Structure-Activity Studies on Narcotic Antagonists. 2. N-Substituted Ethyl 3-(*m*- or *p*-Hydroxyphenyl)nipecotates

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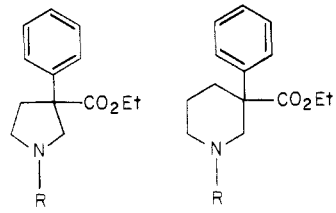
A number of N-substituted ethyl 3-(*m*- or *p*-hydroxyphenyl)nipecotates were synthesized to evaluate the role of a *m*- or *p*-hydroxy substituted β-phenethylamine moiety in narcotic antagonist action. Ethyl *m*- or *p*-methoxyphenylcyanoacetate was alkylated with 1-bromo-3-chloropropane. The resultant chloronitriles were hydrogenated (Raney Ni) to amines and cyclized to yield the N-substituted ethyl 3-(*m*- or *p*-methoxyphenyl)nipecotates. These were N-benzylated, O-demethylated using BBr_3, N-debenzylated, and then N-alkylated. The following N-substituted derivatives were prepared: methyl, allyl, cyclopropylmethyl, and *n*-propyl. No significant morphine-like analgesic activity was found in mice by the tail-flick method. The acetic acid writhing assay showed several compounds to possess analgesic activity. *N*-*n*-Propyl and *N*-(cyclopropylmethyl) *m*-hydroxy derivatives were marginally active antagonists by the mouse tail-flick method. Surprisingly, the *N*-methyl *m*-hydroxy derivative, 11m, was found to be an antagonist.

In our preceding paper,[1] we described a number of *N*-alkyl derivatives of ethyl 3-phenylpyrrolidine-3-carboxylate (1) and ethyl 3-phenylnipecotate (2). These



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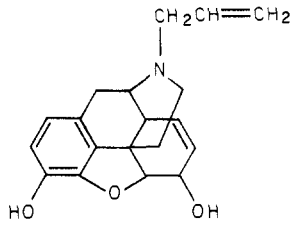

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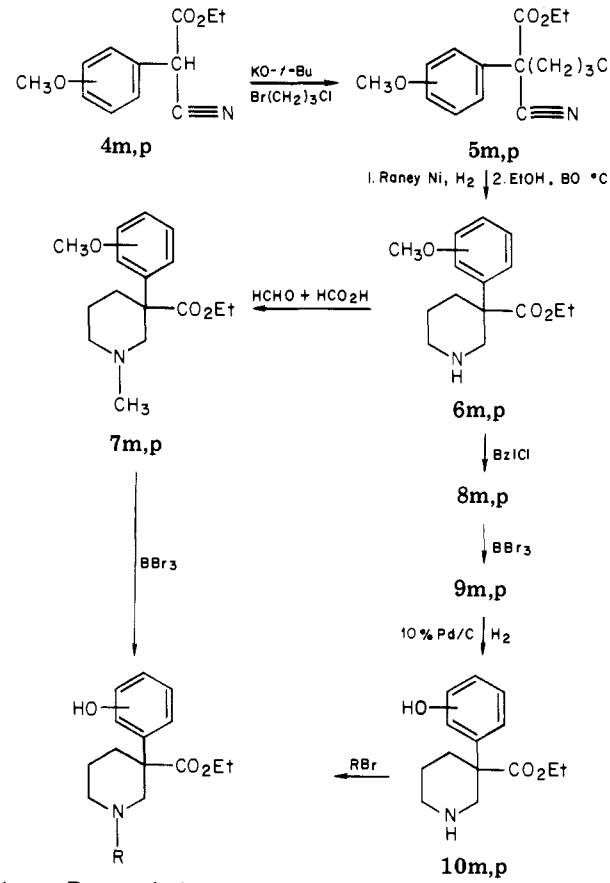
represent two series of analgesic analogues that possess the β-phenethylamine moiety, a structural feature that is present in most opioid antagonists.[2] Yet, the appropriately N-substituted (allyl, *n*-propyl, cyclopropylmethyl) derivatives of 1 and 2 lacked significant opioid antagonist activity.

