	0.5		≤4
26	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Pal(3)^3,D-Arg^6,D-Ala^{10}]LH-RH[k]		0.50	0.19	0.47	0.5	0.3	
27	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Pal(3)^3,D-hArg(Et$_2$)^6,D-Ala^{10}]LH-RH	−20.8 (1.0)	0.88	0.18	0.43	0.6		>4
28	[*N*-Ac-D-Nal(2)^1,D-pF-Phe^2,D-Pal(3)^3,D-hArg(Et$_2$)^6,D-Ala^{10}]LH-RH	−16.0 (1.1)	0.73	0.15	0.43	0.7		
29	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Pal(4)^3,D-hArg(Et$_2$)^6,D-Ala^{10}]LH-RH	−13.1 (0.8)	0.94	0.20	0.40	1.7		
30	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Pr$_2$)^6,D-Ala^{10}]LH-RH	−17.5 (0.4)	2.17	0.33	0.60	2.6	2.0	14
31	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Pr$_2$)^6]LH-RH	−25.0 (0.4)	1.67	0.31	0.60	2.6	2.3	16
32	[*N*-Ac-D-Nal(2)^1,D-pF-Phe^2,D-Trp^3,D-hArg(Pr$_2$)^6]LH-RH		3.04			2.4	4.7	12
33	[*N*-Ac-D-Nal(2)^1,D-pF-Phe^2,D-Nal(2)^3,D-hArg(Pr$_2$)^6]LH-RH	−15.2 (0.2)		0.30	0.61	7.5		
34	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-hArg(iPr$_2$)^6,D-Ala^{10}]LH-RH					16		>16
35	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Bu$_2$)^6,D-Ala^{10}]LH-RH	−21.7 (1.0)	4.77	0.38	0.64	3.4		>16
36	[*N*-Ac-D-Ser^1,D-pF-Phe^2,D-Trp^3,D-hArg(hexyl$_2$)^6]LH-RH	−4.2 (0.1)	2.26	0.33	0.62	16		
37	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,Orn^4,D-hArg(hexyl$_2$)^6,D-Ala^{10}]LH-RH	−19.7 (0.3)	1	0.32	0.63	>16		
38	[*N*-Ac-D-Nal(2)^1,D-pF-Phe^2,D-Trp^3,D-hArg(Et,Me$_2$NPr)^6]LH-RH	−28.3 (0.6)	4.97	0.11	0.20	>2		
39	[*N*-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et,Me$_2$NPr)^6]LH-RH	−17.9 (0.3)	4.98	0.12	0.20	>2		
40	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6,aza-Gly^{10}]LH-RH	−29.6 (0.3)		0.25	0.66	0.55		9.5
41	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6,Phe^7,D-Ala^{10}]LH-RH	−16.5 (0.5)		0.28	0.68	0.75		8.0
42	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^6,Trp^7,D-Ala^{10}]LH-RH	−17.2 (0.5)		0.26	0.66	1.2		
43	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^5,Tyr^6,D-Ala^{10}]LH-RH	−44.6 (0.1)		0.27	0.66	1.7		
44	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,D-hArg(Et$_2$)^5,D-Nal(2)^6,D-Ala^{10}]LH-RH	−25.7 (1)		0.28	0.66	1.6		
45	[*N*-Ac-D-hArg(Et$_2$)^1,D-pCl-Phe^2,D-Trp^3,D-Nal(2)^6,D-Ala^{10}]LH-RH	−13.0 (0.5)		0.28	0.68	8.0		
46	[*N*-Ac-D-hArg(Et$_2$)^1,D-pCl-Phe^2,D-Trp^3,hArg(Et$_2$)^5,D-Nal(2)^6,D-Ala^{10}]LH-RH	−18.4 (0.4)		0.19	0.51	>16		
47	[*N*-Ac-D-Nal(2)^1,D-pCl-Phe^2,D-Trp^3,hArg(Et$_2$)^5,D-Tyr^6,Trp^7,D-Ala^{10}]LH-RH	−20.3 (0.3)		0.29	0.66	11.5		
48	[*N*-Ac-D-hArg(Et$_2$)^1,D-pCl-Phe^2,D-Trp^3,hArg(Et$_2$)^5,D-Tyr^6,Trp^7,D-Ala^{10}]-LH-RH	−11.2 (0.4)		0.20	0.53	>16		

