Antiovulatory Antagonists of LHRH Related to Antide

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We report 104 analogues of the potent antiovulatory antagonist of LHRH, N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Lys(Nic)-D-Lys(Nic)-Leu-Ilys-Pro-D-Ala-NH$_2$, Antide. We replaced the Nic group in Antide with other acyl substituents to modulate size, hydrophilicity or basicity of the molecule, we also replaced the Lys residues with shorter basic amino acids, and made cyclic 5/6 analogues as well as position 5 or 6 dimers. We substituted Ilys^8 with other alkyl groups and acyl derivatives. When injected in 0.1% DMSO in water in a typical antiovulatory (AO) assay, Antide gives six rats ovulating out of eight (6/8) at 2 µg, 4/8 at 4 µg, and in the histamine release assay (HRA), ED$_{50}$ is >300 µg/ml; [Lys(N-Isobutyl)^8]Antide gave 2/8 at 2 µg/rat; [Lys (8-Qis)^5]Antide gave 1/8 at 1 µg, and 0/8 at 2 µg, and in the HRA ED$_{50}$, 22 µg/ml; [D-Lys(8-Qis)^6]Antide gave 4/8 at 1 µg and 0/8 at 2 µg, and in the HRA, ED$_{50}$ was 27 µg/ml; [Lys(8-Qic)^8] gave 5/8 at 1 µg, 1/8 at 2 µg/ [Lys(2-Pyc)^5]Antide gave 5/8 at 1 µg and 0/8 at 2 µg, and in the HRA ED$_{50}$ was 116 µg/ml; [D-Lys (2-Pyc)^6]Antide gave 3/8 at 1 µg, and in the HRA, ED$_{50}$ was 100 – >300 µg/ml; [Lys(2-Pyc)^5,D-Lys(2-Pyc)^6]Antide gave 2/8 at 1 µg. The substitutions of the Nic groups of Antide at Lys^5 or D-Lys^6 with 8-Qis or with 2-Pyc groups seem to give highly potent antiovulatory antagonists of LHRH and constitute significant new leads to generate potent antiovulatory compounds endowed with moderate or low histamine release.

Keywords: Antiovulatory; antagonists; LHRH; antide; rat

Abbreviations

Antide, A, N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Lys(Nic)-D-Lys(Nic)-Leu-Ilys-Pro-D-Ala-NH$_2$; Acap, 6-aminocaproyl; AcOH, acetic acid; Ac$_2$O, acetic anhydride; 6-Anic, 6-amino-nicotinoyl; 4-Aphe, 4-amino-Phe; Boc, t-butyloxycarbonyl; 5-Br-Nic, 5-bromo-Nic; BuOH, 1-butanol; Carb, carbamoyl; 4-Cboyl, 4-Cl-benzoyl; 5-Cl-Nic, 5-Chloro-Nic; Cpa, 4-Cl-Phe; Dab, 2,4-diaminobutyryl; DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; 3-Dmab, 3-dimethylamino-benzoyl; Dpr, 2,3-diaminopropionyl; 6,6'-Dt-di-Nic, 6,6'-dithio-di-Nic; 5-Fic, 5-fluoro-2-indolecarbonyl; Glyl, glycolyl; 2-Hynic, 2-hydroxy-Nic; 4-Iaphe, 4-Aphe(N-iPr); 2-Inc, indole-2-carbonyl; Idab, 2,4-diaminobutyryl(N-iPr); Idpr, 2,3-diaminopropionyl (N-iPr); Igly, N-Ipr-Gly; Ilys, lysine(N-iPr); 4-Imac, imidazole-4-acetyl; Imda, iminodiacetyl; Ilc, indoline-2-carbonyl; 2-kimc, 2-ketoimidazolidine-4-carbonyl; LHRH, luteinizing hormone-releasing hormone; MBHA, 4-methylbenzhydrylamine; 5-Mic, 5-methoxy-2-Inc; Moc, morpholinyl-N-carbonyl; Nal, 3-(2-naphthyl)alanine; Nal-Arg, N-Ac-D-Nal-D-4-F-Phe-D-Trp-Ser-Tyr-D-Arg-Leu-Arg-Pro-Gly-NH$_2$; Nic, nicotinoyl; Nico, Nic-N-oxide; 2-Noyl, 2-naphthoyl; ONp, 4-nitrophenyl ester; 4-Pac, Pyrazole-4-carbonyl; Pal,

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3-(3-pyridyl)alanine; PEA, α-phenylethyl-amine; PITC, phenylisothiocyanate; PTC, phenylthiocarbamyl; 2-Pyc, pyrrole-2-carbonyl; 4-Pydc, pyridazine-4-carbonyl; Pyr, pyridine; 3,5-Pyrdc, pyridine-3,5-dicarbonyl; 3-Pyrs, 3-Pyridinesulphonyl; 2-Pyzc, pyrazine-2-carbonyl; 2-Qic, quinoline-2-carbonyl; 8-Qis, 8-quinolinesulphonyl; TFA, trifluoroacetyl; Thprn, tetrahydropyranyl; 5-Urc; Uracil 5-carbonyl.

A large number of analogues of the luteinizing hormone-releasing hormone (LHRH) have been prepared during the last two decades in attempts to design highly potent and reversible antagonists which would be useful for fertility control by inhibiting the release of pituitary gonadotropins [1].

Most of the analogues prepared to date feature a strongly cationic amino acid at position 8, since it has been suggested that this is an important requirement for interaction of LHRH with its anterior pituitary cell receptors [2], although potent antiovulatory antagonists have been prepared recently by translocation of the basic amino acid to other loci [3, 4]. Potent LHRH antagonists usually have four or five D-amino acids, e.g. N-Ac-D-Nal-D-4-F-Phe-D-Trp-Ser-Tyr-D-Arg-Leu-Arg-Pro-Gly-NH$_2$, Nal-Arg [5], and N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Arg-Leu-Arg-Pro-D-Ala-NH$_2$ [6] which inhibit ovulation in a range of 1–3 μg/rat. Unfortunately, the topography derived by having the sequence of lipophilic N-terminal D-amino acids, and the cationic D-Arg^6 and Arg^8, led to side effects caused by histamine release (HR) from mast cells [7–9]; for example Nal-Arg in the HR assay (HRA) gave an effective dose, ED$_{50}$, of 0.17 μg/ml, compared to 328 μg/ml for LHRH [9]. The initial observation that substitution of some LHRH antagonists with Ilys^8 decreased the release of histamine as a side effect [10] led to the design of N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Lys-(Nic)-D-Lys(Nic)-Leu-Ilys-Pro-D-Ala-NH$_2$, Antide [11]. Whereas in the rat antiovulatory assay (AOA) [12] Antide shows full inhibition of ovulation at 1.0 μg, it is among the weakest of the LHRH antag-onists in the HRA [9], with an ED$_{50}$ of >300 μg/ml for histamine release from rat mast cells in vitro. Because of these favourable properties, Antide has been undergoing clinical investigations [13], although it has limited solubility in saline solutions.

We set out to explore the possibility of enhancing the antiovulatory potency of Antide with substituents that should preserve the low levels of HR of Antide. We explored the replacement of Ilys^8 with shorter basic amino acids substituted with an N-isopropyl group, hydrophilic alkyl or acyl derivatives in the side-chain amino group. The presence in Antide of Lys (Nic)^5 and D-Lys(Nic)^6 residues allowed the synthe sis of cyclic analogues bridging positions 5 and 6 intra-molecularly, as well as the preparation of dimers of Antide by bridging two molecules at position 5 or two molecules at position 6 by the use of bifunctional reagents.

We also investigated the substitution of the Nic group in Antide with several types of aminoacyl, carbamoyl, glycyl, peptidyl, heterocyclic carbonyl or sulphonyl groups chosen to attain a gradation of overall size, hydrophilicity or basicity of the molecule. Another factor investigated was decreasing the length of the Lys residues, which was explored by replacing the Lys residue of Antide with Orn, Dab or Dpr, to establish the importance of the distance of the Nic group, or its substituents, from the peptide backbone. It was hypothesized that the shorter side-chains would lead to an increase in solubility of the analogues as compared to Antide.

We report here the physicochemical properties (Table 1) for analogues (1–104) of LHRH, and precursors (Tables 2 and 3), as well as their antiovulatory and histamine releasing data (Table 4).

Peptide synthesis

Suitably protected peptides were assembled manually by solid-phase peptide synthesis (SPPS) using the Boc-strategy as previously described [4]. The starting resin was either a standard polystyrene resin 1% cross-linked with divinylbenzene or a 4-methyl-benzhydrylamine (MBHA) resin [14], both esterified with Boc-D-Ala [4]. The methods of peptide synthesis may best be classified as follows.

Method A

After assembly by SPPS, protected peptides were removed from resins by ammonolysis, which also removes the formyl group from Trp(For) or α-methyl-Trp(For), and were freed from remaining blocking groups by treatment with HF/anisole [4]. When the Nic groups in Lys(Nic)^5 or D-Lys(Nic)^6 groups in Antide were replaced by another heterocyclic acid, the precursor Boc-Lys(X) or Boc-D-Lys(X) was prepared by reacting Boc-Lys or Boc-D-Lys with the heterocyclic acid activated as the 4-nitrophenyl ester [4], or for sulphonyl compounds, 3-Pyrs-Cl [15] or 8-Qis-Cl and, for carbamoyl compounds, by reaction with potassium cyanate [16]. The method was applicable to other basic amino acids. For substitutions at position 8, analogues of Ilys were prepared by

Table 1 Physicochemical Properties of LHRH Analogues

Antide Analogue	Compund No.[a] (Method)	MW	Yield[c] (aa an.)	OR[d] (deg)	TLC[e] R_fA	R_fB	R_fC	R_fD	HPLC Ret.[f]
(a) Substituents at position 8									
[Idpr^8]A	1(E)	1549^b	38	−27	0.23	0.09	0.76	0.29	8.4^h
[Idab^8]A	2(E)	1563^b	50	−14	0.19	0.06	0.78	0.31	8.0^h
[Iaphe^8]A	3(E)	1625^b	40^l	−15	0.30	0.11	0.80	0.33	8.2^h
[Dpr(Igly)^8]A	4(E)	1606^b	18	−28	0.17	0.06	0.69	0.28	6.8^h
[Dab(Igly)^8]A	5(E)	1620^b	38	−23	0.17	0.05	0.69	0.29	7.2^h
[Orn(Igly)^8]A	6(E)	1634^b	25	−35	0.17	0.05	0.68	0.28	7.0^h
[Lys(Igly)^8]A	7(E)	1648^b	41	−2	0.19	0.06	0.71	0.29	7.8^h
[Dpr(Thprn)^8]A	8(E)	1591^b	3	−5	0.35	0.09	0.74	0.23	8.2^h
[Lys(Thprn)^8]A	9(E)	1633^b	33	−25	0.18	0.04	0.69	0.28	7.0^h
[Lys(Ibut)^8]A	10(A)	1607	10	−25	0.18	0.07	0.70	0.40	8.4^h
[Dpr^8]A	11(E)	1507^b	63	−25	0.37	0.10	0.72	0.25	6.6^h
[4-Aphe^8]A	12(A)	1585	17	−36	0.20	0.08	0.69	0.42	7.0^h
[Lys(2-Ilc)^8]A	13(A)	1694	30	−30	0.59	0.29	0.80	0.56	12.6^h
[Dpr^8]A	14(B)	1564^b	16	−12	0.35	0.08	0.66	0.20	6.0^h
[Dpr(Gly-Gly)^8]A	15(B)	1621^b	62	−23	0.29	0.05	0.59	0.18	5.4^h
[Dpr(Gly-Acap)^8]A	16(B)	1677	42	−24	0.28	0.05	0.63	0.16	6.0^h
[Lys(Nico)^8]A	17(B)	1670^b	80^m	−27	0.35	0.08	0.66	0.22	9.2^h
(b) Lys^5 and/or D-Lys^6 dimers and cyclic structures									
Bis-(5-[Lys^5]A) -CO- bridge	18(C)	2,998^b	38^n	−27	0.01	0.00	0.50	0.24	5.2^k
Bis-(6-(D-Lys^6]A) -CO- bridge	19(C)	2,998^b	23^n	−31	0.01	0.00	0.49	0.26	5.4^k
Bis-(5-Lys^5]A) -3,5-Pyrdc- bridge	20(C)	3,104^b	23	−29	0.19	0.07	0.66	0.34	6.0^k
Bis-(6-[D-Lys6]A) -3,5-Pyrdc- bridge	21(C)	3,104^b	14	−18	0.20	0.10	0.66	0.36	5.6^k
[Bis-(5[Lys^5]A) -6,6′-Dt-di-Nic- bridge	22(C)	3,245^b	25	−19	0.03	0.00	0.52	0.29	10.4^k
Bis-(6[D-Lys^6]A) -6,6′-Dt-di-Nic- bridge	23(C)	3,245^b	17	−18	0.03	0.00	0.56	0.30	12.0^k
(cyclo5/6 -CO- bridge[Lys^5, D-Lys^6]A	24(D)	1,407^b	35^n	−10	0.03	0.00	0.56	0.30	4.6^k
(cyclo5/6 -3,5 Pyrdc- bridge) [Lys^5, D-Lys^6]A	25(D)	1,512^b	55	−19	0.04	0.00	0.38	0.31	4.8^k
(Cyclo 5/6 -2,2′-dithio-diNic- bridge) Lys^5, D-Lys^6]A	26(D)	1,653^b	68°	−21	0.21	0.08	0.64	0.39	8.4^k
(Cyclo 5/6 -Imda- bridge) [Lys^5, D-Lys^6]A	27(D)	1,478^b	26	−13	0.04	0.03	0.53	0.21	3.0^k
(c) Dpr or Dab Antide substituents at positions 5 or 6									
[Dpr(Nic)^5]A	28(E)	1549^b	42	−34	0.19	0.07	0.72	0.26	8.8^h
[D-Dpr(Nic)^6]A	29(E)	1549^b	48	−26	0.18	0.05	0.72	0.26	8.0^h
[Dpr(Pyzc)^5]A	30(E)	1550^b	43	−19	0.33	0.07	0.68	0.22	9.6^h
[D-Dpr(Pyzc)^{6}A	31(E)	1550^b	37	−28	0.19	0.04	0.72	0.28	9.0^h
[Dab(Pyzc)^5]A	32(E)	1564^b	41	−26	0.19	0.05	0.72	0.26	8.4^h
[D-Dab(Pyzc)^6]A	33(E)	1564^b	38	−14	0.18	0.05	0.72	0.27	9.6^h
[Dpr^5]A	34(E)	1444^b	59	−33	0.30	0.03	0.65	0.16	6.4^h
[D-Dpr^6]A	35(E)	1444^b	46	−13	0.26	0.03	0.56	0.16	5.0^h
[Dpr(Gly)^5]A	36(B)	1501^b	36	−34	0.24	0.03	0.57	0.14	6.0^h
[D-Dpr(Gly)^6]A	37(B)	1501^b	44	−30	0.17	0.02	0.53	0.14	4.4^h
Dpr(Gly-Gly)^5]A	38(B)	1558^b	60	−33	0.17	0.03	0.53	0.13	6.0^h
[D-Dpr(Gly-Gly)^6]A	39(B)	1558^b	51	−20	0.15	0.02	0.47	0.13	4.2^h
(d) D-Cit^6-related substituents									
[DD-Dpr(Carb)^6]A	40(B)	1487^b	18	−18	0.20	0.07	0.66	0.30	7.0^h
[D-Dab(Carb)^6]A	41(B)	1501^b	20^p	−11	0.20	0.06	0.67	0.29	6.8^h