This prompted us to prepare the phenolic hydroxyl derivatives of 2 to evaluate the importance of this functional group for opioid antagonist activity in this series. The phenolic hydroxyl has long been associated with potent morphine-like in vivo biological activity and opiate receptor binding activity.[3] A meta-positioned phenolic hydroxyl has been cited as a structural feature which is required for optimum opioid antagonist activity.[4-6] If one views these arylnipecotates as β-phenethylamine derivatives of N-substituted opioids, then a *p*-hydroxyl rather than a *m*-hydroxyl would correspond to that of nalorphine, 3. Thus, we prepared both the *m*- and *p*-hydroxy derivatives of 2.

Scheme I[a]



11m,p, R = methyl
12m,p, R = allyl
13m,p, R = cyclopropylmethyl
14m,p, R = *n*-propyl

[a] m = meta position; p = para position (see X of Table I).

Chemistry. The two key intermediates, ethyl 3-(*m*- or *p*-methoxyphenyl)nipecotate (**6m,p**), were synthesized by a pathway similar to that which we had previously used for the preparation of 2 and is illustrated in Scheme I.[1,7]

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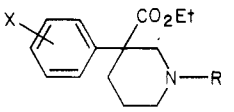
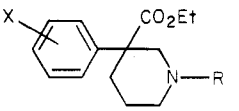
Table I. N-Substituted Ethyl Arylnipecotates, 6-14



no.	R	X	mp, °C	recrystn solvent[a]	yield %	formula[b]
6m	H	m-OCH_3	121-122	A	61	$C_{15}H_{21}NO_3 \cdot C_2H_2O_4$[c]
6p	H	p-OCH_3	158-159	A	64	$C_{15}H_{21}NO_3 \cdot C_2H_2O_4$[c]
7m	Me	m-OCH_3	131-132	B	85	$C_{16}H_{23}NO_3 \cdot HCl$
7p	Me	p-OCH_3	138-140	B	48	$C_{16}H_{23}NO_3 \cdot HCl$
8m	Bzl	m-OCH_3	174-176	B	87	$C_{24}H_{27}NO_3 \cdot HCl$
8p	Bzl	p-OCH_3	185-188	B	87	$C_{24}H_{27}NO_3 \cdot HCl$
9m	Bzl	m-OH	174-175	B	69	$C_{23}H_{25}NO_3 \cdot HCl$
9p	Bzl	p-OH	216-218 dec	B	42	$C_{23}H_{25}NO_3 \cdot HCl$
10m	H	m-OH	197-198	B	92	$C_{14}H_{19}NO_3 \cdot HCl$
10p	H	p-OH	219-222 dec	B	83	$C_{14}H_{19}NO_3 \cdot HCl$
11m	Me	m-OH	176-177	B	52	$C_{15}H_{21}NO_3 \cdot HBr$
11p	Me	p-OH	203-205 dec	C	41	$C_{15}H_{21}NO_3 \cdot HBr$
12m	allyl	m-OH	166-169	B	79	$C_{17}H_{23}NO_3 \cdot 1.5H_3PO_4$
12p	allyl	p-OH	187-189 dec	B	28	$C_{17}H_{23}NO_3 \cdot HCl$
13m	CPM[d]	m-OH	136-138	B	85	$C_{18}H_{25}NO_3 \cdot 2H_3PO_4$
13p	CPM[d]	p-OH	184-186 dec	C	61	$C_{18}H_{25}NO_3 \cdot HCl$
14m	n-Pr	m-OH	163-165	B	92	$C_{17}H_{25}NO_3 \cdot 1.5H_3PO_4$
14p	n-Pr	p-OH	182-183 dec	B	58	$C_{17}H_{25}NO_3 \cdot HCl$

[a] A = EtOAc-i-PrOH, B = EtOAc-EtOH, C = MEK-EtOH. [b] Analyses for C, H, and N were within ±0.4% of the theoretical values. [c] Oxalate salt. [d] CPM = cyclopropylmethyl.