[a] Unnatural amino acid abbreviations are given in ref 1. The analogues are grouped according to the type of substitution in position 6. Acceptable amino acid analyses were obtained for all LH-RH analogues (see Experimental Section). [b] k' = (retention volume − void volume)/void volume; conditions in Experimental Section. [c] BAW = 1-BuOH/HOAc/H_2O, 4:1:5 (upper phase); BEAW = 1-BuOH/EtOAc/HOAc/H_2O, 1:1:1:1. [d] Based on 10 animals per dose group. [e] Compound was administered either at noon on proestrus (PE) or 24 h earlier on diestrus II (D_{II}). [f] Compound administered as a solution in 50% propylene glycol/0.9% saline (pg) or corn oil (co) vehicle. [g] Reference 25. [h] Reference 26. [i] Reference 42. [j] US Adopted Name (USAN) Council designation. [k] Reference 43. [l] Does not elute under these conditions.

34 may be too shielded to exert its effect. This interpretation is supported by the observation that the corresponding *n*-propyl-substituted analogue **30**, an analogue with similar hydrophobicity (k') but a less sterically hindered guanidine function, is substantially more potent. Analogues containing the *n*-propyl substitution (**32, 33**) show high potency, acutely and in the duration of action assay, but do not appear to offer an improvement over the D-Arg substitution in this assay.

Analogues containing D-hArg(Me$_2$), which has a much less sterically hindered guanidine function than the D-hArg(Pr$_2$) system, were prepared (**13, 14**). These analogues had ED_{50}'s similar to those of their corresponding parent structures (**2** and **11**, respectively). Although it appears that **14** has a greater duration of action than **11** (compare D_{II} data) but not **2** in this assay, another duration of action

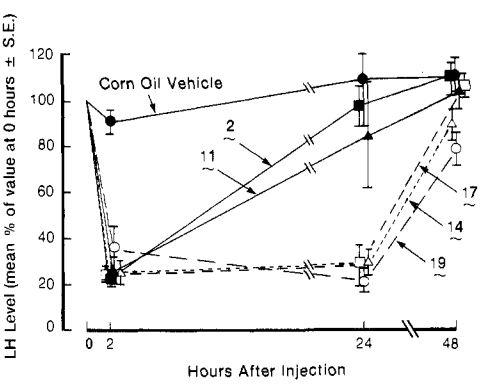

Figure 2. LH levels in castrated male rats following injection of LHRH antagonists at a dose of 50 μg/kg (n = 11 in control group and 5 or 6 in treated groups).[40]

assay (to be discussed below, Figure 2) showed a clear-cut prolongation of action for the D-hArg(Me_2) substitution compared to the standards.

Increasing the length and hydrophobicity of the guanidino substituent [i.e., to D-hArg(Et_2)] resulted in some analogues that had significantly increased potency both acutely and in the duration of action assay. In the *N*-Ac-D-pCl-Phe series, **15** has about equal potency to **2** in the PE assay but is more potent in the D_{II} assay. Presence of the D-Ala substitution in position 10, a modification that presumably blocks the action of post-proline cleaving enzyme, confers substantially increased duration of action (analogue **15** vs **16**). In the *N*-Ac-D-Nal(2) series, the D-hArg(Et_2) substitution gives a significant increase in potency, both on PE and D_{II} (see also Figure 2). Increasing the hydrophobicity in this series by the replacement of D-pF-Phe with D-pCl-Phe gave **18**, an analogue with slightly decreased acute potency, but which exhibits a further increase in its duration of action (D_{II} data). Incorporation of the D-Ala substitution[39] for protection from post-proline cleaving enzyme resulted in **19** (RS-68439; U.S. Adopted Name Council designation: detirelix), which has high potency in both the acute and duration of action assays.[40]