(continued)

Table 1. *(continued)*

Antide Analogue	Compound No.[a] (Method)	MW	Yield[c] (aa an.)	OR[d] (deg)	TLC[e] R_fA	R_fB	R_fC	R_fD	HPLC Ret.[f]
D-Cit⁶]A	42(B)	1515[b]	25	−35	0.19	0.07	0.67	0.28	6.8[h]
[D-Lys(Carb)⁶]A	43(B)	1529[b]	39	−18	0.19	0.06	0.70	0.28	7.0[h]
[D-Trp³, D-Dpr(Carb)⁶]A	44(B)	1525[b]	19[q]	−25	0.49	0.24	0.74	0.41	28.2[h]
[D-Trp³, D-Dab(Carb)⁶]A	45(B)	1539[b]	219	−28	0.47	0.23	0.74	0.39	25.6[h]
(e) Pyridine or quinoline carboxylic acid substituents									
[Lys(2-Cl-Nic)⁵]A	46(A)	1626	35	−32	0.21	0.05	0.75	0.42	7.4[i]
[D-Lys(2-Cl-Nic)⁶]A	47(A)	1626	34	−26	0.22	0.07	0.72	0.42	7.4[i]
[Lys(6-Cl-Nic)⁵]A	48(A)	1626	19	−31	0.30	0.11	0.76	0.47	10.2[i]
[D-Lys(6-Cl-Nic)⁶]A	49(A)	1626	51	−27	0.31	0.11	0.75	0.46	9.8[i]
[Lys(5-Br-Nic)⁵]A	50(A)	1670[b]	33	−24	0.28	0.11	0.79	0.31	11.5[i]
[D-Lys(5-Br-Nic)⁶]A	51(A)	1670[b]	26	−26	0.28	0.11	0.80	0.31	11.2[i]
[Lys(Nico)⁵]A	52(B)	1607	18[m]	−25	0.15	0.04	0.43	0.34	9.6[g]
[D-Lys(Nico)⁶]A	53(B)	1607	20[m]	−25	0.15	0.04	0.43	0.34	10.0[g]
[Lys(8-Qis)⁵]A	54(A)	1677[b]	35	−24	0.34	0.11	0.58	0.39	20.0[g]
[D-Lys(8-Qis)⁶]A	55(A)	1677[b]	58	−18	0.35	0.12	0.61	0.41	20.4[g]
[Lys(3-Pyrs)⁵]A	56(A)	1627[b]	59	−18	0.27	0.09	0.61	0.40	6.4[g]
[D-Lys(3-Pyrs)⁶]A	57(A)	1627[b]	38	−30	0.27	0.09	0.61	0.40	5.6[g]
[Lys(2-Qic)⁵]A	58(B)	1641[b]	27	−18	0.39	0.14	0.67	0.43	8.0[g]
D-Lys(2-Qic)⁶]A	59(B)	1641[b]	27	−17	0.40	0.14	0.67	0.43	7.8[g]
Lys(3-Qic)⁵]A	60(B)	1641[b]	12	−24	0.24	0.08	0.68	0.32	14.4[h]
[Lys(8-Qic)⁵]A	61(B)	1641[b]	13	−27	0.24	0.07	0.67	0.34	17.4[h]
[D-Lys(8-Qic)⁶]A	62(B)	1641[b]	12	−25	0.27	0.08	0.59	0.36	21.6[h]
[Lys(4-Pydc)⁵]A	63(B)	1592[b]	12	−28	0.17	0.05	0.58	0.29	8.0[h]
(f) Hydroxy or amino-pyridine carboxylic acids substituents									
[Lys(2-Hynic⁵)]A	64(B)	1607[b]	13[r]	−13	0.24	0.08	0.57	0.38	14.4[g]
[D-Lys(2-Hynic)⁵]A	65(B)	1607[b]	15[r]	−25	0.25	0.08	0.57	0.38	13.6[g]
[Lys(5-Urc)⁵)]A	66(B)	1624[b]	6	−39	0.21	0.06	0.63	0.31	11.6[g]
[D-Lys(5-Urc)⁶)]A	67(B)	1624[b]	20	−37	0.25	0.05	0.53	0.37	15.2[g]
[Lys(6-Urc)⁵)]A	68(B)	1624[b]	18	−30	0.21	0.05	0.63	0.27	12.0[g]
[D-Lys(6-Urc)⁶)]A	69(B)	1624[b]	17	−25	0.13	0.04	0.54	0.26	12.8[g]
[Lys(6-Hynic)⁵)]A	70(B)	1607[b]	5[r]	−40	0.18	0.05	0.61	0.26	13.2[g]
[Lys(6-Anic)⁵)]A	71(B)	1606[b]	15[s]	−21	0.12	0.03	0.57	0.25	8.8[g]
(g) Indole or pyrrole carboxylic acid substituents									
[Lys(2-Inc)⁵]A	72(A)	1629	40	−20	0.41	0.13	0.75	0.46	20.2[i]
[D-Lys(2-Inc)⁶]A	73(A)	1629	53	−17	0.41	0.13	0.75	0.48	18.2[i]
[Lys(Mic)⁵]A	74(A)	1659	52	−30	0.41	0.13	0.75	0.47	17.4[i]
[D-Lys(Mic)⁶]A	75(A)	1659	53	−19	0.39	0.11	0.74	0.46	16.0[i]
[Lys(Fic)⁵]A	76(A)	1647	47	−16	0.46	0.13	0.77	0.48	25.4[i]
[D-Lys(Fic)⁶]A	77(A)	1647	80	−11	0.45	0.13	0.78	0.49	22.6[i]
[Lys(2-Ilc)⁵]A	78(A)	1631	12	−22	0.34	0.11	0.76	0.50	12.4[i]
[D-Lys(2-Ilc)⁶]A	79(A)	1631	9	−26	0.40	0.11	0.76	0.50	12.4[i]
[Lys(3-Inc)⁵]A	80(A)	1629	37	−36	0.36	0.12	0.73	0.44	10.8[i]
[D-Lys(3-Inc)⁶]A	81(A)	1629	46	−22	0.35	0.12	0.74	0.44	10.2[i]
[Lys(4-Inc)⁵]A	82(B)	1629[b]	15	NR	0.30	0.09	0.68	0.37	18.2[i]
[Lys(5-Inc)⁵]A	83(B)	1629[b]	9	NR	0.30	0.09	0.68	0.36	18.8[i]
[Lys(2-Pyc)⁵]A	84(A)	1579	57	−37	0.35	0.13	0.75	0.43	8.6[i]
[D-Lys(2-Pyc)⁶]A	85(A)	1579	62	−22	0.34	0.11	0.73	0.43	8.0[i]
[D-Lys(2-Pyc)⁵, [D-Lys(2-Pyc)⁶]A	86(A)	1567[b]	37	−28	0.50	0.19	0.76	0.40	18.8[i]

Table 1. (continued)

Antide Analogue	Compund No.[a] (Method)	MW	Yield[c] (aa an.)	OR[d] (deg)	TLC[e]				HPLC Ret.[f]
					R_fA	R_fB	R_fC	R_fD	
(h) Heterocyclic acid substituents with five-membered rings									
[Lys(4-Imac)^5]A	87(B)	1594[b]	61	−31	0.10	0.01	0.37	0.28	6.8[g]
[D-Lys(4-Imac)^6]A	88(B)	1594[b]	55	−15	0.12	0.02	0.28	0.32	8.4[g]
[Lys(4-Pac)^5]A	89(B)	1580[b]	67	−20	0.28	0.12	0.61	0.38	9.6[g]
[D-Lys(4-Pac)^6]A	90(B)	1580[b]	79	−13	0.27	0.12	0.59	0.38	10.4[g]
[Lys(2-Kimc)^5]A	91(B)	1598[b]	19	−37	0.16	0.03	0.54	0.32	6.4[g]
[Lys(D-2-Kimc)^5]A	92(B)	1598[b]	7	−31	0.15	0.03	0.55	0.30	4.4[g]
[DLys(2-Kimc-5)^6]A	93(B)	1598[b]	9	−25	0.18	0.04	0.61	0.30	8.4
(i) Miscellaneous substituents									
[Lys(Gly)^5]A	94(A)	1544[b]	20	−44	0.24	0.06	0.64	0.31	5.2[g]
[D-Lys(Glyl)^6]A	95(A)	1544[b]	23	−16	0.20	0.06	0.66	0.29	5.0[g]
[Lys(Moc)^5]A	96(B)	1599[b]	50	−12	0.24	0.08	0.56	0.37	13.2[g]
[D-Lys(Moc)^6]A	97(B)	1599[b]	30	−19	0.24	0.08	0.56	0.37	13.6[g]
[D-4-Aphe^6]A	98(A)	1585	40	−42	0.11	0.04	0.69	0.35	4.8[g]
[D-Glu(NHNH$_2$)^6]A	99(E)	1501	8	−22	0.12	0.04	0.62	0.36	4.8[g]
[Lys(3-Dmab)^5]A	100(B)	1633[b]	10[t]	−17	0.21	0.05	0.70	0.35	10.8[j]
[D-Dpr(2-Noyl)^1]A	101(E)	1634[b]	44	−21	0.18	0.06	0.71	0.29	7.6[j]
[D-Dpr(4-Cboyl)^2]A	102(E)	1634[b]	47[u]	−10	0.18	0.06	0.70	0.29	5.8[j]
[D-Dpr(Nic)^3]A	103(E)	1634[b]	39	−2	0.30	0.11	0.71	0.33	10.8[j]
[α-Methyl-Trp^7]A	104(A)	1678[b]	7[v]	−12	0.17	0.05	0.64	0.38	7.0[j]

[a] The letter in parenthesis indicates the synthetic method (A–E) used.
[b] Low-resolution mass spectrometry using fast atom bombardment gave the molecular ion (MW or MW + 1) where indicated.
[c] The yields % reported are based on milliequivalents of starting amino acid-resin, on milliequivalents of amino groups for MBHA resin or, for methods B–D, on milliequivalents of starting peptide. Unless otherwise indicated, amino acid analysis (aa an.) gave the expected molar ratios within 10% of predicted molar ratios.
[d] OR = Optical rotation, OR was determined as $[α]D^{27}$, in degrees (c 1, 5 N AcOH).
[e] The composition of solvents A–D for TLC is described in the Experimental section.
[f] HPLC retention times are given for samples run isocratically. From the HPLC patterns, it was estimated that the analogues had a purity of at least 95%. The composition of these solvents were: solvent A = 0.05% TFA, solvent B = x% acetonitrile −100−−x% solvent A, flow rate 1.5 ml/min.
[g] 50% solvent B.
[h] 55% solvent B.
[i] 58% solvent B.
[j] 60% solvent B.
[k] 65% solvent B.
[l] The PTC derivative for Iaphe could not be resolved from late lipophilic eluents and could not be estimated.
[m] Nico did not yield a PTC derivative, but was detected and quantified in the HPLC analysis.
[n] An unknown peak may be the PTC derivative for unhydrolyzed carbonyl-bis-Lys (L,L or L, D); similarly, citrulline is hydrolysed only partially to ornithine.
[o] An unknown peak is probably due to the PTC derivative for a lysine derivative of unknown structure.
[p] The PTC derivatives for Dab(carb) = 0.22, for Dab 0.65.
[q] Trp = 0.80 for analogue 44 and Trp = 0.91 for analogue 45.
[r] 2-Hynic did not give a PTC derivative, and 6-Hynic also did not give a PTC derivative but was detected and quantified in the HPLC analysis.
[s] 6-Anic did not give a PTC derivative but was detected and quantified in the HPLC analysis.
[t] 3-Dmab did not give a PTC derivative but was detected and quantified in the HPLC analysis.
[u] Partial hydrolysis of Dpr(Cboyl) gives a low value for the PTC derivative for Dpr.
[v] α-Methyl–Trp = 0.84.