Table II. Pharmacology of N-Substituted Ethyl 3-(m- or p-Hydroxyphenyl)nipecotates 11-14 in Mice



no.	R	X	analgesia ED_{50}, μmol/kg (95% CL): tail flick	analgesia ED_{50}, μmol/kg (95% CL): acetic acid writhing	antagonism of morphine analgesia AD_{50}, μmol/kg (95% CL): tail flick
11m[c]	Me	m-OH	>290	5.66 (0.12-25.5)	24.4 (6.13-85.4)[d]
11p[c]	Me	p-OH	>290	22.9 (8.13-93.8)	>290
12m[e]	allyl	m-OH	>229	7.22 (1.60-32.3)	>92
12p[f]	allyl	p-OH	>307	10.4 (2.98-36.5)	>307
13m[g]	CPM	m-OH	68 (34-76)	4.0 (0.66-24)	18.8 (4.95-71.5)
13p[f]	CPM	p-OH	100 (60-182)	4.65 (0.77-27.9)	>294
14m[e]	n-Pr	m-OH	>91	5.59 (1.60-19.6)	59.3 (25.8-136)
14p[f]	n-Pr	p-OH	>305	62.5 (21.7-193)	140 (54.0-363)
morphine[h]			4.9 (2.8-8.7)	0.72 (0.27-2.08)	
naloxone[f]					0.08 (0.014-0.52)

[a] Subcutaneous administration of salt dissolved in distilled H_2O. [b] Antagonism measured 30 min after 9.7 μmol/kg (ED_{80}) of morphine sulfate. [c] HBr salt. [d] Additional test results: 27.6 (8.10-93.8) and 15.1 (4.9-42.4). [e] $1.5H_3PO_4$ salt. [f] HCl salt. [g] $2H_3PO_4$ salt. [h] H_2SO_4 salt.

The starting methoxycyano ester **4** was prepared by known procedures. Thus, the required methoxybenzyl alcohol was reacted with thionyl chloride to yield the analogous chloride.[8] The crude methoxybenzyl chloride was converted to a nitrile with NaCN in Me_2SO[9] and then reacted with diethyl carbonate using sodium hydride as the basic reagent[10] to yield the required cyano ester, **4**. The cyano ester **4** was alkylated[11] with 1-bromo-3-chloropropane to give the chloronitrile **5**, which was reduced[1] to the corresponding primary amine. The amine was not isolated but cyclized to give the intermediate **6**.

Direct O-demethylation of **6** with 48% HBr or BBr_3 gave only 8–11% of **10**. However, when **6** was first N-benzylated and then O-demethylated using BBr_3[12] followed by hydrogenolysis of the *N*-benzyl group, **10** was obtained in overall yields of 30–55%. The N-substituted target compounds (**12–14**) were prepared by N-alkylation of **10** with the appropriate alkyl bromide.[13] Only in the case of the very reactive halide, allyl bromide, was dialkylation a problem, yielding the *O,N*-diallyl derivative (ca. 20%). Quaternization was minimal when only a small excess of alkyl bromide was used. It was found that **10** could not be directly N-methylated with formaldehyde and formic acid. An alternate pathway was used to prepare **11** which involved, first, N-methylation[7] of **6** and then BBr_3 O-demethylation.

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As we had previously described with ethyl 3-phenylnipecotate (2),[1] the ^1H NMR spectra of these compounds also showed nonequivalence of the protons on the 2- and 4-methylenes of the piperidine ring. Decoupling experiments on the unsubstituted methoxy esters **6m** and **6p** verified our earlier tentative assignment for these nonequivalent protons. In both **6m** and **6p** only the C-2 protons were sufficiently resolved to allow assignment of coupling constants. The C-2 protons were analyzed as AB systems with δ and coupling constants given under Experimental Section. The couplings were unchanged either after D_2O treatment or in the *N*-methyl derivatives, **11**. This is indicative of diaxial coupling[14] and indicates that the axial C-2 proton of **6**, which is almost 1-ppm downfield, is cis to the equitorial phenyl.