Several of the compounds described above were evaluated in an assay that was designed to more closely represent a therapeutic model and to more precisely assess a compound's duration of action.[41] The analogues were injected (sc, co vehicle) into castrated adult male rats, and the degree and duration of the suppression of circulating blood levels of LH were determined. This assay monitored the effect of a compound on tonic LH secretion. All of the analogues tested maximally suppressed LH values by 2 h after administration (50 μg/kg, co), but the standards (**2**, **11**) had allowed the return of pulsatile LH secretion to the control range by 24 h (Figure 2). In contrast, for the rats receiving the three analogues containing *N,N'*-dialkylhomoarginine residues (**14**, **17**, **19**), LH levels were still maximally suppressed 24 h after dosing. In addition, detirelix caused full suppression at 24 h at only 25 μg/kg.[40]

Additional analogues in the D-hArg(Et_2) series were prepared in order to further investigate structure–activity relationships. When D-Trp is replaced by D-Nal(2) in position 3 (**21**, **23**) or D-Nal(2) is replaced by D-Trp in position 1 (**22**, **23**), significantly less potent analogues result. The D-Ala substitution in position 10 of detirelix was replaced by NHEt or aza-Gly, modifications that are thought to block post-proline cleaving enzyme action and that were effective in some LH-RH antagonists.[10,42-44] The hydrophobic NHEt causes reduced potency (**24**) compared to detirelix. It may be that this analogue is beyond the optimum hydrophobicity for antagonists in this series (at least in this assay). Substitution of the hydrophilic aza-Gly residue in position 10 caused an increase in potency acutely (**40**), but decreased duration of action.

Replacement of the D-Trp residue in position 3 by a 3-(3-pyridyl)-D-alanine [Pal(3)] residue but not by a 3-(4-pyridyl)-D-alanine [Pal(4)] residue leads to increased antiovulatory potency in some antagonists.[45-49] Thus, standard **26** was reported to be twice as potent as the corresponding D-Trp^3 analogue. We incorporated Pal(3) into the detirelix structure to yield **27** and found that this analogue may be slightly less potent than the standard **26** but slightly more potent than detirelix, when assessed in the acute assay. However, **27** is clearly less long-acting than detirelix (D_{II} data and unpublished results), again demonstrating that the perception of "potency" for these LH-RH antagonists may be dependent on the time point examined.

Several other alkyl substitutions on the guanidine function were examined. Further lengthening the alkyl substituents to hArg(Bu_2) and hArg($hexyl_2$) resulted in less potent analogues. Thus **35**, a homologue of detirelix, is significantly less potent in the acute assay, again suggesting either steric hindrance at the guanidine function or a global hydrophobicity of the molecule beyond the optimum for this series. It was anticipated that the hArg($hexyl_2$)-containing homologues of detirelix would be too hydrophobic, so these were combined with hydrophilic modifications in position 1[25] or position 4,[42] which had previously given potent LH-RH antagonists, but without success (**36**, **37**).

Since the addition of one positive charge to the LH-RH antagonist structure had beneficial effects (**2**), we prepared D-hArg(Et,Me_2NPr) from the corresponding "water-soluble carbodiimide". Incorporation of this amino acid, which has two positive charges on the side chain, led to decreased potency, however (**38**, **39**).

Although the residue on position 7 of the LH-RH so far characterized from mammalian species is Leu, in the corresponding "chicken II"[50] and salmon gonadotropin-releasing hormones,[51] this position is occupied by the more hydrophobic Trp. On the basis of this observation, amino acid substitutions were made in position 7 of LH-RH antagonists,[46,52] and potency increases resulted. This may have been due to a modulation of the global hydrophobicity of the molecules since in the analogue series containing the relatively hydrophilic D-Pal(3)[3] substitution the most hydrophobic natural amino acid (Trp) resulted in optimum potency (i.e., [*N*-Ac-D-$Nal(2)^1$,D-$pCl\text{-}Phe^2$,D-$Pal(3)^3$,D-Arg^6,Trp^7,D-Ala^{10}]LH-RH).[46] In the more hy-

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drophobic D-Trp[3] series, the less hydrophobic Phe[7] substitution was optimal (i.e., [*N*-Ac-D-Nal(2)[1],D-pCl-Phe[2],D-Trp[3],D-Arg[6],Phe[7],D-Ala[10]]LH-RH).[52] However, the presently described series containing the D-hArg(Et_2) residue is already more hydrophobic than the parent structure for those analogues, and incorporation of hydrophobic position 7 substitutions (**41**, **42**) results in decreased potency.

