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Luteinizing Hormone-Releasing Hormone and Analogs. Synthesis and Biological Activity

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A fragment synthesis of LH-RH is described which lends itself to large-scale preparation. Fragment 1–3 is coupled with fragment 4–6 followed by reaction with the tetrapeptide 7–10 to yield the unprotected decapeptide. The preparation of analogs follows the same synthetic pattern. The biological activity of the analogs is compared with that of synthetic LH-RH.

The isolation and structure elucidation of the porcine hypothalamic luteinizing hormone-releasing hormone (LH-RH) by Schally and collaborators[1] and its apparent diagnostic and therapeutic value[2] have caused a flurry of synthetic activity.[3-17] The choice of our synthetic approach to LH-RH was influenced by two considerations: the synthesis should be simple enough to lend itself to large-scale preparation, and extensive separation procedures should be avoided after the final coupling step. The fragment synthesis selected permitted considerable scale up. Since most of the partial sequences were crystalline, time-consuming purifications of the intermediates could be minimized. Only one separation step was required to obtain the LH-RH in the desired state of purity.[18] The synthesis of the LH-RH analogs was quite similar to that of LH-RH.

Chemistry. The synthesis of LH-RH, Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, is represented in Scheme I. The partial sequence 1–3 (A) was coupled with the partial sequence 4–6 (B) and the hexapeptide (C) was coupled with fragment 7–10 (D) to yield directly the unprotected decapeptide.

Fragment A was prepared by coupling of Pyr-His-$NHNH_2$[19] with H-Trp-OMe[20] by the azide method. The resulting tripeptide methyl ester was hydrazinolyzed to give A. B was obtained by hydrolysis of Z-Tyr(Bzl)-Gly-OMe[21] to the acid and conversion of the acid by the mixed anhydride method with *tert*-butyl carbazate to Z-Tyr(Bzl)-Gly-NHNHBoc, which was hydrogenolyzed to H-Tyr-Gly-NHNHBoc. This intermediate was condensed with Z-Ser-ODnp[22] to give Z-Ser-Tyr-Gly-NHNHBoc which was hydrogenolytically transformed into B. For the partial sequence D, H-Pro-Gly-NH_2 was coupled with Boc-Arg(NO_2)-OH[23] to Boc-Arg(NO_2)-Pro-Gly-NH_2. After removal of the Boc group and coupling with Z-Leu-OTcp,[24] the tetrapeptide was hydrogenolyzed with concomitant removal of Z and NO_2 to yield D.

The condensation of A and B to yield C, followed by reaction with D and chromatographical purification of the product gave LH-RH (AY-24,031).

LH-RH Analogs. The procedures employed were essentially the same as those used in the LH-RH synthesis. The methods for preparing the 7–10 tetrapeptide amides were governed by the individual changes in amino acid composition and are described in detail in the Experimental Section. Table I provides a summary of the analogs prepared and analytical and biological data.

Endocrinology. Induction of Ovulation in Hamsters. The method used was similar to that described in the literature by Arimura, *et al.*[25]

The estrus cycle of Syrian hamsters (*ca.* 100 g) was checked by inspecting vaginal discharge which occurred every 4 days in most of the animals. Experiments were started after the animals had shown two regular 4-day cycles. At 1:00 p.m. on the day of proestrus, 13 mg of phenobarbital/100 g of body weight was injected ip to block