Pharmacology. Compounds **11–14** were tested for analgesic and antagonist activity by sc administration of aqueous solutions of the amine salts to mice (Table II). No significant morphine-like analgesic activity was found by the tail-flick method.[15] This result was not entirely unexpected, since the tail-flick test is generally insensitive to antagonist analgesics such as nalorphine or pentazocine. In the acetic acid writhing test,[16] a method which detects antagonist analgesics, **11–14** demonstrated analgesic activity. These results are not conclusive though, since the writhing test does give positive results with many drugs that are not effective analgesics.[17]

Compounds **11–14** were tested for antagonist activity against morphine sulfate by the mouse tail-flick method.[15] Weak antagonist action was observed with four compounds as shown in Table II. Interestingly, **11m** (R = CH_3, X = *m*-OH) was found to be another example of a *N*-methyl derivative with antagonist activity.[5,18,19]

The marginal antagonist activity exhibited by these compounds indicates that structural features in addition to the β-(hydroxyphenyl)ethylamine moiety are required for a compound to be a potent antagonist. These preliminary pharmacological data also tends to support the conclusion of previous investigators[4-6] that a meta-position aryl hydroxyl enhances analgesic and/or antagonist action. We are continuing to study the analgesic activity of these arylnipecotates in an effort to evaluate the significance of these results.

Experimental Section

The structures of all compounds are supported by their IR (Perkin-Elmer 467) and ^1H NMR (Perkin-Elmer R-12B) (tetramethylsilane) spectra. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements (performed by Baron Consulting Co., Orange, CT) were within 0.4% of theoretical values.

Ethyl 5-Chloro-2-cyano-2-(*m*-methoxylphenyl)valerate (5m). Ethyl *m*-methoxyphenylcyanoacetate[10] (**4m**; 110 g, 0.5 mol) was slowly added to a cold solution of commercial potassium *tert*-butoxide (61.7 g, 0.55 mol) in *t*-BuOH (500 mL). The resultant suspension was stirred and cooled while 1-bromo-3-chloropropane (158 g, 1.0 mol) was rapidly added. The mixture was stirred at room temperature for 24 h and then refluxed for an additional 1 h. The mixture was cooled, diluted with H_2O (250 mL), made slightly acidic with 4 N AcOH, and extracted three times with Et_2O. The combined Et_2O extracts were washed with 5% Na_2CO_3 and saturated NaCl, dried (Na_2SO_4), filtered, concentrated in vacuo, and distilled. The desired cyano ester **5m** distilled at 168–172 °C (0.7 mm) as a colorless oil (132 g, 89%). Anal. ($C_{15}H_{18}ClNO_3$) C, H, N.

Ethyl 5-Chloro-2-cyano-2-(*p*-methoxyphenyl)valerate (5p). This compound was prepared by the procedure described above for **5m** from ethyl *p*-methoxyphenylcyanoacetate (**4p**;[20] 143.4 g, 0.65 mol), potassium *tert*-butoxide (80.2 g, 0.72 mol), *t*-BuOH (650 mL), and 1-bromo-3-chloroproapane (204.8 g, 1.3 mol). The crude reaction mixture was distilled to yield **5p** (110 g, 57%), bp 140–142 °C (0.1 mm). Anal. ($C_{15}H_{18}ClNO_3$) C, H, N.

Ethyl 3-(*m*- or *p*-Methoxyphenyl)nipecotate (6m,p). Ethyl 5-chloro-2-cyano-2-(*m*- or *p*-methoxyphenyl)valerate (**5m** or **5p**; 10 g, 0.034 mol), EtOH (50 mL), Raney Ni (two tablespoons, Strem Chemicals, Inc.), and saturated NH_3–ETOH (50 mL) were placed in a Parr low-pressure hydrogenation bottle. Just before reduction was started, platinic chloride, 0.16 g of $H_2PtCl_6 \cdot 6H_2O$ in 10 mL of EtOH, was added to the hydrogenation mixture. The above mixture was shaken in a Parr low-pressure hydrogenation apparatus at 50 psi until hydrogen uptake stopped (ca. 8 h). After the Ni was removed by filtration, the solution was concentrated in vacuo. The residue was dissolved in anhydrous EtOH (200 mL) and refluxed for 8 h to afford the cyclized product. The reaction mixture was cooled and concentrated in vacuo. The residual oil was dissolved in 4 N HCl and extracted with ether. The water fraction was made strongly basic with 25% NaOH. The separated oil was extracted with Et_2O, dried (Na_2SO_4), filtered, concentrated in vacuo, and distilled. Ester **6m** distilled at 130–132 °C (0.1 mm) as a colorless oil and **6p** distilled at 131–133 °C (0.2 mm). Final purification of **6m** and **6p** was accomplished by conversion of the free bases to their respective oxalate salts. An EtOAc solution of **6m** or **6p** was slowly added to a 1 mol excess of oxalic acid dissolved in *i*-PrOH. See Table I for details. Free base **6m**: NMR ($CHCl_3$) δ 1.16 (t, 3, CH_3), 1.40–2.20 [m, 4, one 4-H, two 5-H and N-H (s, 1.67)], 2.30–3.25 (m, 3, one 4-H and two 6-H), 2.75 (d, 1, ^2J = 11 Hz, 2-H), 3.74 [d, 1, ^2J = 11 Hz (showing long-range coupling, ^2J = 2.5 Hz), 2-H], 3.76 (s, 3, OCH_3), 4.16 (q, 2, OCH_2), 6.68–7.48 (m, 4, aromatic). Free base **6p**: NMR ($CDCl_3$) δ 1.18 (t, 3, CH_3), 1.45–2.10 (m, 3, one 4-H, two 5-H), 2.22 (s, 1, N-H), 2.40–3.20 (m, 3, one 4-H and two 6-H), 2.73 (d, 1, ^2J = 11.5 Hz, 2-H), 3.73 [d, 1, ^2J = 11.5 Hz (showing long-range coupling, ^2J = 2.5 Hz), 2-H], 3.78 (s, 3, OCH_3), 4.17 (q, 2, OCH_2), 7.14 (q, 4, aromatic).