Table 2 Physicochemical Properties of Protected Amino Acids

	MW	Yield (%)	OR[a] (deg.)	mp. (°C)	Elemental Analyses[b]
Boc-Idpr	246.3	80	+5	197	C,H,N
Boc-Idpr(Z).PEA(-)	501.6	71	-18	159	C,H,N
Boc-Idab	260.3	69	+3	206 dec	C,H,N
Boc-Idab(Z).PEA(-)	515.7	34	+8	135-137	C,H,N
Boc-4-Iaphe(Z)	456.5	47	-18	141-143	C,H,N
Igly	117.1	67	-	196-198	C,H;N[c]
Z-Igly.DCHA	432.7	61	-	141-143	C,H,N
Boc-Dpr(Z-Igly).PEA(+)	558.7	71	+43	95-100	C,H,N
Boc-Dab(Z-Igly).PEA(+)0.5H2O	527.7	99	-10	101-105	C,H,N
Boc-Orn(Z-Igly).PEA(-)0.5H2O	586.7	79	0	116-118	C,H,N
Boc-Lys(Z-Igly).PEA(+)	600.8	72	+9	95-98	C,H,N
Boc-Dpr(Thprn)	288.3	60	0	210 dec.	H,N;C[d]
Boc-Dpr(Thprn, Z).PEA(-)	543.7	40	-17	118-119	C,H;H[e]
Boc-Lys(Thprn, Z).PEA(+)	585.7	50	+17	118-119	C,H,N
Z-2-Ilc	297.3	79	-65	117-119	C,H,N
Boc-Lys(Z-2-Ilc)	525.6	83	-31	112-116	C,H,N
Boc-lys(Ibut, Z).PEA(-)	515.7	29	-54	128-130	C,H,N
Boc-Dpr(Fmoc)	426.5	66	-5	149-151	C,H,N
2,2'-Dt-di-Nic	308.3	99	-	232-233	C,H,N
Z-Imda.2DCHA	628.9	39	-	178-180	C,H,N
Boc-Dpr(Nic)	309.3	52	-7	171-173	C,H,N
Boc-Dpr(Pyzc)	310.3	73	-21	225 dec.	C,H,N
Boc-Dab	200.2	53	+4	216 dec.	C,H,N
Boc-Dab(Pyzc)	324.3	86	-12	236 dec.	C,H,N
Boc-D-Dpr(Carb).4H2O.DCHA	429[e]	67	-15	157-161	C,H;N[e]
BOC-D-Dab(Carb).PEA(+)0.5H2O.0.6 EtOH	383[f]	60	+25	undefined	C;H,N[f]
Boc-Lys(2-Cl-Nic)	385.9	53	+2	189-192	C,H,N;Cl[g]
Boc-Lys(6-Cl-Nic)	385.9	39	-11	121-124	C,H,N,Cl
Boc-Lys(5-Br-Nic)	430.4	71	-12	167-169	H,N,Br;C[h]
Boc-Lys(8-Qis).d(+)MBA	558.7	44	-18	124-125	C,H,N,S
Boc-Lys(3-Pyrs).DCHA	568.8	83	+40	52-55	C,H,N,S
Boc-Lys(2-Inc).DCHA	570.7	77	+16	205-206	C,H,N
Boc-Lys(5-Mic)	600.8	79	+17	197-199	C,H,N
Boc-Lys(5-Fic)	588.7	86	+25	198-200	C,H,N,F
Boc-Lys(3-Inc)	389.5	62	-11	undefined	C,H,N
Boc-Lys(2-Pyc)DCHA	520.7	58	-11	190-195	C,H,N
Tos-4-Imac.DCHA	461.6	79	-	170-173	C,H,N,S
Tos-4-Pac	266.3	48	-	145-146	C,H; N[i]
2-Kimc(3-Z)	264.2	48	-28	192-194[j]	C,H,N
Boc-Lys(O-Bzl-Glyl).DCHA	575.8	76	+8	110-113	C,H,N
Boc-D-Dpr(2-Noyl).DCHA	539.7	54	-22	182-184	C,H,N
Boc-D-Dpr(4-Cboyl)	342.8	44	+18	140-143	C,H,N
Boc-Dpr(Fmoc)	426.5	66	-5	149-151	C,H,N
Boc-α-Me-Trp(For).DCHA	527.7	42	+25	229-230	N

[a] Optical rotations were determined as $[\alpha]D^{27}$, in degrees (c 1, DMF). The OR for the D-analogues were approximately equal, but opposite in sign, to the ones for the L-isomers. [b] Unless indicated, elementary analyses were with 0.4% for the elements noted. [c]N, calculated 12.0; found 11.5. [d]C, calculated 54.2; found 53.7. [e]N, calculated 12.8; found 12. FABMS gave molecular ion 429 (MW). [f] Calculated H, 8.15 N, 13.4; found 7.70; N, 12.9. FABMS gave a molecular ion 383 (MW). [g]Cl calculated 9.19; found 9.64. [h]C calculated 47.4; found 46.8. [i]N, calculated: 10.5; found 11.3. [j]Lit. m.p. 164-168°C after crystallization from H2O and 194°C after crystallization from MeOH, no optical rotation reported [32].

Table 3 Physicochemical Properties of Nitrophenyl(ONp)esters

	MW	Yeild (%)	mp. (°C)	Elemental Analyses[b]
Z-Igly-ONp	372.4	73	88–90	C,H,N
Z-Ilc-ONp[b]	418.4	84	143–144	C,H,N
Nico-ONp	260.2	97	240–242	C,H,N
3,5-Pyrdc-di-ONp	409.3	98	243–245	C,H,N
6,6′-Dt-di-Nic-di-ONp	550.5	85	230–232	C,H,N,S
2,2′Dt-di-Nic-di-ONp	550.5	82	239–240	C,H,N,S
Z-Imda-di-ONp	509.4	31	128–130	C,H,N
2-Pyzc-ONp	254.2	55	179–182	C,H,N[c]
2-Cl-Nic-ONp	278.7	65	134–136	C,H,N,Cl
6-Cl-Nic-ONp	278.7	65	172–173	C,H,N,Cl
5-Br-Nic-ONp	323.1	44	130–132	C,H,N,Br
2-Qic-ONp	294.3	100	186–189	C,H,N
3-Qic-ONp	294.3	90	208–209	C,H,N
8-Qic-ONp	294.3	80	126-128	C,H,N
4-Pydc-ONp	245.2	78	160–162	C,H,N
2-Hynic-ONp	260.2	94	242–244	C,H,N
5-Urc-ONp	277.2	92	310 dec.	C,H,N
6-Urc-ONp	277.2	100	314 dec.	C,H,N
6-Hynic-ONp	260.2	94	282–284	C,H,N
6-TFA-Anic-ONp	355.2	77	149–151	C,H,N,F
Tos-4-Imac-ONp	401.4	81	119–120	C,H,N,S
Tos-4-Pac-ONp	387.4	85	149–151	C,H,N,S
2-Inc-ONp	282.3	79	209–212	C,H,N
5-Mic-ONp	312.3	64	189–193	C,H,N
5-Fic-ONp	300.2	75	221–223	C,H,N,F
3-Inc-ONp	282.3	37	217–219	C,H,N
4-Inc-ONp	282.3	79	178–180	C,H,N
5-Inc-ONp	282.3	74	227–230	C,H,N
2-Pyc-ONp	232.2	68	180–185	C,H,N
2-Kimc(3-Z)-Onp[d]	385.3	42	186–187	C,H,N
3-Dmab-OMp	286.3	76	108–110	C,H,N

[a] Unless indicated, elementary analyses were with 0.4% for the elements noted.
[b] $[\alpha]D^{27°} = -68°(c\ 1,\ DMF)$.
[c] Calculated: N, 17.1; found: N, 16.6.
[d] $[\alpha]\ D^{27°} = -62°(\ 2,\ DMF)$.

alkylation of α-Boc–dibasic amino acids [17] followed by carbobenzoxylation for amino group protection.

Method B

Starting from [AA⁵]Antide, [D-AA⁶]Antide, or [AA⁸]Antide, where AA = Lys, Orn, Dab or Dpr, the desired peptides were made by acylation of the side-chain amino group in solution with a suitable acylating intermediate. The conversion of heterocyclic acids of low solubility to their 4-nitrophenyl esters proved troublesome. In the case of Nic-N-oxide (Nico), its conversion to Nico-ONp failed when using N,N-dimethylformamide (DMF) or pyridine and dicyclohexylcarbodiimide (DCC). The main product appeared to be an adduct of DCC and Nico. However the Nico-ONp was successfully made by reacting Nico

with 4-nitrophenyl tri-fluoroacetate in pyridine solution [18]. This method was easily applicable to other heterocyclic acids. Analogue 17 was prepared by direct acylation of [Lys⁸]-Antide with Nico-ONp. The advantage of this method included the ease of rapidly making and purifying acyl analogues at position 5, 6 or 8, as well as the preparation of sensitive analogues without exposure to DCC, trifluoroacetyl (TFA) or HF.

Method C

Analogues were made from [Lys⁵]Antide or [D-Lys⁶]-Antide by dimerization with bifunctional reagents.

Method D

Cyclic peptides were made from [Lys⁵, D-Lys⁶]Antide

Table 4 Antiovulatory and Histamine release data for Antide analogues[a]

| | | AOA (rats ovulating/8 rats) dose in μg | | | | | | HRA[c] ED$_{50}$ ± SEM |
| | | Corn Oil (μg) | | | 0.1% DMSO in H$_2$O (μg) | | | |
Analogue	Compound No.	0.5	1	2	1	2	4	(μg/ml)
Antide[b]			1/8	0/8	−	6/8	4/8	>300
(a) Substituents at position 8								
[Idpr^8]A	1		6/8					
[Idab^8]A	2				6/8			
[Iaphe^8]A	3		7/8					
[Dpr(Igly)^8]A	4				7/8			
[Dab(Igly)^8]A	5				6/8			
[Orn(Igly)^8]A	6				5/8	6/8		
[Lys(Igly)^8]A	7				7/8			
[Dpr(Thprn)^8]A	8				5/8	8/8		
[Lys(Thprn)^8]A	9				8/8			
[Lys(N-iBut)^8]A	10					2/8		
[Dpr^8]A	11					7/8		
[4-Aphe^8]A	12					7/8		
[Lys(2-Ilc)^8]A	13		8/8					
[Dpr(Gly)^8]A	14					8/8		
Dpr(Gly-Gly)^8]A	15					7/8		
[Dpr(Gly-Acap)^8]A	16					7/8		
[Lys(Nico)^8]A	17					6/8		
(b) Lys^5 and/or D-Lys^6 dimers and cyclic structures								
Bis-(5-[Lys^5]A) -CO- bridge	18		7/8					
Bis-(6-[D-Lys^6]A) -CO- bridge	19		6/8		8/8			
Bis(5-[Lys^5]A) -3,5-Pyrdc- bridge	20		4/8		6/8			
Bis(6-[D-Lys^6]A) -3,5-Pyrdc- bridge	21		7/8					
Bis-(5-[Lys^5]A) -6,6'-Dt-di-Nic- bridge	22		7/8					
Bis-(6-[D-Lys^6]A) -6,6'-Dt-di-Nic- bridge	23		8/8					
(Cyclo 5/6 -CO- bridge)[Lys^5,D-Lys^6]A	24		7/8					
(Cyclo 5/6 -3,5-Pyrdc- bridge)[Lys^5,D-Lys^6]A	25		8/8					
(Cyclo 5/6 -2,2'-Dt-di-Nic- bridge) [Lys^5,D-Lys^6]A	26		7/8					
(Cyclo 5/6 -Imda- bridge) [Lys^5,D-Lys^6]A	27				7/8			
(c) Dpr or Dab substituents at positions 5 or 6								
[Dpr(Nic)^5]A	28		6/8					
[D-Dpr(Nic)^6]A	29		5/8					
[Dpr(2-Pyzc)^5]A	30		8/8					
[D-Dpr(2-Pyzc)^6]A	31		5/8					
[Dab(2-Pyzc)^5]A	32		6/8					
[D-Dab(2-Pyzc)^6]A	33		6/8					
[Dpr^5]A	34					8/8		
[D-Dpr^6]	35					5/8		
[Dpr(Gly)^5]A	36					7/8		
[D-Dpr(Gly)^6]A	37					7/8		
[Dpr(Gly-Gly)^5]A	38					7/8		
[D-Dpr(Gly-Gly)^6]A	39					8/8		

(continued)

Table 4. (continued)

Analogue	Compound No.	Corn Oil (μg) 0.5	1	2	0.1% DMSO in H$_2$O (μg) 1	2	4	HRA^c ED$_{50}$ ± SEM (μg/ml)
(d) D-Cit^6-related substituents								
[D-Dpr(Carb)^6]A	40				5/8			
[D-Dab(Carb)^6]A	41				8/8			
[D-Cit^6]A	42				7/8			
[D-Lys(Carb)^6]A	43				7/8			
[D-Trp^3, D-Dpr(Carb)^6]A	44				8/8			
[D-Trp^3, D-Dab(Carb)^6]A	45				8/8			
(e) Pyridine or quinoline acid substituents at positions 5 or 6								
[Lys(2-Cl-Nic)^5]A	46		7/8					
[D-Lys(2-Cl-Nic)^6]A	47		7/8					
[Lys(6-Cl-Nic)^5]A	48		8/8					
[D-Lys(6-Cl-Nic)^6]A	49		4/8					
[Lys(5-Br-Nic)^5]A	50		5/8					
[D-Lys(-Br-Nic)^6]A	51		6/8					
[Lys(Nico)^5]A	52		7/8					
[D-Lys(Nico)^6]A	53		8/8					
[Lys(8-Qis)^5]A	54		2/8		1/8	0/8		22 ± 3.5^d
[D-Lys(8-Qis)^6]A	55		2/8		4/8	0/8		27 ± 3.5^e
[Lys(3-Pyrs)^5]A	56		8/8		5/8	5/8		
[D-Lys(3-Pyrs)^6]A	57		7/8		8/8	7/8		
[Lys(2-Qic)^5]A	58				6/8			
[D-Lys(2-Qic)^6]A	59				7/8			
[Lys(3-Qic)^5]A	60				6/8			
[Lys(8-Qic)^5]A	61				5/8	1/8		
[D-Lys(8-Qic)^6]A	62				8/8			
[Lys(4-Pydc)^5]A	63				8/8			
(f) Hydroxy- or amino-pyridine carboxylic acid substituents at positions 5 or 6								
[Lys(2-Hynic)^5]A	64				6/8			
[D-Lys(2-Hynic)^6]A	65				8/8			
[Lys(5-Urc)^5]A	66				7/8			
[D-Lys(-Urc)^6]A	67				8/8			
[Lys(6-Urc)^5]A	68				8/8			
[D-Lys(6-Urc)^6]A	69				5/8			
[Lys(6-Hynic)^5]A	70				7/8			
[Lys(6-Anic)5]A	71				8/8			
(g) Indole or pyrrole carboxylic acid substituents at positions 5 and/or 6								
[Lys(2-Inc)^5]A	72		6/8	4/8				
[D-Lys(2-Inc)^6]A	73		7/8	8/8				
[Lys(5-Mic)^5]A	74		5/8					
[D-Lys(5-Mic)^6]A	75		7/8					
[Lys(5-Fic)]A	76		5/8					
[D-Lys(5-Fic)^6]A	77		6/8					
[Lys(2-Ilc)^5]A	78		6/8					
[D-Lys(2-Ilc)^6]A	79		6/8					
[Lys(3-Inc)^5]A	80		3/8					47 ± 18e
[D-Lys(3-Inc)^6]A	81		5/8					100->300^e