Side Effects. During toxicology studies with **11**, the ability of the "D-Arg[6] class" of LH-RH antagonists to cause edema was discovered.[53] These compounds are potent agents for rat peritoneal mast cell degranulation (MCD) in vitro,[54] and this assay has been used to predict the potency of LH-RH antagonists for the production of systemic effects. The rate of entry of a compound into the circulation after sc dosing appears to be important, however. Although **11** and detirelix have similar ED_{50} values for rat mast cell degranulation in vitro, **11** rapidly caused edema in rats at doses of 1.25 mg/animal (sc) while the longer acting, more hydrophobic detirelix shows no edematogenic effects at that dose when administered sc. For detirelix, edema is only found occasionally at doses ≥20 mg/animal (sc) or when administered intravenously at lower doses (C.-H. Lee, unpublished). Evidence for occasional mild, localized effects of histamine release has been observed in clinical trials with **11** (J. E. Hall and W. F. Crowley, Jr., personal communication) and with detirelix (S. Monroe and R. Jaffe, personal communication).

These observations have recently led to the study of modifications designed to minimize the mast cell degranulating potency of these compounds while retaining their antiovulatory potency. A common feature of mast cell degranulating agents (including many neuropeptides) is the presence of multiple positive charges and hydrophobic regions. The "D-Arg[6] class" of analogues tested contained basic residues in positions 6 and 8.

An initial approach toward antagonists with reduced MCD potency was the synthesis of analogues with increased distance between the charges.[55] Thus, the switching of position 5 and position 6 substitutions led to [*N*-Ac-D-Nal(2)[1], D-αMe,pCl-Phe[2],D-Trp[3],Arg[5],D-Tyr[6],D-Ala[10]]LH-RH. This analogue had 10% of the MCD potency of its parent compound with only a 50% decrease in its antiovulatory potency.[56] A similar switch in detirelix results in **43**. This analogue had decreased MCD potency as expected but also suffered a substantial drop in antiovulatory potency. The corresponding analogue with D-Nal(2) in position 6 (**44**) showed a similarly decreased antiovulation potency.

When the hArg(Et_2) residue was moved to the N-terminus, a region characterized in recent analogue series by hydrophobic substitutions, a further drop in antiovulatory potency resulted (**45**). When this N-terminal modification was combined with the position 5, 6 switch, the resulting analogue **46** had a severely reduced antiovulatory potency.

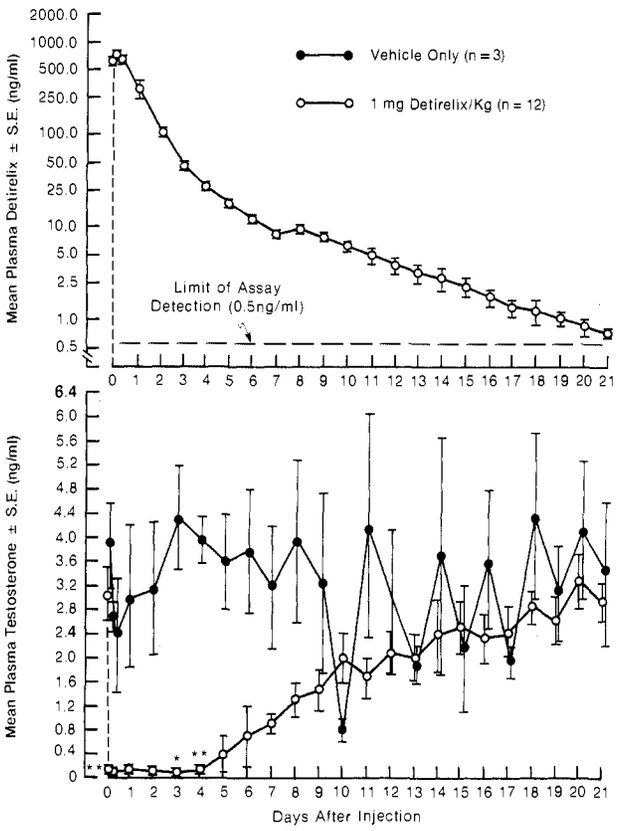

Figure 3. Plasma levels of detirelix and testosterone in male dogs following a single subcutaneous dose of 1 mg/kg of detirelix in aqueous propylene glycol vehicle (*$p < 0.005$; **$p < 0.01$ compared to vehicle group at same time point by t test).