Scheme I

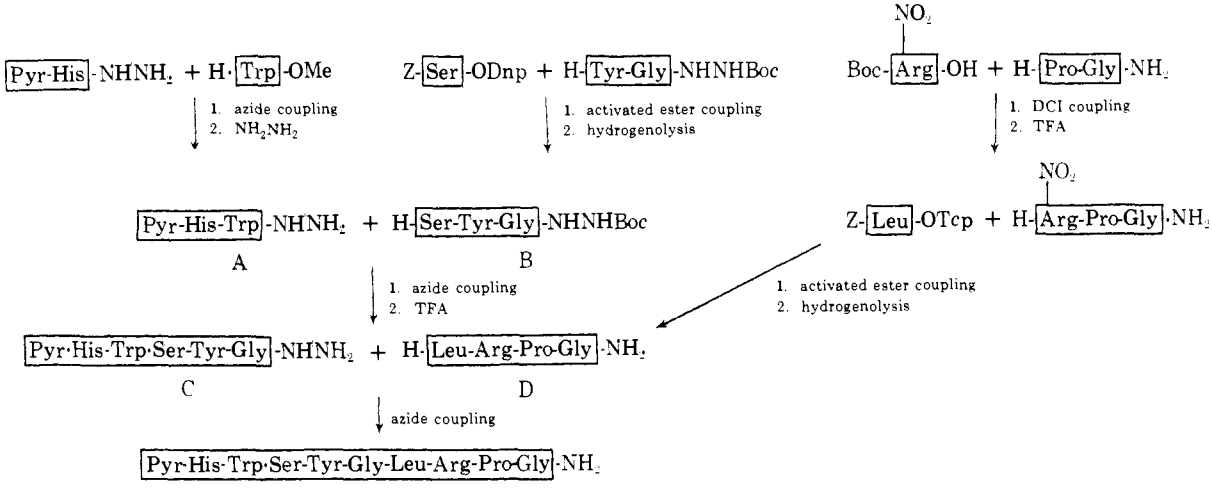

Table I. Amino Acid Analyses

	Glu	His	Ser	Tyr	Gly	Leu	Arg	Pro	Dose per hamster, μg/sc	No. ovulating	Mean ± S.E., no. of ova per ovulating hamster
1 LH-RH	1.07	1.00	0.90	1.12	2.01	0.86	0.95	0.96	0.12	0/6	0
									0.23	3/3	9.6 ± 1.1
									0.45	5/6	9.0 ± 1.3
			Ala								
2 [Ala⁴]-LH-RH	1.04	0.85	1.11	0.98	1.99	1.04	0.93	1.09	1.0	3/5	5.7 ± 3.2
									5.0	4/4	10.5 ± 0.3
				Ala							
3 [Ala⁵]-LH-RH	1.15	0.89	1.23	1.24	2.11	1.01	0.97	0.95	1.0	1/5	11
									5.0	4/5	10 ± 0.5
				Phe							
4 [Phe⁵]-LH-RH	1.04	0.98	0.92	0.98	2.00	0.97	0.93	0.81	0.25	0/3	0
									0.5	4/6	9.0 ± 0.5
									1.0	3/3	11 ± 0.7
5 [Ser⁷]-LH-RH	1.00	0.99	1.79	0.96	1.99		1.12	1.05	0.5	0/5	0
									1.0	2/3	6.5 ± 0.5
									2.5	5/5	6.4 ± 0.6
							Lys				
6 [Lys⁸]-LH-RH	1.03	0.95	1.05	1.04	2.07	0.99	0.99	1.13	0.1	0/4	0
									0.25	7/8	4.6 ± 0.9
									0.50	9/10	7.7 ± 0.7
7 [Glu⁸]-LH-RH	2.06	0.81	0.82	0.97	1.89	1.00		1.02	10.0	4/7	2.6 ± 1.1
									25.0	9/10	5.9 ± 0.8
			Ala	Phe							
8 [Ala⁴,Phe⁵]-LH-RH	1.05	0.94	1.05	0.97	2.07	0.98	1.01	0.97	1.0	3/5	2.7
									2.5	3/4	3.7
									5.0	5/5	6.4 ± 1.2
			Ala	Phe			Lys				
9 [Ala⁴,Phe⁵,Lys⁸]-LH-RH	0.87	0.86	0.88	0.78	2.00	0.95	1.01	0.84	5.0	0/2	0
									10.0	1/4	3.0
									25.0	5/5	6.8 ± 1.2
10 LH-RH-NH(CH₂)₄NH₂	1.00	0.97	0.81	0.97	2.00	1.01	1.00	1.03	25.0	0/4	0
									100.0	0/5	0

spontaneous ovulation. On the same day half the total dose of either LH-RH or the analogs was injected sc at 3:00 p.m. and the other half at 4:00 p.m. Control animals received 0.5 ml of water according to the same schedule. The following morning, the hamsters were killed and their oviducts inspected under a microscope for the presence of ova. The number of animals which had ovulated as well as the number of freshly shed ova in their oviducts was recorded.