(continued)

Table 4. (continued)

Analogue	Compound No.	AOA (rats ovulating/8 rats) dose in μg Corn Oil (μg)			0.1% DMSO in H₂O (μg)			HRA[c] ED50±SEM (μg/ml)
		0.5	1	2	1	2	4	
[Lys(4-Inc)⁵]A	82				8/8			
[Lys(5-Inc)⁵]A	83				8/8			
[Lys(2-Pyc)⁵]A	84	6/8	0/8		5/8	0/8		116 ± 25[d]
[D-Lys(2-Pyc)⁶]A	85		1/8		3/8			100->300[e]
[Lys(2-Pyc)⁵, D-Lys(2-Pyc)⁶]A	86				2/8			
(h) Substituents with heterocyclic acids with five-membered rings at positions 5 or 6								
[Lys(4-Imac)⁵]A	87				6/8			
[D-Lys(4-Imac)⁶]A	88				8/8			
[Lys(4-Pac)⁵]A	89				5/8			
[D-Lys(4-Pac)⁶]A	90				6/8			
[Lys(2-Kimc)⁵]A	91				6/8			
[Lys(D-2-Kimc)⁵]A	92				7/8			
[D-Lys(2-Kimc-5)⁶]A	93				7/8			
(i) Miscellaneous substituents								
[Lys(Gly)⁵]A	94		6/8					
[D-Lys(Glyl)⁶]A	95		6/8					
[Lys(Moc)⁵]A	96		7/8					
[D-Lys(Moc)⁶]A	97		6/8					
[D-Aphe⁶]A	98					5/8		
[D-Glu(NHNH₂)⁶]A	99					8/8		
[Lys(3-Dmab)⁵]A	100					7/8		
[D-Dpr(2-Noyl)¹]A	101				8/8			
[D-Dpr(4-Cboyl)²]A	102				6/8			
[D-Dpr(Nic)³]A	103				5/8	8/8		
[α-Methyl-Trp⁷]A	104					8/8		

[a] Preliminary results were presented at the 22nd European Peptide Symposium held on Sept 13–19, 1992 in Interlaken, Switzerland [33].

[b] Typical values for Antide's AOA.

[c] The HRA assays were run with a standard of LHRH and a standard of Nal-Arg which were evaluated for each set of analogues. The results are given, where possible, as a mean ± standard error of the mean (SEM).

[d] ED50 ± SEM for LHRH = 181 ± 18, Nal-Arg = 0.21 ± 0.02.

[e]ED50 ± SEM for LHRH = 146 ± 12, Nal-Arg = 0.20 ± 0. Analogues 81 and 85 were so weak in histamine release that they did not give a maximal response, hence an ED50 could only be estimated.

by intramolecular reaction with bifunctional reagents.

Method E

Peptides were assembled by SPPS on an MBHA resin and then were freed from blocking groups and were removed from the resin simultaneously by the usual HF/anisole treatment. In the case of analogue 99, the peptide resin was treated first with hydrazine, to convert the D-Glu(Bzl)⁶ residue to D-Glu(NHNH₂)⁶, the resin was treated then with HF/anisole to liberate directly the free peptide [D-Glu(NHNH₂)⁶]Antide, 99. For analogues 11, 34 and 35, the final peptide resin was treated first with piperidine in DMF, to convert the appropriate isomer of the Dpr(Fmoc) residues to

the respective Dpr⁵, Dpr⁸ or D-Dpr⁶, residues, followed by the usual HF/anisole treatment.

Analogues were purified by preparative HPLC, and purity was assessed by high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), fast atom bombardment mass spectrometry (FABMS) and/or amino acid analysis [19] (Table 1). The amino acid analyses were usually within 10% of theory, except for Trp and α-methyl-Trp which were independently estimated from their UV absorption at 280 nm and often gave somewhat low values due to the presence of several moles of water or trifluoroacetic acid in the lyophilized peptide [4]. In most cases where a substituting residue could not be detected by amino acid analyses and it was deemed

desirable, FABMS was used to determine the molecular ion.

Bioassays

A standard AOA on four-day-cycle rats [12] was performed for each analogue (Table 4) by Dr Jerry Reel of BioQual Inc., under contract NO1-HD-1-3130 with the Contraceptive Development Branch (CDB) of the National Institutes of Health (NIH). A standard HRA was performed for some of the most active analogues (Table 4) by Dr William A. Hook, of the Clinical Immunology Section, NIH, in duplicate with the mast cells from three different rats [9].

Structure Activity Relationships

The AOA are reported in two different injection vehicles (Table 4). Initially, analogues were dispersed in corn oil. Subsequently the standard vehicle for the assay became 0.1% dimethyl sulphoxide (DMSO) (0.1% DMSO in water). The table of AOA shows the reference values for Antide for one or both of these two assay vehicles. The HR potencies are reported only for the most potent antagonist types (Table 4).

(a) Analogues with Substituents at Position 8

Since a previous study on LHRH analogues [10] was done on a very small number of alkyl derivatives of Lys^6 or Lys^8, we thought that other alkyl or acyl group might more favourably adjust the physicochemical properties of the Antide molecule. Reducing the distance between the N-iPr group and the peptide backbone led to analogues weaker than Antide in the AOA, as did the use of Igly as the N-iPr group carrier, even though $Orn(iPr)^8$ analogues are often potent analogues [11, 20]. The tetrahydropyranyl group was also not active, although $[Lys(N-Isobutyl)^8]$Antide (analogue 10) in the AOA gave 2/8 at a dose of 2 µg, at which dose Antide gives 6/8 (0.1% DMSO vehicle). Although this analogue compares favourably with Antide in the AOA, it probably would not have a higher solubility in aqueous medium than that of Antide.

(b) Analogues with Lys^5 and $D-Lys^6$ Dimers and Cyclic Structures

Previous studies had shown that dimerization of a somatostatin analogue by coupling its lysyl residue to a number of bifunctional reagents had given di-

mers which were much more highly potent inhibitors of the active uptake of cholate in isolated rat liver cells [21]. In the case of the Antide dimers, an added attraction was that the use of the -3,5-Pyrdc- bridge (analogues 20, 21) maintained a 'Nic' residue to either monomeric chain, and the use of the -6,6'-Dt-di-Nic- bridge (analogues 22, 23) entailed a disulphide bridge between the Nic moieties. In the case of the 5/6 cyclo structures, the use of the -3,5-Pyrdc-bridge (analogue 25) maintained a 'Nic' residue to either Lys^5 or $D-Lys^6$ which was easily accommodated in space-filling molecular models. Similar dimers or 5/6 cyclic peptides with a carbonyl bridge, could be regarded as having a carbamyl group, of interest as described further below. However, all of these analogues were weaker than Antide in the AOA.

(c) Analogues with Dpr and Dab Substituents at Positions 5 or 6

Since other structure activity relationships (SAR) findings [11] showed that substitution of the nicotinoyl (Nic) groups of Antide with picolinoyl (Pic) groups gave potent AO antagonists, one could regard Lys^5 and $D-Lys^6$ as 'carriers' for Nic or Pic groups and it should be possible to substitute them with other acyl groups more advantageously. Since the $Orn(Nic)^5$ substitution also maintains AO potency [11], we also explored the effect of reducing the distance of the Nic group to the peptide backbone by introducing the $Dpr(Nic)^5$ and $D-Dpr(Nic)^6$ (analogues 28, 29), but these analogues were weaker than Antide. The substitution with $Lys(Pyzc)^5$ or $D-Lys(Pyzc)^6$ had been found to give also potent antagonists [22]. However, analogues (30–33), exploring the introduction of either Dpr or Dab or their D-isomers as carriers for the Nic group were also weaker than Antide. The unsubstituted Dpr^5 or $D-Dpr^6$ analogues or their Gly- or Gly-Gly substituted forms (analogues 34–39) also were weaker than Antide.

(d) Analogues with $D-Cit^6$-related Substituents

Previous studies had shown that N-Ac-D-Nal-DCpa-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH$_2$ caused 100% inhibition of ovulation in cycling rats in doses of 3 µg and had no detectable edematogenic effects even at 1.5 mg/kg, as did similar Pal^3 antagonists [23]. Some of our analogues preserve most of that sequence but feature $D-Pal^3$, $Lys(Nic)^5$, $Ilys^8$ and either $D-Dpr(carb)^6$ or $D-Dab(carb)^6$ (analogues 40 and 41 respectively) or $D-Cit^6$ or $D-Lys(Carb)^6$ (analogues 42 and 43 respectively). Other analogues introduced $D-Trp^3$ (analogues 44 and 45 respec-

tively). More recently [24], LHRH antagonists, some of them active in the AOA at 0.75 μg/rat, have been made of this type of analogue by substitution with D-Trp[3] or D-Pal[3], Tyr[5] or Arg[5], and D-Lys(Dpr)[6] or D-Lys(Dab)[6]. None of our analogues (No. 40–45) substituted with shorter or longer carbamoyl amino acids were of higher potency than Antide. It is not apparent whether these analogues have lower potency than Antide in the AOA due to the multiple substitutions, or whether they would have exhibited AOA at higher doses.

(e) Analogues with Pyridine or Quinoline Acid Substituents at Positions 5 or 6

In the AOA employing corn oil, [Lys(8-Qis)[5]]Antide gave 2/8 at 1 μg/rat, which makes it almost as potent as Antide, and in 0.1% DMSO, it gave 1/8 at 1 μg and 0/8 at 2 μg, which indicates higher potency than that of Antide in this vehicle (Table 4). In the AOA (corn oil), [D-Lys(8-Qis)[6]]Antide gave 2/8 at 1 μg/rat, but in 0.1% DMSO, it gave 4/8 at 1 μg and 0/8 at 2 μg, which indicates also higher potency than that of Antide in this vehicle. Analogue [Lys(8-Qic)[5]]Antide 61, the carboxamido analogue of 54, gave 1/8 when tested at 2 μg in the AOA using 0.1% DMSO, which is a higher potency than that of Antide. On the other hand, analogues 56 and 57, [Lys(Pyrs)[5]]Antide and [D-Lys(Pyrs)[6]]Antide, which are the sulphonamido analogues of Antide were unexpectedly inactive at 2.0 μg/rat in 0.1% DMSO or at 1 μg in corn oil. All other analogues in this series seemed to be inactive or weaker than Antide at the doses tested. In the HRA (Table 4), [Lys(Qis)[5]]Antide gave an ED_{50} at 22 μg/ml and [D-Lys(Qis)[6]]Antide gave an ED_{50} at 27 μg/ml. These potencies are a great improvement over that of Nal-Arg, which is approximately 0.17 μg/ml, but are not as good as the potency of Antide, which is >300 μg/ml.

(f) Analogues with Hydroxy- or Amino-pyridine Acid Substituents at Positions 5 or 6

Whereas these analogues were more soluble than Antide in an aqueous medium, none of them was more potent than Antide in the AOA.

(g) Analogues with Indole or Pyrrole Carboxylic Acid Substituents at Positions 5 and/or 6

Most of the indole carboxylic acid analogues were not as potent as Antide. Only [Lys(3-Inc)[5]]Antide was

active in the AOA, 3/8 at 1.0 μg/rat in corn oil vehicle, but it was weaker than Antide. On the other hand [Lys(2-Pyc)[5]]Antide (analogue 84), when tested in the AOA in corn oil, gave 6/8 at 0.5 μg, 0/8 at 1 μg, which is approximately equipotent with Antide; however, in 0.1% DMSO-H_2O, it gave 5/8 at 1 μg and 0/8 at 2 μg, which indicates higher potency than Antide. In the AOA [D-Lys(2-Pyc)[6]]Antide (analogue 85), gave 1/8 when tested in corn oil at 1 μg which is at least as potent as Antide, and 3/8 in 0.1% DMSO-H_2O at 1 μg, somewhat more potent than Antide. [Lys(2-Pyc)[5], D-Lys(2-Pyc)[6]]Antide (analogue 86) when tested in 0.1% DMSO-H_2O gave 2/8 at 1 μg, also somewhat more potent than Antide.

In the HRA [Lys(3-Inc)[5]]Antide (analogue 81) gave an estimated ED_{50} at 12–105 μg/ml and [D-Lys(3-Inc)[6]]Antide (analogue 82) gave an estimated ED_{50} at 100–300 μg/ml. This suggests that the 3-Inc substitution is not as good as the Nic-substitution for HR but is better than that of Nal-Arg. [Lys(2-Pyc)[5]]Antide (analogue 84) gave an ED_{50} at 116 μg/ml, and [Lys(2-Pyc)[5]]Antide (analogue 85) gave an estimated ED_{50} at 100–300 μg/ml. These findings suggest that the 2-Pyc substitutions lead to antagonists with low degree of HR, although somewhat less favourable than that of the Nic group of Antide.