When the hArg(Et_2)[5],D-Tyr[6] substitution pattern was coupled with the more hydrophobic position 7 substitution described above, a substantial antiovulatory potency decrease occurred (**47**). Coupling this substitution pattern with the hArg(Et_2)[1] modification led to a further decreased potency (**48**).

Duration of Action. Members of the "D-Arg[6] class" of LH-RH antagonists have a very long duration of action compared to native LH-RH ($t_{1/2}$ ~ 3 min in rats; ~30 min in humans) and to earlier LH-RH antagonists (e.g., **1**). Thus, while a single dose of 1 mg/kg of **1** to dogs caused suppression of testosterone for 24 h, the same dose of detirelix resulted in castrate levels for 1 week (Figure 3). Moreover, compound could still be detected in the circulation by a specific radioimmunoassay for 3 weeks (C. Nerenberg, unpublished). From clinical studies in men, an apparent $t_{1/2} > 41$ h was calculated.[57]

Although LH-RH antagonists have a slower[58,59] off-rate from the LH-RH receptor ($t_{1/2}$ = 120 min or more) than the LH-RH agonists ($t_{1/2}$ ~ 30 min), the exceptionally prolonged residence time of these agents in the body is probably due to the hypothesized depoting of these agents in phospholipid membranes in the body. The combination of positively charged residues (for electrostatic interaction with phosphate head groups) and very hydrophobic residues (for interaction with the membrane core) should re-

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sult in high membrane affinity. Studies designed to evaluate the membrane affinity of members of this class are under way (P. Felgner, unpublished).

Summary

Although the family of LH-RH antagonists with a nonpolar, hydrophobic amino acid in position 6 exhibited high acute potency (1), the duration of action was unexceptional. The antagonists containing a positively charged, polar amino acid in position 6, in contrast, showed high and sustained potency (2, 11, 12). The introduction of alkyl chains on the guanidine function on the side chain in position 6, which was designed to increase affinity for phospholipid cell membranes, also rendered the side chains more lipophilic. In fact, the most potent members of this "polar" family of LH-RH antagonists, in spite of their additional positive charge, have an apparent lipophilicity similar to that of the earlier hydrophobic antagonists (compare k' of 1 and 19), but higher water solubility. Although it is known that LH-RH antagonists are more slowly released from the pituitary cell receptors than are LH-RH agonists,[58,59] we believe that the remarkably long duration of action of these antagonists is also due in part to a hydrophilic type of depot effect in which binding to membranes prevents rapid clearance of the compound by glomerular filtration. As the guanidine function becomes more hindered by the length or branching of the alkyl chains, however, the duration of action begins to decrease and the analogue behaves more like the earlier "hydrophobic" analogues (e.g., 34, 35).

The most potent members of the polar class of LH-RH antagonists prepared in this study were D-hArg(Et_2)- or D-Arg(Et_2)-containing analogues (19, 20, 25, 27). Because of its high acute potency and very prolonged duration of action (see Figure 2), detirelix (19) was chosen for detailed biological and clinical pharmacology studies. The ability of members of this class of LH-RH antagonists to cause mast cell degranulation has removed them from consideration as candidates for full commercial development. Potential clinical candidates with negligible histamine-releasing potency are the subject of current studies.[54,60]

Experimental Section

General Methods. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter in a 1-dm microcell at 25 °C at the concentration indicated (w/v %). Thin-layer chromatography (TLC) was performed in a solvent vapor saturated chamber on 5 × 20 cm glass plates coated with a 250-μm layer of silica gel GF (Analtech). The plates were visualized by UV absorption followed by chlorination (Cl_2) and 1% KI/starch spray. N,N'-Dialkylcarbodiimides were purchased from Aldrich (R, R' = *i*-Pr; cyclohexyl; Et, Me_2NPr) or prepared as described.[61,62]

Analytical HPLC was performed under isocratic conditions on a Spectra-Physics Model 8700 instrument equipped with a 20-μL loop injector (Rheodyne) and an Altex Ultrasphere 5-μm C-18 reversed-phase column (4.6 × 250 mm). The eluent contained 60% CH_3CN (Burdick and Jackson, UV) and was 0.03 M in NH_4OAc (Fluka, puriss) at pH 7. The column effluent was monitored at 215 nm with a Spectra-Physics Model 8400 detector.