Ethyl 3-(*m*- or *p*-Hydroxyphenyl)nipecotate (10m,p). Free base **6** (10 g, 0.028 mol), benzyl chloride (5.32 g, 0.042 mol), and $KHCO_3$ (28 g, 0.28 mol) in butanone (250 mL) were stirred and refluxed for 6 h. The mixture was filtered and concentrated in vacuo. The residue was dissolved in 4 N HCl and extracted with ether. The water fraction was made basic with $NaHCO_3$, extracted with Et_2O, washed with NaCl brine, dried ($CaSO_4$), filtered, concentrated in vacuo, dissolved in anhydrous Et_2O, and treated with HCl gas to yield **8**·HCl. See Table I for details.

BBr_3 (14.0 g, 0.056 mol) dissolved in CH_2Cl_2 (10 mL) was slowly added to a stirred solution of **8** (10.5 g, 0.028 mol) in CH_2Cl_2 (100 mL) at −60 °C over a 15-min period. The mixture was stirred for 1 h at −60 °C and then allowed to warm to room temperature. The mixture was cooled to −40 °C and EtOH (50 mL) was added. The mixture was evaporated in vacuo and the residue was dissolved in 3 N NaOH using minimal heat and then extracted with Et_2O, dried ($CaSO_4$), filtered, evaporated in vacuo, dissolved in anhydrous Et_2O, and treated with HCl gas to yield **9**·HCl. See Table I for details.

9·HCl (10 g, 0.027 mol), EtOH (100 mL), and 10% Pd/C catalyst (5.0 g) were shaken in a Parr low-pressure hydrogenation apparatus at 50 psi for 2 h. The hydrogenolysis mixture was

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filtered and concentrated in vacuo to give crude 10·HCl. The hydrochloride salt was purified by recrystallization. See Table I for details.

General Procedures for the Preparation of N-Substituted Ethyl 3-(*m*- or *p*-Hydroxyphenyl)nipecotates (11m,p–14m,p). The *N*-methyl derivative 11 was prepared by methylation of free base 6 with HCHO and HCO_2H using a known procedure[7] to yield 7, which was subsequently O-demethylated as described for the preparation of 10. All other N-substituted compounds (12–14) were prepared as follows: Ethyl 3-(*m*- or *p*-hydroxyphenyl)nipecotate hydrochloride (10·HCl; 3.00 g, 0.0105 mol), alkyl bromide (0.012 mol), $KHCO_3$ (8.4 g, 0.084 mol), and butanone (100 mL) were refluxed with stirring for 6 h or until the reaction was complete as indicated by GC (3% OV-17). The cooled mixture was filtered and concentrated in vacuo, and the residue was dissolved in dilute HCl. The solution was washed with Et_2O (discarded), made basic with excess solid $NaHCO_3$, and extracted with Et_2O. The Et_2O extract was dried ($CaSO_4$) and concentrated in vacuo to yield a residual oil, which was converted to the indicated salt and purified by recrystallization. See Table I for details of 12–14.