(h) Analogues with Heterocyclic Acids with Five-membered Rings at Positions 5 or 6

The discovery of the high AO potency of the 2-Pyc derivatives led us to explore the effect of substituting with other five-membered ring heterocyclic acids. All of these analogues were weaker than Antide in the AOA.

(i) Analogues with Miscellaneous Substituents

We also explored the introduction of other functional groups at positions 5 or 6 (analogues 94–100) with the view of increasing solubility or altering basicity. All of these analogues were weaker than Antide. Attempts were made to use Dpr as a carrier of the 2-naphthyl, 4-Cl-phenyl or Pyridyl groups for D-Nal, D-Cpa, D-Pal, respectively (analogues 101–103). None of these compounds seems to be active at the dose tested.

Since potent AO analogues of LHRH had been prepared by replacement of Leu[7] with Trp[7] [25], we substituted with α-methyl-Trp[7] [26], which would also be expected to reduce breakdown at this site, e.g.

by angiotensin-converting enzyme [27]. However, this compound did not match the potency of Antide in the AOA.

Conclusions

Our finding with [Lys(N-Isobutyl)^8]Antide (analogue 10) suggests that it should be possible to devise further alkyl substitutions on Lys^8 that may enhance AO potency. The finding of high AO potency with [Lys(8-Qis)^5]Antide and [D-Lys(8-Qis)^6]Antide with moderate HR, constitute a new lead for the choice of substituents. The most significant lead was generated with [Lys(2-Pyc)^5]Antide (analogue 84) and [D-Lys(2-Pyc)^6]Antide (analogue 85) which were at least as potent as Antide or somewhat more potent in the AOA in mineral oil or in aqueous medium. Analogue 86, [Lys(2-Pyc)^5, D-Lys(2-Pyc)^6]Antide featuring the dual replacement with the 2-Pyca substituent may be equipotent or more potent than analogues 84 and 85. The HRA for [Lys(2-Pyca)^5]Antide and for [D-Lys(2-Pyca)^6]Antide indicate that the HR potency for analogues substituted with the 2-Pyc group is low, although it is not as favourable as that of the Nic group.

EXPERIMENTAL

Conventional Boc-amino acids were purchased from Bachem Inc. The other derivatives, Boc-D-Nal, Boc-D-Cpa, Boc-D-Pal, Boc-Lys(Nic), Boc-D-Lys(Nic), Boc-Ilys(Z), Boc-Dpr and Boc-D-Dpr, were provided by P. N. Rao, of the Southwest Foundation for Biomedical Research, under contract NO1-HD-1-3137 with the CDB, NIH. Additionally, Boc-Lys(X) and Boc-D-Lys(X), the corresponding Orn, Dab, Dpr derivatives and N-isopropyl–basic amino acids were prepared in our laboratories as shown below. The MBHA resin was purchased from Applied Biosystems and chloromethylated resins and ion exchange resins were supplied by Bio-Rad. HPLC solvents were supplied by Fisher Scientific. Other reagents were of analytical grade and were purchased from Aldrich Chemical Co., Pierce Chemical Co., Lancaster or Chemical Dynamics. Peptides were treated with liquid HF in an all-Teflon apparatus (Protein Research Foundation, Osaka, Japan). The purity of peptides was verified at 220 nm by analytical HPLC in a Millipore apparatus using a µBondapak (Millipore) C_{18} column (30 × 0.39 cm) as previously described [4]. The peptides were finally purified by preparative HPLC in a Rainin apparatus previously described [4], using a column module, 2.14 × 25 cm, with a guard

module, 2.14 × 5 cm, both packed with Dynamax-60A, 8 µm, C_{18} (Rainin). The solvent systems used both for analytical or preparative HPLC were: (A) 0.05% TFA; (B) 60% MeCN–40% solvent A. TLC was performed on silica gel G pre-coated TLC plates (Analtech Uniplates, 0.25 mm). The following solvent systems were used (ratios given by volume): (A) n-BuOH:AcOH:H$_2$O (3:1:1); (B) n-BuOH:AcOH:H$_2$O (5:1:1); (C) n-BuOH:AcOH:H$_2$O:Pyr (5:1:1:1); (D) n-BuOH:AcOH:H$_2$O (4:1:5, upper phase). After development, peptides were visualized with chlorine-tolidine. For amino acid analysis, peptides were hydrolysed with 6 N HCl for 24 h at 110°C and the amino acids in the hydrolysis product were derivatized with phenylisothiocyanate. The resulting phenylthiocarbamyl (PTC)–amino acids were determined by the Picotag method (Millipore) in an analytical HPLC set up, described previously [28]. The optical rotations for amino acid derivatives and peptides were measured in a Rudolph Polarimeter (precision ±0.01°). Microanalyses were performed by Galbraith Labs. Inc. (Knoxville, TN). FABMS was performed in a VG 70-250SE Mass Spectrometer, VG An-alytical, Manchester, UK, by the Analytical Department of Northwestern University, Evanston, Illinois, USA.

2-N-Boc-3-N-iPr-2,3-diaminopropionic Acid, Idpr

Boc-Dpr (1.02 g, 5 mmol) in water (10 ml) was dissolved by the addition of 4M NaOH to pH 10.5, acetone (10 ml) and 5% Pd-C (0.25 g) were added and the mixture was agitated under a hydrogen atmosphere in a Parr shaker for 35 h. After filtration the solution was neutralized to pH 7 with 2 N HCl and evaporated to dryness in vacuo. The solid was extracted with iPrOH yielding 0.837 g (80%), m.p. 197 °C, $[\alpha]^{27}_D = + 5°$ (c = 1, DMF). Anal. $C_{11}H_{22}N_2O_4$ (Table 2).

N-Isopropyl–amino Acids

These intermediates were prepared as exemplified above for Boc-Idpr, by minor modifications of a procedure for the synthesis of Boc-δ-N-iPr-α-N-Boc-Orn [16]. Suitable ketones were used such as tetrahydropyranone, for analogues 8 and 9, and isobutyraldehyde for analogue 10 (Table 2).

Boc–Amino Acids

These were prepared by reaction of the amino acid with di-tert-butyl-carbonate as previously described [29] (Table 2).

Bod-Dab

This intermediate was purchased from Bachem, or was prepared by reduction of Boc-Gln as described by Waki *et al.* [30] for the synthesis of Boc-Dpr (Table 2).

Z-Amino Acids

These were prepared by treatment with Z-Cl by standard methods [31] (Table 2).

2-Kimc(3-Z)

This intermediate was prepared from Boc-Asn by Hoffman rearrangement [32] (Table 2).

2,2'-Dithio-di-nicotinic acid, 2,2'-Dt-di-Nic

2-Mercaptonicotinic acid (1.552 g, 10 mmol) suspended in water (10 ml) was dissolved by adding concentrated ammonium hydroxide to pH 7.0. The solution was treated with potassium ferricyanide (3.29 g) dissolved in water (10 ml) while maintaining the pH at 7.0 by the addition of ammonium hydroxide. When reaction ceased, slight excess of 0.2 M potassium ferricyanide (5 ml) was added and the pH was decreased to 3.8 by the addition of AcOH (5 ml). The precipitate that formed was collected, washed with water, ethanol, ether, yielding 1.55 g (99%), m.p. 232–233. Anal. $C_{12}H_8N_2O_4S_2$ (Table 2).

α-N-Boc-Dpr(Nic)

To a solution of α-N-Boc-Dpr (2.04 g, 10 mmol) in 2 N NaOH (5 ml) was added Nic-4-nitrophenyl ester (2.44 g, 10 mmol) in DMF (40 ml) with vigorous magnetic stirring. This procedure is a slight modification of the procedure for preparing Boc-Lys(Picolinoyl)·DCHA salt [4]. The product crystallized from EtOH-pet ether, yielding 1.61 g (52%), m.p. 171–173 °C, $[\alpha]^{27}D = -7$ ° ($c = 1$, DMF). Anal. $C_{14}H_{19}N_3O_5$ Table 2).

α-N-Boc-D-Dpr(Carbamoyl),dycyclohexylamine salt, Boc-D-Dpr(Carb)·DCHA

Potassium cyanate (487 mg, 6 mmol) was added to a mixture of Boc-Dpr (613 mg, 3 mmol) in H_2O (3 ml) and DMF (3 ml). After heating for 5 h, the solution was diluted with NaCl saturated, acidified with 20% citric acid and extracted with ethyl acetate. The pooled and dried (MgSO$_4$) ethyl acetate extracts were treated with dicyclohexylamine (DCHA) (0.6 ml, 3 mmol), ether was added to the mixture, and the resulting solid was collected, yielding 0.86 g, m.p. 157–161°C, $[\alpha]^{27}D = -15$ ° ($c = 1$, DMF). Anal. $C_{21}H_{40}N_4O_5 \cdot 0.4$ H_2O (Table 2).

α-N-Boc-Lys(3-Pyridinesulphonyl)·DCHA Salt, Boc-Lys(3-Pyrs)·DCHA

To a solution of Boc-Lys (2.46 g, 10 mmol) in 2 N NaOH (5 ml) was added tetrahydrofuran (THF) (5 ml) and 3-pyridylsulphonyl chloride·HCl (3.2 g, 15 mmol) was added in small portions over 20 min, while maintaining the pH at 7.5–8.0 by the addition of TEA. When the addition of reagents was complete, the reaction mixture was stirred for another 10 min. The THF was removed under vacuum, and the resulting mixture was acidified with 10% citric acid, and the mixture was extracted with ether, the ether extracts were washed with H_2O, dried (MgSO$_4$) and then were evaporated under reduced pressure. The residual oil was dissolved in ether and the solution was treated with dicyclohexylamine (2 ml, 10 mmol) yielding 4.7 g (83%) of crystalline product, m.p. 52–55 °C, $[\alpha]^{27}D = +40°$ ($c=1$, DMF). Anal. $C_{28}H_{48}N_4O_6S$ (Table 2).

Z-N-iPr-Gly-ONp, Z-Igly-ONp

Z-Igly·DCHA (2.6 g, 6 mmol) was converted to the free acid by extraction with ethyl acetate and citric acid. The organic phase was washed with H_2O and dried (MgSO$_4$) and the solvent was removed under reduced pressure. The residual oil was dissolved in ethyl acetate (30 ml) cooled in ice with magnetic stirring. After adding p-nitrophenol (1.25 g, 9 mmol) the ice-cooled solution was treated with DCC (1.86 g, 9 mmol). After 5 h the dicyclohexyl urea was removed by filtration and the solution was evaporated in a vacuum. The residue was crystallized from EtOH yielding 2.4 g (73%) m.p. 88–89 °C. Anal. $C_{19}H_{20}N_2O_6$ (Table 3).

Nicotinic Acid N-oxide-ONp, Nico-ONp

To a solution of nicotinic acid N-oxide (139 mg, 1 mmol) in pyridine (1.5 ml) was added 4-nitrophenyl trifluoroacetate (700 mg, 3.0 mmol), giving a clear solution. Overnight crystals formed, which were collected and washed with iPrOH, yielding 253 mg (97%), m.p. 240–242 °C. Anal. $C_{12}H_8N_2O_5$ (Table 3).

Solid-phase Synthesis of Protected Peptides

Boc-amino acids were used for the syntheses, and for protection of side-chain functionalities, Boc-Ser(Bzl), Boc-Ilys(Z), were used. The starting Boc-D-Ala-Resin (0.64 mmol of D-Ala/g), was prepared on a 200–400 mesh, chloromethylated resin (BioRad), 1% cross-linked with divinylbenzene, by esterification with Boc-D-Ala [4]. The Boc-amino acid-resin was subjected to the required number of coupling cycles by SPPS. In each cycle, resins were treated with 30% trifluoroacetic acid in DCM to remove Boc groups and, after neutralization with 10% DIEA in DCM, the resin was treated with a threefold excess of the appropriate Boc-amino acid and DCC. Where possible, completion of each coupling step was monitored by the ninhydrin test. If a test was faintly positive, the unreacted peptide was acetylated by treatment with Ac_2O:DIEA:DCM (1:1:8) for 10 min. After introduction of Boc-D-Nal at position 1, acidolysis and neutralization as for a normal peptide cycle, followed by acetylation with Ac_2O:DIEA:DCM (1:1:8), gave the N-Ac-D-Nal-substitution. Following method A, protected peptides were removed from the resin either by ammonolysis with NH_3/MeOH (1:1, 35 ml). After three days, the resin was removed by filtration, and extracted three times with hot DMF. The peptide amide was isolated from the pooled extracts by precipitation with water or Et_2O–petroleum ether, usually yielding 400–600 mg of protected peptide, which was used directly for preparation of the free analogues. Protected peptides were freed from blocking groups by treatment with liquid HF/anisole and were purified as previously described [4].

N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Lys(5-Br-Nic)-D-Lys(Nic)-Leu-Ilys-Pro-D-Ala-NH₂, (Lys(5-Br-Nic)⁵)Antide (50, Table 4)

N - Ac - D - Nal - D - Cpa - D-Pal-Ser(Bzl) - Lys(5-Br-Nic)-D-Lys(Nic)-Leu-Ilys(Z)-Pro-D-Ala-NH₂ (300 mg) prepared by SPPS, method A, was treated with anisole (1 ml) and liquid HF (9 ml) for 45 min at 0°C. After removal of HF in a vacuum, the residue was extracted with 30% AcOH (10 ml) three times, and the aqueous extract was washed with petroleum ether (20 ml) three times. The aqueous extract was treated with AG1-X2, acetate (2 g) and after filtration, the solution was lyophilized, yielding 250 mg of product. This product was dissolved in water with enough AcOH to dissolve the sample (15% AcOH) and the solution was applied to a preparative column (2.14 × 25 cm) with

a guard module (5 cm), both packed with Dynamax-60A (8 μm, C_{18}, Rainin). Elution was accomplished by running a gradient with 0–45% B over a period of 120 min, at a rate of 3 ml/min, monitoring at 280 nm. Fractions corresponding to the main peak were pooled and lyophilized, yielding 83 mg (33% yield) of analogue 50 (Table 1). This analogue was homogeneous on TLC with four solvent systems, and gave one single peak on analytical HPLC, $[\alpha]^{27}D= -24°$ ($c = 1$, 5 N HOAc). Amino acid analysis by the HPLC–Picotag method [19], using standards of Nal, Cpa, Pal, Ilys, gave Nal 0.97, Cpa 1.03, Pal 0.96, Ser 0.90, Lys 2.06, Leu 1.02, Ilys 1.00, Pro 1.06, Ala 0.99. Analysis by FABMS gave a molecular ion 1671 (MW + 1).