Amino Acid Analysis. Amino acid analyses were performed on a Beckman 119CL analyzer in the single-column mode after 18–24-h hydrolysis in 4 N $MeSO_3H$/0.2% 3-(2-aminoethyl)indole reagent[63] (Pierce). The difficult resolution of pF-Phe, Trp, Arg, hArg(Me_2), and hArg(Et_2) made the analyses less precise than usual, but convincing evidence for the presence of these amino acids was obtained in each case (usually within ±10% but in all cases within ±20%). The buffer sequence pH 3.25 (50 min), pH 4.12 (67 min), pH 6.25 (100 min) was used. Satisfactory amino acid analyses were obtained for compounds 12–39.

Peptide Synthesis. Protected peptides were prepared on a Beckman 990 synthesizer by using a standard program, with 50% CF_3CO_2H/CH_2Cl_2 and 10% Et_3N/CH_2Cl_2 as deprotection and neutralization reagents, respectively. Final deprotection/cleavage was performed with anhydrous (CoF_3), redistilled liquid HF containing 10% anisole as scavenger for 1 h at 0 °C. The crude product was converted to the AcO^- form by passage through a column of the weakly basic anion exchanger AG3 (AcO^- form) in H_2O and was lyophilized. This material was purified by reversed-phase preparative HPLC as described,[8] with a 2.5 × 100 cm column (Altex) packed with 25–40-μm Lichroprep RP-18 (E. Merck) or 20–30-μm Vydac C18 TP. The eluent contained various proportions of CH_3CN (~60%) and H_2O, depending on the hydrophobicity of the compound (k'), but in each case the eluent was 0.03 M in NH_4OAc (pH 7).

The fractions containing the product (analytical HPLC) were pooled and concentrated to dryness. The bulk of the NH_4OAc was sublimed under vacuum from the flask into a Kjeldahl head by use of a 40 °C water bath. The residual traces were removed by lyophilization three times from H_2O to yield the pure product as a fluffy white powder.

Rat Antiovulatory Assay.[38] Adult female Simonsen Albino rats (Sprague–Dawley strain; >180 g) were acclimatized to laboratory conditions (14:10 light/dark with lights on at 5:00 a.m.) for at least 10 days. Daily vaginal lavages were taken from each rat between 7:30 and 9:00 a.m. for at least 12 days. Cytology of vaginal lavages was examined microscopically to determine the stage of the estrous cycle. Rats with at least two normal 4-day cycles preceding the test cycle were selected. The rats were injected subcutaneously with a solution of the analogue in vehicle (50% propylene glycol/0.9% saline or Mazola corn oil).

On the morning of expected estrus, the rats were sacrificed, and the oviducts were removed and examined under a dissecting microscope for the presence of freshly ovulated eggs. The eggs were teased out of the oviducts and counted. The percent of females ovulating was plotted against the log–dose to calculate the ED_{50} for antiovulatory activity. Usually three or more dose groups (data from a total of 10 animals per dose group) were used to determine the ED_{50}, but occasionally two dose groups were found to be sufficient.

General Amino Acid Synthesis. Benzyl N^{α}-(Benzyloxycarbonyl)-N^G,$N^{G'}$-di-*n*-propyl-D-homoargininate·TsOH (6c). A solution of benzyl N^{α}-(benzyloxycarbonyl)-D-lysinate·TsOH[64] (4.87 g, 9 mmol) and N,N'-di-*n*-propylcarbodiimide[61] (1.70 g, 13.5 mmol) in *tert*-butyl alcohol (50 mL) was heated at 100 °C for 18 h. The reaction mixture was concentrated, and the residue was passed through a silica gel column using a CH_2Cl_2/MeOH (19:1) to CH_2Cl_2/MeOH (9:1) gradient. The product was obtained as a homogeneous (TLC; R_f(A) 0.59) white foam (5.4 g; 90% yield).