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Some Analogues of Luteinizing Hormone-Releasing Hormone with Substituents in Position 10

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As part of our studies on the design of agonists of the luteinizing hormone-releasing hormone (LH-RH), we have synthesized the [des-Gly-NH_2^{10}]-LH-RH *N*-methylhydrazide (1), the corresponding thiosemicarbazide (2), and the *N*-formyl- (3), *N*-acetyl- (4) and *N*-(trifluoroacetyl)hydrazide (5). Analogue 1 may be regarded as isosteric with [des-Gly-NH_2^{10}]-LH-RH *N*-alkylamides which are, in general, potent agonists. Analogues 2–5 may be regarded as isosteric with [aza-Gly-NH_2^{10}]-LH-RH, which is equipotent with the hormone. The required protected intermediates were prepared by solid-phase synthesis, and the free peptides were prepared from them by deprotection with HF, followed by purification on Sephadex G-25. Bioassay of these analogues with rat hemipituitaries in vitro showed the following values as percentages of the hormonal values for the release of LH and FSH respectively: *N*-methylhydrazide (1), 17 and 11%; semithiocarbazide (2), 6.5 and 4.6%; *N*-formylhydrazide (3), 15.3 and 10%; *N*-acetylhydrazide (4), 1.2 and 0.6%; *N*-(trifluoroacetyl)hydrazide (5), 1.0 and 0.9%. Thus, these types of isosteric substitutions are inimical to the preservation of the high biological activity of LH-RH.

In attempting to explore the usefulness of isosteric substitutions[1] in the design of agonists of the luteinizing hormone-releasing hormone (LH-RH), we synthesized analogues featuring the substitution of glycinamide-10 with hydrazine derivatives. Thus, we prepared the [des-Gly-NH_2^{10}]-LH-RH *N*-methylhydrazide (1) the thiosemicarbazide (2), and the *N*-formyl- (3), *N*-acetyl- (4), and *N*-(trifluoroacetyl)hydrazide (5). The *N*-methylhydrazide analogue may be regarded as an isostere of the highly potent [des-Gly-NH_2^{10}]-LH-RN *N*-alkylamides,[2] whereas the other four analogues may be regarded as isosteric with [aza-Gly-NH_2^{10}]-LH-RH, which is equipotent with LH-RH.[3] Of additional interest was the observation of Coy et al. that several [des-Gly-NH_2^{10}]-LH-RH *N*-(fluoroalkyl)amides were more potent than the hormone itself,[4] which was an additional consideration in the preparation of the trifluoroacetyl derivative 5. Our choice of derivatives related to azaglycine, itself an *N*-carbamylhydrazide, stems from the reported difficulty of several enzymes in hydrolyzing peptide linkages involving an aza amino acid.[5] Thus, we hoped to design analogues that might be metabolized more slowly and, because of the gradation in polarity of acyl substitutents in going from thiocarbamyl to formyl, acetyl, and trifluoroacetyl, we hoped to see a trend in affinity for receptors which would be evidenced in the potency of these analogues.

Peptide Synthesis. The desired protected peptides were synthesized by the solid-phase method of synthesis[6] as previously reported,[7] yielding pGlu-His-Trp-Ser-(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-O-resin. Methanolysis of this resin ester with MeOH–triethylamine led to the protected nonapeptide methyl ester. Treatment of the ester with CH_3NHNH_2 and deprotection of the resulting peptide with liquid HF, as previously described,[9] gave 1. LH-RH$^{1-9}$ acid, prepared by direct treatment of the above peptide resin with HF, was coupled to thiosemicarbazide or formyl hydrazide with dicyclohexylcarbodiimide as the coupling agent, giving 2 and 3, respectively. Treatment of protected nonapeptide resin with hydrazine and deprotection of the product by treatment with HF gave LH-RH$^{1-9}$ hydrazide. Acylation of the latter with acetic anhydride or *p*-nitrophenyl trifluoroacetate yielded 4 and 5, respectively. All analogues were purified by ion-exchange chromatography and then by gel filtration[10] and/or

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