N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Lys(Nic)-D-Lys(Nic)-Leu-Lys(Nico)-Pro-D-Ala-NH₂, [Lys(Nico)⁸ Antide (14, Table 4)

N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Lys(Nic)-D-Lys(Nic)-Leu-Lys-Pro-D-Ala-NH₂ (80 mg, 0.05 mmol) prepared by SPPS, method A, as described above, was dissolved in DMF (0.5 ml). To this solution was added diisopropylethylamine (43 μl, 0.25 mmol) and Nico-ONp (13 mg, 0.05 mmol). After 16 h, the solvent was removed in a vacuum, the residue was purified on HPLC as above, yielding 75 mg (80% yield) of analogue 14, homogeneous on TLC and showing a single peak on HPLC (Table 1), $[\alpha]^{27}D = -27°$ ($c = 1$, 5 N HOAc). Amino acid analysis gave Nal 0.95, Cpa 0.91, Pal 0.96, Ser 0.90, Lys 2.06, Leu 1.02, Ilys 1.00, Pro 1.06, Ala 0.99, Nico 1.02. Nico does not give a PTC derivative but it is detected unchanged in our HPLC amino acid analysis. A FABMS determination gave a molecular ion 1671 (MW + 1).

Bis-(5-N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Lys-D-Lys(Nic)-Leu-Ilys-Pro-D-Ala-NH₂ -CO- bridge, Bis(5-(Lys⁵)Antide) -CO- bridge (18, Table 4)

N-Ac-D-Nal-D-Cpa-D-Pal-Ser(Bzl)-Lys(Fmoc)-D-Lys(Nic)-Leu-Ilys(Z)-Pro-D-Ala-Resin was assembled by method A. N-Ac-D-Nal-D-Cpa-D-Pal-Ser(Bzl)-Lys-D-Lys(Nic)-Leu-Ilys(Z)-Pro-D-Ala-NH₂ (68 mg, 0.04 mmol), obtained after ammonolysis of the preceding resin, was dissolved in DMF (400 μl), diisopropylethylamine (34 μl) was added with strong agitation with a vortex mixer. After 20 min, the mixture was cooled in ice and bis-4-nitrophenyl carbonate (6 mg, 0.02 mmol) was added. After 16 h at room temperature, HPLC indicated almost complete reaction. A new portion of diisopropylethyla-

mine (17 μl) and starting decapeptide (34 mg, 0.02 mmol) was added. The next day, the reaction mixture was dissolved in 1 ml of DMF and was added to ether–petroleum ether (1:3) and the mixture was kept at 0 °C for 1 h. The solid was collected by filtration, yielding 100 mg. After treatment with HF/anisole as for analogue 50, 70 mg of free peptide was obtained. Purification was accomplished by HPLC as for analogue 50, with a gradient from 0 to 50% B for 90 min. Fractions corresponding to the main peak were pooled and lyophilized yielding 45 mg (38% yield) of analogue 18, homogeneous on TLC and showing a single peak on HPLC (Table 1), $[\alpha]^{27}D = -27$ ° ($c = 1$, 5 N HOAc). Amino acid analysis gave Nal 0.90, Cpa 0.97, Pal 1.01, Ser 0.99, Lys 1.08, Leu 1.00, Ilys 0.93, Pro 1.02, Ala 1.07. A FABMS analysis gave a molecular ion 2999 (MW + 1).

(Cyclo 5/6 -Imda- bridge)(N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Lys-D-Lys-Leu-Ilys-Pro-D-Ala-NH₂], (Cyclo 5/6 - Imda- bridge) [Lys⁵, D-Lys⁶)]Antide (27, Table 4)

N-Ac-D-Nal-D-Cpa-D-Pal-Ser(Bzl)-Lys(Fmoc)-D-Lys(Fmoc)-Leu-Ilys(Z)-Pro-D-Ala–resin was assembled by method A. N-Ac-D-Nal-D-Cpa-D-Pal-Ser(Bzl)-Lys-D-Lys-Leu-Ilys(Z)-Pro-D-Ala-NH₂ (96 mg, 0.06 mol), obtained after ammonolysis of the preceding resin, was dissolved in DMF (96 ml), Z-Imda-di-ONp (36 mg, 0.02 mmol) and then diisopropylethylamine (52 μl, 0.3 mmol) were added with strong agitation with a vortex mixer. After 22 h at room temperature, HPLC showed almost complete reaction. The reaction mixture was evaporated to dryness in a vacuum, and the residue was dissolved in 50% AcOH and applied to the preparative HPLC column as for analogue 50. Elution was accomplished with a gradient from 20 to 70% solvent B for 100 min. Appropriate fractions were collected and lyophilized, yielding 60 mg of protected product. After treatment with HF/anisole and HPLC purification with a gradient 0–40% solvent B for 80 min, fractions corresponding to the main peak were pooled and lyophilized yielding 23 mg (26% yield) of analogue 27, homogeneous on TLC and showing a single peak on HPLC (Table 1), $[\alpha]^{27}D = -13$ ° (c 1, 5 N HOAc). Amino acid analysis gave Nal 1.02, Cpa 0.97, Pal 0.97, Ser 1.02, Lys 1.80, Leu 1.03, Ilys 0.95, Pro 1.03, Ala 1.06, iminodiacetic acid 0.92. A FABMS analysis gave a molecular ion 1478 (MW).

N-Ac-D-Nal-D-Cpa-D-Pal-Ser-Dpr-D-Lys(Nic)-Leu-Ilys -Pro-D-Ala-NH₂, ((Dpr)⁵)Antide (34, Table 4)

N-Ac-D-Nal-D-Cpa-D-Pal-(Ser(Bzl)-Dpr(Fmoc)-D-Lys

(Nic)-Leu-Ilys(Z, Pro-D-Ala-MBHA resin (1.17 mmol) was assembled by SPPS. This product was treated with piperidine (50 ml) in DMF (50 ml) for 23 h. After washing to neutral pH and drying, the resin was treated with anisole (1 ml) and liquid HF (9 ml) for 60 min at 0 °C. Proceeding as for peptide 50, yielded 1.39 g of product. HPLC purification with a gradient 0–43% solvent B, yielded 1.0 g (59%) of analogue 34 (Table 1). This analogue was homogeneous on TLC and gave one single peak on HPLC, $[\alpha]^{27}D = -33$ ° ($c = 1$, 5 N HOAc). Amino acid analysis gave Nal 0.98, Cpa 0.99, Pal 0.96, Ser 0.90, Lys 0.94, Leu 1.00, Ilys 0.94, Pro 1.00, Ala 1.00, Dpr 1.00. A FABMS analysis gave a molecular ion 1444 (MW).

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Appendix to "Antiovulatory Antagonists of LHRH Related to Antide" in minipoint

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This miniprint section, containing elemental analysis data of Tables 2 and 3 and amino acid analysis data of Antide analogues, can be read with the help of a standard magnifying glass.

APPENDIX 1: Elemental Analyses

Table 2 elemental analyses

Compound	Analysed for	Analysis
Boc-Idpr	$C_{11}H_{22}N_2O_4$	Calc.: C, 53.6; H, 9.00; N, 11.4 / Found: C, 53.3; H, 8.59; N, 11.3
Boc-Idpr(Z) PEA(-)	$C_{27}H_{39}N_3O_6$	Calc.: C, 64.7; H, 7.84; N, 8.38 / Found: C, 64.7; H, 7.95; N, 8.52
Boc-Idab	$C_{12}H_{24}N_2O_4$	Calc.: C, 55.4; H, 9.29; N, 10.8 / Found: C, 55.4; H, 9.23; N, 10.5
Boc-Idab(Z) PEA(-)	$C_{28}H_{41}N_3O_6$	Calc.: C, 65.2; H, 8.01; N, 8.15 / Found: C, 64.9; H, 7.95; N, 8.19
Boc-4-Iaphe(Z)	$C_{25}H_{32}N_2O_6$	Calc.: C, 65.8; H, 7.07; N, 6.14 / Found: C, 65.7; H, 7.12; N, 6.05
Igly	$C_5H_{11}N_1O_2$	Calc.: C, 51.3; H, 9.47; N, 12.0 / Found: C, 51.0; H, 9.51; N, 11.5
Z-Igly DCHA	$C_{25}H_{40}N_2O_4$	Calc.: C, 68.5; H, 9.15; N, 6.48 / Found: C, 68.7; H, 9.45; N, 6.08
Boc-Dpr(Z-Igly) PEA(+)	$C_{29}H_{42}N_4O_7$	Calc.: C, 62.4; H, 7.58; N, 10.0 / Found: C, 62.2; H, 7.65; N, 9.77
Boc-Dab(Z-Igly) PEA(+)	$C_{30}H_{44}N_4O_7$ 0.5H2O	Calc.: C, 61.9; H, 7.80; N, 9.63 / Found: C, 62.2; H, 7.61; N, 9.47
Boc-Orn(Z-Igly) PEA(-)	$C_{31}H_{46}N_4O_7$ 0.5H2O	Calc.: C, 62.5; H, 7.95; N, 9.40 / Found: C, 62.9; H, 7.80; N, 9.41
Boc-Lys(Z-Igly) PEA(+)	$C_{32}H_{48}N_4O_7$	Calc.: C, 64.0; H, 8.05; N, 9.33 / Found: C, 63.6; H, 8.02; N, 9.07
Boc-Dpr(Thprn)	$C_{13}H_{24}N_2O_5$	Calc.: C, 54.2; H, 8.39; N, 9.72 / Found: C, 53.7; H, 8.27; N, 9.80
Boc-Dpr(Thprn, Z) PEA(-)	$C_{29}H_{41}N_3O_7$	Calc.: C, 64.1; H, 7.60; N, 7.73 / Found: C, 64.3; H, 7.66; N, 7.74
Boc-Lys(Thprn, Z) PEA(+)	$C_{32}H_{47}N_3O_7$	Calc.: C, 65.6; H, 8.09; N, 7.17 / Found: C, 65.8; H, 8.17; N, 7.17
Z-Ilc	$C_{17}H_{15}NO_4$	Calc.: C, 68.7; H, 5.09; N, 4.71 / Found: C, 69.0; H, 5.14; N, 4.72
Boc-Lys(Z-2-Ilc)	$C_{28}H_{35}N_3O_7$	Calc.: C, 64.0; H, 6.71; N, 7.99 / Found: C, 63.8; H, 6.96; N, 7.59
Boc-Lys(Ibut, Z) PEA(-)	$C_{31}H_{47}N_3O_6$	Calc.: C, 66.8; H, 8.49; N, 7.54 / Found: C, 66.8; H, 8.39; N, 7.40
Boc-Dpr(Fmoc)	$C_{23}H_{26}N_2O_6$	Calc.: C, 64.8; H, 6.14; N, 6.57 / Found: C, 64.7; H, 6.07; N, 6.43
2.2'-Dt-di-Nic	$C_{12}H_8N_2O_4S_2$ 0.5H2O	Calc.: C, 44.2; H, 3.31; N, 8.58; S, 19.6 / Found: C, 43.9; H, 3.07; N, 8.54; S, 19.6
Z-Imda 2DCHA	$C_{36}H_{58}N_3O_6$	Calc.: C, 68.4; H, 9.44; N, 6.67 / Found: C, 68.6; H, 9.38; N, 6.54
Boc-Dpr(Nic)	$C_{14}H_{19}N_3O_5$	Calc.: C, 54.4; H, 6.20; N, 13.6 / Found: C, 54.5; H, 6.27; N, 13.6
Boc-Dpr(Pyzc)	$C_{13}H_{18}N_4O_5$	Calc.: C, 50.3; H, 5.85; N, 18.1 / Found: C, 50.3; H, 5.77; N, 18.1
Boc-Dab	$C_9H_{18}N_2O_4$	Calc.: C, 49.5; H, 8.31; N, 12.8 / Found: C, 49.7; H, 8.36; N, 12.9
Boc-Dab(Pyzc)	$C_{14}H_{20}N_4O_5$	Calc.: C, 51.9; H, 6.22; N, 17.3 / Found: C, 51.8; H, 6.37; H, 17.0
Boc-D-Dpr(Carb) DCHA	$C_{21}H_{40}N_4O_5$ 0.4H2O	Calc.: C, 57.9; H, 9.41; N, 12.8 / Found: C, 58.1; H, 9.28; N, 12.1
Boc-D-Dab(Carb) PEA(+)	$C_{18}H_{30}N_4O_5$ 0.5H2O, 0.6EtOH	Calc.: C, 55.1; H, 8.15; N, 13.4 / Found: C, 55.1; H, 7.70; N, 12.9
Boc-Lys(2-Cl-Nic)	$C_{17}H_{24}N_3O_5Cl$	Calc.: C, 52.9; H, 6.27; N, 10.9; Cl, 9.19 / Found: C, 52.9; H, 6.24; N, 10.7; Cl, 9.64
Boc-Lys(6-Cl-Nic)	$C_{17}H_{24}N_3O_5Cl$	Calc.: C, 52.9; H, 6.27; N, 10.9; Cl, 9.19 / Found: C, 52.7; H, 6.30; N, 10.8; Cl, 9.07
Boc-Lys(5-Br-Nic)	$C_{17}H_{24}N_3O_5Br$	Calc.: C, 47.4; H, 5.62; N, 9.79; Br, 18.6 / Found: C, 46.8; N, 5.63; N, 9.38; Br, 18.3
Boc-Lys(8-Qis) d(+)MBA	$C_{28}H_{38}N_4O_6S_1$	Calc.: C, 60.2; H, 6.86; N, 10.0; S, 5.74 / Found: C, 60.6; H, 6.97; N, 9.98; S, 5.30
Boc-Lys(3-Pyrs) DCHA	$C_{28}H_{48}N_4O_6S_1$	Calc.: C, 59.2; H, 8.45; N, 9.86; S, 5.63 / Found: C, 59.5; H, 8.79; N, 9.67; S, 5.45
Boc-Lys(2-Inc) DCHA	$C_{32}H_{50}N_4O_5$	Calc.: C, 67.3; H, 8.83; N, 9.81 / Found: C, 67.2; H, 8.98; N, 9.60
Boc-Lys(5-Mic)	$C_{33}H_{52}N_3O_4$	Calc.: C, 66.0; H, 8.72; N, 9.33 / Found: C, 66.4; H, 9.02; N, 9.29
Boc-Lys(5-Fic) DCHA	$C_{32}H_{49}N_4O_5F$	Calc.: C, 65.3; H, 8.39; N, 9.52; F, 3.23 / Found: C, 65.7; H, 8.44; N, 9.47; F, 3.59
Boc-Lys(3-Inc)	$C_{20}H_{27}N_3O_5$	Calc.: C, 61.7; H, 6.99; N, 10.8 / Found: C, 61.9; H, 6.82; N, 10.5
Boc-Lys(2-Pyc)	$C_{28}H_{48}N_4O_5$	Calc.: C, 64.6; H, 9.29; N, 10.8 / Found: C, 64.8; H, 9.58; N, 10.8
Tos-Imac DCHA	$C_{24}H_{35}N_3O_4S$	Calc.: C, 62.4; H, 7.86; N, 8.43; S, 6.45 / Found: C, 62.3; H, 7.69; N, 8.79; S, 6.65
Tos-4-Pac	$C_{11}H_{10}N_2O_4S$	Calc.: C, 49.6; H, 3.79; N, 10.5 / Found: C, 49.9; H, 3.97; N, 11.3
Boc-Lys(O-Bzl-Glyl)	$C_{32}H_{53}N_3O_6$	Calc.: C, 66.8; H, 9.28; N, 7.30 / Found: C, 66.7; H, 9.45; N, 7.30
Boc-D-Dpr(2-Noyl)	$C_{31}H_{45}N_3O_5$	Calc.: C, 69.9; H, 8.40; N, 7.79 / Found: C, 69.6; H, 8.37; N, 7.89
Boc-D-Dpr(4-Cboyl)	$C_{15}H_{19}N_2O_5Cl$	Calc.: C, 52.6; H, 5.59; N, 8.17 / Found: C, 52.3; H, 5.57; N, 7.91
2-Kimc(3-Z)	$C_{12}H_{12}N_2O_5$ 0.5H2O	Calc.: C, 52.7; H, 4.80; N, 10.3 / Found: C, 52.6; H, 4.69; N, 10.4
Boc-α-Me-Trp(For) DCHA	$C_{30}H_{45}N_3O_5$	Calc.: N, 7.96 / Found: N, 8.16