N^G,$N^{G'}$-Di-*n*-propyl-D-homoargininate·TsOH (8c). A mixture of 6c (4.9 g, 7.3 mmol) and 0.8 g of 10% Pd/C in 100 mL of EtOH was treated with H_2 at atmospheric pressure for 4 h. The reaction mixture was filtered through Celite and concentrated to give the product as a white foam (3.25 g, 100% yield).

N^{α}-(*tert*-Butoxycarbonyl)-N^G,$N^{G'}$-di-*n*-propyl-D-homoargininate·AcOH (10c). A solution of 8c (2.25 g, 5.1 mmol) in a mixture of 10.2 mL of 1 N NaOH, 10 mL of H_2O, and 20 mL of dioxane was treated at 0 °C with MgO (0.21 g, 5.1 mmol) and $(Boc)_2O$ (1.21 g, 5.6 mmol). The reaction mixture was stirred at 0 °C or 1 h and room temperature for 1 h. The mixture was filtered and concentrated in vacuo to give an oil. The oil was partitioned between Et_2O and H_2O. The aqueous layer was adjusted to pH 4 with 1 N HCl and concentrated to dryness in vacuo. The pure product was obtained by silica gel chromatography using CH_2Cl_2/MeOH/AcOH (9:1:1) as eluent. The appropriate fractions (TLC) were pooled and concentrated, and the product was stirred with AG3 (AcO^- form) in MeOH solution.

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The MeOH solution was filtered and concentrated to yield the product as a white foam (2 g, 77% yield).

N^{α}-(*tert*-Butoxycarbonyl)-N^{G},$N^{G'}$-dimethyl-D-arginine (9a). N^{G},$N^{G'}$-Dimethyl-D-arginine (**7a**) was synthesized as described.[28] The crude product was not purified, but was converted directly to the N-protected compound **9a** as described for **10c**.

Registry No. 1, 82778-58-3; **2**, 81608-49-3; **4**, 89662-37-3; **6b**, 98500-63-1; **6c**, 98500-64-2; **6d**, 89662-39-5; **6e**, 98500-66-4; **6f**, 98500-70-0; **6g**, 98500-61-9; **7a**, 110797-83-6; **8b**, 110798-01-1; **8c**, 110798-02-2; **8d**, 110798-03-3; **8e**, 110798-04-4; **8f**, 110798-05-5; **8g**, 110798-06-6; **9a**, 110797-84-7; **10a**, 98500-76-6; **10b**, 110798-07-7; **10c**, 110798-08-8; **10d**, 110798-09-9; **10e**, 110798-10-2; **10f**, 110798-11-3; **10g**, 110798-12-4; 11, 86855-16-5; **12**, 90684-94-9; **13**, 89662-32-8; 14, 89662-33-9; **15**, 89662-29-3; **16**, 89662-27-1; **17**, 89662-28-2; **18**, 89680-24-0; **19**, 89662-30-6; **20**, 110797-85-8; **21**, 110797-86-9; **22**, 110797-87-0; **23**, 110850-65-2; **24**, 110797-88-1; **25**, 110797-89-2; **26**, 93128-18-8; **27**, 110797-90-5; **28**, 110825-66-6; **29**, 110797-91-6; **30**, 89662-20-4; **31**, 89662-21-5; **32**, 89662-22-6; **33**, 89662-18-0; **34**, 89662-13-5; **35**, 110797-92-7; **36**, 89662-25-9; **37**, 110797-93-8; **38**, 89680-25-1; **39**, 89662-31-7; **40**, 106916-57-8; **41**, 106881-68-9; **42**, 110797-94-9; **43**, 110797-95-0; **44**, 110797-96-1; **45**, 110797-97-2; **46**, 110797-98-3; **47**, 110797-99-4; **48**, 110798-00-0; DCC, 538-75-0; EtN=C=NEt, 693-29-8; PrN=C=NPr, 821-79-4; *i*-PrN=C=NPr-*i*, 693-13-0; BuN=C=NBu, 693-64-1; $H_3C(CH_2)_5N{=}C{=}N(CH_2)_5CH_3$, 13296-55-4.