Table 3 elemental analyses

Compound	Analysed for	Analysis
Z-Igly-ONp	$C_{19}H_{20}N_2O_6$	Calc.: C, 61.3; H, 5.41; N, 7.52 / Found: C, 61.0; H, 5.44; N, 7.16
Z-Ilc-ONp	$C_{23}H_{18}N_2O_6$	Calc.: C, 66.0; H, 4.34; N, 6.70 / Found: C, 66.2; H, 4.36; N, 6.73
Nico-ONp	$C_{12}H_8N_2O_5$	Calc.: C, 55.4; H, 3.10; N, 10.8 / Found: C, 55.1; H, 3.28; N, 10.8
3,5-Pyrdc-di-ONp	$C_{19}H_{11}N_3O_8$	Calc.: C, 55.8; H, 2.71; N, 10.3 / Found: C, 55.9; H, 2.93; N, 10.3
6,6'-Dt-di-Nic-di-ONp	$C_{24}H_{14}N_4O_8S_2$	Calc.: C, 52.4; H, 2.56; N, 10.2; S, 11.6 / Found: C, 52.7; H, 2.58; N, 10.3; S, 11.9
2,2'-Dt-di-Nic-di-ONp	$C_{24}H_{14}N_4O_8S_2$	Calc.: C, 52.4; H, 2.56; N, 10.2; S, 11.6 / Found: C, 52.5; H, 2.58; N, 10.5; S, 11.7
Z-Imda-di-ONp	$C_{24}H_{19}N_3O_{10}$	Calc.: C, 56.6; H, 3.76; N, 8.25 / Found: C, 56.7; H, 3.80; N, 8.21
Pyzc-ONp	$C_{11}H_7N_3O_4$	Calc.: C, 53.9; H, 2.88; N, 17.1 / Found: C, 54.0; H, 3.13; N, 16.6

2-Cl-Nic-ONp	C12H7N2O4Cl	Calc.: C, 51.7; H, 2.53; N, 10.1; Cl, 12.7
		Found: C, 51.5; H, 2.74; N, 10.2; Cl, 12.8
6-Cl-Nic-ONp	C12H7N2O4Cl	Calc.: C, 51.7; H, 2.53; N, 10.1; Cl, 12.7
		Found: C, 51.7; H, 2.75; N, 10.3; Cl, 12.5
5-Br-Nic-ONp	C12H7N2O4Br	Calc.: C, 44.6; H, 2.18; N, 8.67; Br, 24.7
		Found: C, 44.6; H, 2.22; N, 8.71; Br, 24.5
2-Qic-ONp	C16H10N2O4	Calc.: C, 65.3; H, 3.43; N, 9.52
		Found: C, 65.7; H, 3.35; N, 9.63
3-Qic-ONp	C16H10N2O4	Calc.: C, 65.3; H, 3.43; N, 9.52
		Found: C, 65.7; H, 3.46; N, 9.51
8-Qic-ONP	C16H10N2O4	Calc.: C, 65.3; H, 3.43; N, 9.52
		Found: C, 65.6; H, 3.41; N, 9.73
4-Pydc-ONp	C11H7N3O4	Calc.: C, 53.9; H, 2.88; N, 17.1
		Found: C, 53.6; H, 3.13; N, 16.9
2-Hynic-ONp	C12H8N2O5	Calc.: C, 55.4; H, 3.10; N, 10.8
		Found: C, 54.9; H, 3.04; N, 10.7
5-Urc-ONp	C11H7N3O6	Calc.: C, 47.7; H, 2.55; N, 15.2
		Found: C, 47.8; H, 2.93; N, 15.0
6-Urc-ONp	C11H7N3O6	Calc.: C, 47.7; H, 2.55; N, 15.2
		Found: C, 47.9; H, 2.87; N, 15.2
6-Hynic-ONp	C12H8N2O5	Calc.: C, 55.4; H, 3.10; N, 10.8
		Found: C, 55.7; H, 3.17; N, 10.9
6-TFA-Anic-ONp	C14H8N3O5F	Calc.: C, 47.3; H, 2.27; N, 11.8; F, 16.0
		Found: C, 47.2; H, 2.24; N, 12.2; F, 15.8
Tos-4-Imac-ONp	C18H15N3O6S	Calc.: C, 53.9; H, 3.77; N, 10.5
		Found: C, 54.0; H, 4.21; N, 10.3
Tos-4-Pac-ONp	C17H13N3O6S	Calc.: C, 52.7; H, 3.38; N, 10.8; S, 8.29
		Found: C, 52.5; H, 2.99; N, 10.7; S, 8.19
2-Inc-ONp	C15H10N2O4	Calc.: C, 63.8; H, 3.57; N, 9.93
		Found: C, 63.5; H, 3.81; N, 9.70
5-Mic-ONp	C16H12N2O5	Calc.: C, 61.5; H, 3.87; N, 8.97
		Found: C, 61.9; H, 3.91; N, 8.98
5-Fic-ONp	C15H9N2O4F	Calc.: C, 60.0; H, 3.02; N, 9.33; F, 6.33
		Found: C, 59.7; H, 3.29; N, 9.15; F, 6.12
3-Inc-ONp	C15H10N2O4	Calc.: C, 63.8; H, 3.57; N, 9.93
		Found: C, 64.2; H, 3.59; N, 9.94
4-Inc-ONp	C15H10N2O4	Calc.: C, 63.8; H, 3.57; N, 9.93
		Found: C, 64.0; H, 3.78; N, 9.83
5-Inc-ONp	C15H10N2O4	Calc.: C, 63.8; H, 3.57; N, 9.93
		Found: C, 64.0; H, 3.75; N, 986
2-Pyc-ONp	C11H8N2O4	Calc.: C, 56.9; H, 3.47; N, 12.1
		Found: C, 56.9; H, 3.45; N, 12.1
2-Kimc(Z)-ONp	C18H15N3O7	Calc.: C, 56.1; H, 3.93; N, 10.9
		Found: C, 55.9; H, 4.23; N, 10.8
3-Dmab-ONp	C15H14N2O4	Calc.: C, 62.9; H, 4.93; N, 9.79
		Found: C, 63.2; H, 5.04; N, 9.60

APPENDIX 2: Amino Acid Analyses of Antide Analogues

(a) Substituents at Position 8

Analogue	Nal	Cpa	Pal	Ser	Lys	Leu	Pro	Ala	R
[Idpr8]A	0.93	1.03	1.03	0.92	1.79	1.10	0.98	0.97	0.99a
[Idab8]A	1.05	1.05	1.02	1.05	1.80	1.10	1.08	1.10	1.00b
[Iaphe8]A	0.95	0.93	0.96	0.90	2.00	1.06	1.01	1.03	c
[Dpr(Igly)8]A	0.93	0.90	0.96	0.92	1.86	0.93	1.03	0.99	0.91d, 0.99e
[Dab(Igly)8]A	0.97	0.99	1.02	0.90	2.10	1.03	1.10	1.05	2.14f
[Orn(Igly)8]A	0.91	0.95	1.03	0.93	1.80	1.00	1.02	0.94	1.82g
[Lys(Igly)8]A	0.92	0.95	1.02	0.90	2.92	1.04	1.03	0.90	0.99e
[Dpr(Thprn)8]A	1.10	2.08i	0.94	0.90	1.99	1.02	0.99	1.00	h
[Lys(Thprn)8]A	0.90	0.96	0.93	1.00	1.83	0.93	0.98	1.01	1.04i
[Lys(N-iBut)8]A	1.00	1.01	0.96	0.99	1.84	1.00	0.96	1.10	
[Dpr8]A	1.00	0.96	0.94	0.91	1.87	0.95	0.95	0.95	0.95d
[4-Aphe8]A	0.98	1.00	0.93	0.96	1.91	0.99	1.05	0.96	0.93j
[Lys(2-Ilc)8]A	0.90	0.96	0.95	1.04	2.70	1.04	1.10	1.03	
[Dpr(Gly)8]A	1.00	0.99	0.94	0.98	1.93	0.98	1.00	0.99	0.95d, 0.95k
[Dpr(Gly-Gly)8]A	0.95	1.00	0.98	0.91	1.85	0.97	0.98	1.00	0.97d, 1.82k
[Dpr(Gly-Acap)8]A	1.00	0.99	1.00	0.93	1.92	0.99	0.92	0.97	0.96d, 0.96k,
[Lys(Nico)8]A	0.95	0.91	0.92	1.02	2.70	0.97	1.06	1.00	1.02m

a Idpr. b Idab. c The PTC derivative for Iaphe could not be resolved from late lipophilic eluents and could not be estimated. d Dpr. e Igly. f PTC derivatives for Dab and Igly were not separable. g PTC derivatives for Orn and Igly were not separable. h PTC derivatives of Cpa and Dpr(Thprn) cochromatograph. i Lys(Thprn). j Aphe. k Gly. l 6-Acap = 0.96. m Nico.

(b) Lys5 and/or D-Lys6 Dimers and Cyclic Structures

Analogue	Amino Acid								
	Nal	Cpa	Pal	Ser	Lys	Leu	Ilys	Pro	Ala
Bis-(5-[Lys5]A)-CO-bridgea	0.90	0.97	1.01	0.99	1.08	1.00	0.93	1.02	1.07
Bis-(6-[D-Lys6]A)-CO-bridge	0.90	0.94	0.96	0.94	1.12	1.06	0.90	1.04	1.03
Bis-(5-[Lys5]A)-3,5-Pyrdc-bridge	0.92	0.92	0.95	0.97	1.90	0.98	0.96	1.06	1.00
Bis-(6-[D-Lys6]A)-3,5-Pyrdc-bridge	0.98	0.96	0.96	0.99	2.09	1.00	1.01	1.04	1.00
Bis-(5-[Lys5]A)-6,6'-Dt-di-Nic-bridge	0.96	0.90	1.06	1.02	2.09	1.04	0.96	1.06	1.06
Bis-(6-[D-Lys6]A)-6,6'-Dt-di-Nic-bridge	0.94	0.92	0.99	0.92	2.00	1.08	0.90	1.05	1.08
(cyclo5/6-CO-bridge) [Lys5, D-Lys6]Aa	0.92	0.94	0.96	0.89	0.59	1.02	0.98	1.10	0.94
(cyclo 5/6-3,5-Pyrdc-bridge) [Lys5, D-Lys6]A	0.99	0.96	1.00	1.01	1.96	0.97	1.02	1.03	0.96
(Cyclo 5/6-2,2'-Dt-di-Nic-bridge) [Lys5, D-Lys6]Ab	1.01	0.99	0.98	0.90	0.57	1.10	1.04	0.99	0.98
(Cyclo 5/6-Imda-bridge) [Lys5, D-Lys6]Ac	1.02	0.97	0.97	1.02	1.80	1.03	0.95	1.03	1.06

a An unknown peak may be unhydrolysed carbonyl-bis-Lys (L,L or L,D); similarly, citrulline is hydrolysed only partially to ornithine. b An unknown peak is probably due to a lysine derivative of unknown structure. c Iminodiacetic acid (Imda) = 0.92.

(c) Dpr or Dab Substituents at Positions 5 or 6

Analogue	Amino Acid									
	Nal	Cpa	Pal	Ser	Lys	Leu	Ilys	Pro	Ala	R
[Dpr(Nic)5]A	1.03	1.00	0.97	1.02	1.03	0.98	0.97	1.01	0.95	0.98a
[D-Dpr(Nic)6]A	1.00	0.98	0.92	1.05	1.05	1.00	0.93	1.01	0.99	1.05a
[Dpr(Pyzc)5]A	1.05	0.97	0.99	0.94	1.06	0.97	0.99	1.01	0.92	1.02a
[D-Dpr(Pyzc)6]A	1.05	0.99	0.96	0.91	1.07	1.06	0.94	0.97	0.96	0.92a
[Dab(Pyzc)5]A	0.90	0.99	1.04	0.91	1.04	1.06	1.08	0.91	0.97	0.92b
[D-Dab[(Pyzc6)]A	1.05	1.00	1.02	0.90	1.04	1.08	1.07	0.92	0.99	0.92b
[Dpr5]A	0.98	0.99	0.96	0.90	0.94	1.00	0.94	1.00	1.00	1.00a
[D-Dpr6]A	1.05	0.91	1.00	0.97	1.00	1.00	0.98	0.94	0.97	1.02a
[Dpr(Gly)5]A	1.01	0.98	0.95	0.92	0.95	1.00	0.97	0.93	0.95	0.97a,c
[D-Dpr(Gly)6]A	1.10	1.02	1.08	0.90	1.00	1.06	1.01	1.06	1.05	0.90a,d
[Dpr(Gly-Gly)5]A	1.05	0.98	0.97	0.91	0.98	1.01	0.95	0.90	0.93	0.99a,e
[D-Dpr(Gly-Gly)6]A	1.06	1.00	0.98	0.91	0.98	1.04	1.10	0.92	0.94	0.93a,f

a Dpr. b Dab. c Gly = 0.98. d Gly = 1.02. e Gly = 1.82. f Gly = 1.80.

(d) D-Cit⁶-related Substituents

Analogue	Nal	Cpa	Pal	Ser	Lys	Leu	Ilys	Pro	Ala	R
[D-Dpr(Carb)⁶]A	1.02	0.97	0.96	0.91	1.10	1.04	1.00	0.96	0.96	a
[D-Dab(Carb)⁶]A	0.96	1.01	0.99	0.88	1.07	1.10	0.93	1.10	1.08	b
[D-Cit⁶]A	1.04	0.93	0.95	0.90	1.10	1.05	0.92	1.02	1.00	c
[D-Lys(Carb)⁶]A	0.90	0.92	0.94	0.96	1.24	0.99	0.93	1.79[f]	1.06	d
[D-Trp³, D-Dpr(Carb)⁶]A	1.10	0.95		0.90	1.10	1.08	1.01	1.05	1.03	e
[D-Trp³, D-Dab(Carb)⁶]A	0.99	0.94		0.90	1.03	1.10	0.92	1.07	1.06	f

a Dpr(Carb) = 0.39, Dpr = 0.66. b Dab(Carb) = 0.22, Dab = 0.65. c Cit = 0.64, Orn = 0.39, the latter arises from partial hydrolysis of Cit. d Pit derivatives of Pro and Lys(Carb) cochromatograph. e Trp = 0.80, Dpr(Carb) = 0.32, Dpr = 0.74. f Trp = 0.91, Dab(Carb) = 0.24, Dab = 0.76.

(e) Pyridine or Quinoline Acid Substituents at Positions 5 or 6

Analogue	Nal	Cpa	Pal	Ser	Lys	Leu	Ilys	Pro	Ala	R
[Lys(2-Cl-Nic)⁵]A	0.93	1.02	1.00	0.94	1.84	0.93	1.02	1.01	0.96	
[D-Lys(2-Cl-Nic)⁶]A	1.05	1.03	1.06	0.96	1.78	1.00	1.03	1.06	0.96	
[Lys(6-Cl-Nic)⁵]A	0.95	1.11	0.93	0.99	2.05	0.90	0.93	1.03	1.05	
[D-Lys(6-Cl-Nic)⁶]A	1.00	0.92	0.94	1.01	1.83	0.94	1.03	1.06	1.00	
[Lys(5-Br-Nic)⁵]A	0.97	1.03	0.96	0.90	2.06	1.02	1.00	1.06	0.99	
[D-Lys(5-Br-Nic)⁶]A	0.90	0.98	0.99	0.95	1.95	0.99	1.02	1.07	0.93	
[Lys(Nico)⁵]A	0.92	0.90	1.00	0.95	2.10	0.98	0.91	1.02	1.00	0.96[c]
[D-Lys(Nico)⁶]A	1.04	1.01	0.95	1.00	1.78	0.92	1.00	1.02	0.90	1.10[c]
[Lys(8-Qis)⁵]A	0.90	0.91	0.97	0.94	1.07	0.95	0.92	0.97	0.93	1.01[a]
[D-Lys(8-Qis)⁶]A	0.97	0.98	0.95	1.01	0.95	0.90	1.01	0.94	0.95	1.09[a]
[Lys(3-Pyrs)⁵]A	1.10	0.99	0.95	0.98	0.94	1.09	0.99	0.98	0.93	0.96[b]
[D-Lys(3-Pyrs)⁶]A	0.91	1.01	0.90	0.94	0.95	0.96	0.99	0.93	0.92	1.10[b]
[Lys(2-Qic)⁵]A	1.00	0.95	1.01	1.05	1.95	0.95	0.97	1.09	1.01	
[D-Lys(2-Qic)⁶]A	1.01	1.00	1.01	0.93	1.91	1.00	1.02	1.10	1.04	
[Lys(3-Qic)⁵]A	0.95	1.00	0.98	0.96	1.92	1.10	0.92	0.96	1.00	
[Lys(8-Qic)⁵]A	0.92	0.95	0.94	0.90	1.98	1.04	0.97	1.06	1.06	
[D-Lys(8-Qic)⁶]A	1.06	1.08	1.05	0.90	2.02	1.05	1.09	1.02	1.03	
[Lys(4-Pydc)⁵]A	1.00	1.08	1.06	0.93	1.76	1.10	1.10	1.07	1.08	

a Lys(Qis). b Lys(Pyrs). c Nico is the first peak eluting during the analytical run.

(f) Hydroxy- or Amino-Pyridine Carboxylic Acid Substituents at Positions 5 or 6

Analogue	Nal	Cpa	Pal	Ser	Lys	Leu	Ilys	Pro	Ala	R
[Lys(2-Hynic)⁵]A	1.03	0.97	1.07	1.00	1.80	1.01	1.07	1.06	1.01	
[D-Lys(2-Hynic)⁶]A	1.00	0.97	1.07	1.00	1.80	1.03	1.03	1.10	1.02	
[Lys(5-Urc)⁵]A	1.06	0.93	1.05	0.88	2.05	1.10	1.10	1.06	0.88	
[D-Lys(5-Urc)⁶]A	0.91	1.10	1.09	1.08	1.76	1.11	1.08	1.06	1.06	
[Lys(6-Urc)⁵]A	1.03	0.98	1.01	0.96	1.80	1.10	0.93	1.05	1.07	
[D-Lys(6-Urc)⁶]A	1.02	0.95	0.97	0.91	1.84	0.96	0.99	0.99	0.96	
[Lys(6-Hynic)⁵]A	0.93	1.06	0.94	0.95	2.01	1.02	0.96	1.00	1.02	0.93[a]
[Lys(6-Anic)⁵]A	0.98	0.97	1.01	0.89	2.13	1.10	1.09	1.08	1.08	1.00[b]

a 6-Hynic was not derivatized by phenylisothiocyanate, PITC. b 6-Anic = 1.00 was not derivatized by PITC.

(g) Indole or Pyrrole Carboxylic Acid Substituents at Positions 5 and/or 6

Analogue	Nal	Cpa	Pal	Ser	Lys	Leu	Ilys	Pro	Ala	R
[Lys(2-Inc)⁵]A	1.03	0.90	0.99	1.05	1.90	0.92	0.99	1.00	1.05	
[D-Lys(2-Inc)⁶]A	1.00	1.08	1.00	0.99	1.86	0.93	0.96	0.99	1.00	
[Lys(5-Mic)⁵]A	1.05	0.96	1.00	1.01	1.88	0.97	1.05	1.00	1.00	
[D-Lys(5-Mic)⁶]A	1.08	1.05	1.02	0.98	1.99	0.98	0.91	1.00	1.04	
[Lys(5-Fic)⁵]A	1.08	1.01	0.98	1.00	1.90	0.98	0.94	1.00	1.04	
[D-Lys(5-Fic)⁶]A	1.05	0.99	0.90	1.06	1.90	0.98	1.06	1.03	1.08	
[Lys(2-Ilc)⁵]A	0.90	1.10	1.01	1.01	2.00	1.07	1.05	1.08	1.04	
[D-Lys(2-Ilc)⁶]A	1.08	1.04	0.98	0.92	1.87	1.00	0.97	1.02	0.90	
[Lys(3-Inc)⁵]A	1.04	1.00	0.98	0.98	1.10	0.93	0.98	1.00	0.98	0.87[a]
[D-Lys(3-Inc)⁶]A	1.03	0.98	0.98	1.05	1.18	0.90	0.94	1.01	1.05	0.84[a]
[Lys(4-Inc)⁵]A	1.00	0.98	0.98	0.94	1.06	1.01	0.90	0.98	0.99	1.00[b]
[Lys(5-Inc)⁵]A	1.08	1.07	1.03	0.96	1.80	1.09	1.00	1.10	1.09	
[Lys(2-Pyc)⁵]A	1.00	0.98	1.00	1.01	1.35	1.02	0.90	1.04	1.05	0.79[a]
[D-Lys(2-Pyc)⁶]A	0.99	0.90	0.93	0.90	1.47	0.90	1.06	0.96	0.94	0.64[a]
[Lys(2-Pyc)⁵, D-Lys(2-Pyc)⁶]A	0.97	1.01	1.07	0.93	0.90	0.91	1.00	1.04	1.07	0.91[a]

a Hydrolysis of Lys(2-Pyc)⁵ and D-Lys(2-Pyc)⁶ to Lys was incomplete, and an additional peak was detected on HPLC which corresponded to the PTC-derivatives of D- and L-Lys(2-Pyc). The PTC derivative of Lys(2-Pyc) cochromatographed with the new peak . The values of free Lys plus the values for Lys(2-Pyc) add up to the 2 moles expected for Lys. Hydrolysis of Lys(3-Inc)⁵, 9, and D-Lys(3-Inc)⁶ also gave partial hydrolysis to Lys plus D- and L-Lys(3-Inc). b Lys(4-Inc).

(h) Substituents with Heterocyclic Acids with Five-membered Rings at Positions 5 or 6

Analogue	Nal	Cpa	Pal	Ser	Lys	Leu	Ilys	Pro	Ala	R
[Lys(4-Imac)⁵]A	1.00	1.01	0.96	0.91	1.98	1.00	1.01	0.96	0.92	
[D-Lys(4-Imac)⁶]A	0.93	1.00	0.94	0.90	1.99	0.95	1.02	0.94	0.90	
[Lys(4-Pac)⁵]A	0.99	1.01	1.10	0.90	1.98	1.08	1.02	1.08	1.04	
[D-Lys(4-Pac)⁶]A	0.93	1.01	1.02	0.90	1.90	1.08	1.09	1.06	1.07	
[Lys(2-Kimc)⁵]A	1.06	0.91	1.00	0.87	2.06	1.10	1.00	1.09	1.06	1.09[a]
[Lys(D-2-Kimc)⁵]A	1.00	0.94	1.05	0.87	1.90	1.08	0.99	1.09	1.10	1.03[a]
[Lys(2-Kimc-5)⁶]A	1.10	0.97	0.99	0.96	1.99	1.04	0.90	0.97	1.00	1.05[a]

a Dpr.

(i) Miscellaneous Substituents

Analogue	Nal	Cpa	Pal	Ser	Lys	Leu	Ilys	Pro	Ala	R
[Lys(Glyl)⁵]A	0.94	1.00	1.04	0.92	1.94	0.97	0.97	1.03	1.00	
[D-Lys(Glyl)⁶]A	0.96	1.00	1.04	0.92	1.90	0.98	0.99	1.06	1.00	
[Lys(Moc)⁵]A	1.09	1.05	0.92	1.03	1.20	0.90	1.01	1.01	0.92	0.74[a]
[D-Lys(Moc)⁶]A	1.03	0.97	0.94	0.96	1.24	0.96	0.96	1.07	0.90	0.78[a]
[D-4-Aphe⁶]A	0.97	1.08	0.96	0.96	1.00	1.02	0.91	1.06	1.06	0.90[b]
[D-Glu(NHNH2)⁶]A	0.90	1.07	1.02	0.92	0.97	1.10	0.90	1.10	1.01	0.94[c]
[Lys(3-Dmab)⁵]A	1.04	1.00	0.97	1.01	1.90	1.01	0.90	1.08	1.01	0.97[d]
[D-Dpr(2-Noyl)¹]A		0.90	1.00	0.94	1.98	0.97	1.00	1.03	0.92	0.94[e]
[D-Dpr(4-Cboyl)²]A	1.08		0.98	0.92	1.80	0.97	0.93	0.96	0.94	0.71[f]
[D-Dpr(Nic)³]A	0.93	0.91		0.98	1.98	0.98	0.98	1.10	0.99	0.98[e]
[α-Methyl-Trp⁷]A	1.00	0.91	0.92	0.96	1.80		0.90	1.03	1.08	0.84[g]

a Lys(Moc) was prepared by acid treatment of Boc-Lys(Moc), its value added to that determined for Lys adds up to the expected 2.0 moles. b Aphe. c Glu. d 3-Dmab. e Dpr. f Partial hydrolysis of Dpr(Cboyl) gives low Dpr. g α-Me-Trp.