Ring-Substituted [1,2-Bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) Complexes: Compounds with a Selective Effect on the Hormone-Dependent Mammary Carcinoma

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[1,2-Bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes with one substituent in the 2-position (CH_3, CF_3, F, Cl, Br, I: *meso*- and *d,l*-**1**-$PtCl_2$, *meso*-(**3–5**)-$PtCl_2$, *meso*-(**7** and **8**)-$PtCl_2$) or two substituents in the 2,6-positions (CH_3, Cl: *meso*-**2**-$PtCl_2$, *meso*- and *d,l*-**6**-$PtCl_2$) in both benzene rings were synthesized and tested for estrogenic and cytotoxic activities. Two complexes (*meso*-**6**-$PtCl_2$ and *meso*-**7**-$PtCl_2$) possess both effects. In comparative tests on estrogen receptor positive and negative mammary tumors in cell culture (MCF 7, ER^+ and MDA-MB 231, ER^-) and in animals (MXT, ER^+ and MXT, ER^-, mouse), *meso*-**6**-$PtCl_2$ shows a selective effect on the estrogen receptor positive mammary carcinoma. A further increase of efficacy was achieved with the water-soluble (sulfato)platinum(II) derivative (*meso*-**6**-$PtSO_4$). On the DMBA-induced hormone dependent mammary carcinoma of the SD rat, *meso*-**6**-$PtSO_4$ is significantly more active than its ligand (*meso*-**6**) and cisplatin.

Platinum complexes that contain an estrogen receptor (ER) affinic ligand should be enriched in the nuclei of hormone-dependent breast cancer cells by the receptor system, thereby causing a selective effect on this tumor (Figure 1). The first compounds of this type, the stereoisomeric [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes, possess a marked activity on several ER-negative tumor models, e.g., ADJ/PC 6 plasmacytoma/mouse and P 388 leukemia/mouse,[1] but only a low activity on the 9,10-dimethyl-1,2-benzanthracene (DMBA) induced, hormone-dependent mammary carcinoma of the Sprague–Dawley (SD) rat ((±) compound: 6 × 10 mg/kg per day, ip, duration of therapy 4 weeks, increase of tumor area 238%, control 686%).[2] To achieve a stronger mammary tumor inhibiting activity, we introduced CH_3, CF_3, or Hal residues into positions 2 or 2,6 of both benzene rings of [1,2-bis(4-hydroxyphenyl)-ethylenediamine]dichloroplatinum(II), since by this kind of substitution in the class of 1,2-bis(4-hydroxyphenyl)-ethanes an increase of ER affinity was accomplished.[3] In addition we have shown that the introduction of two Cl atoms into 2,6-positions of *N,N'*-dialkyl-1,2-bis(4-hydroxyphenyl)ethylenediamines leads to compounds that have a high binding affinity to ER and a strong inhibitory effect on the DMBA-induced, hormone-dependent mammary carcinoma of the SD rat.[4]

Chemistry. The diastereomeric dichloroplatinum(II) complexes 1-$PtCl_2$ to 8-$PtCl_2$ were synthesized by reacting K_2PtCl_4 with the 2- and 2,6-substituted 1,2-bis(4-hydroxyphenyl)ethylenediamines **1–8** in *t*-BuOH/H_2O or DMF/H_2O solution at pH 5–6 and a temperature below 40 °C (Scheme I, methods A and B).

Owing to steric facts, the formation of meso-configurated complexes proceeds very slowly and requires a reaction time up to 3 days. The analytical data are listed in Table I. The new compounds 1-$PtCl_2$ to 8-$PtCl_2$ show IR spectra typical for [diamine]dichloroplatinum(II) complexes: (1) the N–H stretching vibration is decreased due to the formation of the metal–nitrogen bond (free ligand ν NH = 3400–3300 cm^{-1}; Pt bond ligand ν NH = 3300–3100 cm^{-1}); (2) two absorption bands appear in the far infrared region, one between 650 and 450 cm^{-1}, indicating a Pt–N stretching vibration, and another between 345 and 320 cm^{-1}, indicating a Pt–Cl stretching vibration.[5] The ^1H

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