Results

The biological activity of LH-RH and various analogs is summarized in Table I. None of the analogs prepared showed a higher potency than LH-RH, but all of them retained activity, at least to some degree, with the exception of compound **10**.

Replacement of tyrosine by phenylalanine (compound **4**) leads to a substance with approximately 50% of the activity of LH-RH. Similar results were obtained by Coy, *et al.*,[26] in a different *in vivo* model. Even replacement of tyrosine by the less bulky and nonpolar alanine (compound **3**) does not cause complete loss of activity. Substitution of alanine for serine in position 4 (compound **2**) has a similar effect, in agreement with findings by other workers.[27,28] Compounds **2**–**4** demonstrate that removal of a polar group in position 4 or 5 leaves the principal biological effect unaltered, since ovulation can, in all cases, be induced although higher doses are required. Surprisingly, the opposite also holds true; on introduction of an additional hydroxyl group, replacement of leucine by serine (compound **5**), 10% of the biological effect is still retained. Compound **6**, having lysine in place of arginine, is the most potent analog of this series. Other workers[10,27] using a different biological model have observed a somewhat more pronounced decrease in potency. Substitution of more than one of the amino acids present in the LH-RH molecule, as in compounds **8**[28] and **9**, reduces activity considerably.

Compound **10** was prepared in the hope of increasing and prolonging the activity.[29] However, the compound was completely inactive at the doses employed. The most remarkable and completely unexpected observation was the retention of approximately 1% of LH-RH activity on substitution of the strongly basic arginine by glutamic acid (compound **7**), which emphasized the considerable lack of fastidiousness of the receptor with respect to alterations in the structure of LH-RH.

Experimental Section

Synthesis of LH-RH. Pyr-His-Trp-OMe. Isopentyl nitrite (5.7 ml, 42 mmol) was added under vigorous stirring at −20° to a solution of 8.4 g (30 mmol) of Pyr-His-N_2H_3[19] in 140 ml of DMF, 100 ml of DMSO, and 74 ml of 2.4 *N* HCl in THF. After 30 min at −20°, 30 ml of triethylamine was dropped in, followed by addition of 8.17 g (32 mmol) of H-Trp-OMe HCl[20] and 4.4 ml of triethylamine dissolved in 20 ml of DMF. The mixture remained for 18 hr in the ice bath, was filtered, and concentrated *in vacuo*. The residue was triturated with Et_2O and the insoluble material was crystallized from CH_3OH: yield 9.9 g (71%); mp 241–243°. Anal. ($C_{23}H_{26}N_6O_5$) C, H, N.

Pyr-His-Trp-N_2H_3 (A). A solution of 7.09 g (15 mmol) of Pyr-His-Trp-OMe and 13.5 ml (270 mmol) of $N_2H_4 \cdot H_2O$ in 1 l. of CH_3OH was stirred at 0° for 10 hr and at room temperature for 2 days. The precipitate was collected by filtration and crystallized from CH_3OH: yield 5.6 g (79%); mp 165–169°; $[\alpha]^{25}D$ −24.6° (*c* 1.0, DMF). *Anal.* ($C_{22}H_{26}N_8O_4 \cdot 0.5H_2O$) C, H, N.

Z-Tyr(Bzl)-Gly-OH. NaOH (1 *N*, 5 ml) was added to 2 g (4.2 mmol) of Z-Tyr(Bzl)-Gly-OMe[21] dissolved in 25 ml of CH_3O-CH_2CH_2OH under vigorous stirring. After dissolution of the precipitate 5.25 ml of HCl (1 *N*) was dropped in under cooling. The H_2O washed precipitate was crystallized from CH_3OH-H_2O:

yield 1.5 g (70%); mp 166–167°; $[\alpha]^{25}D$ −22.6° (c 1.0, DMF). *Anal.* ($C_{26}H_{26}N_2O_6$) C, H, N.

Z-Tyr(Bzl)-Gly-NHNHBoc. To 2.31 g (5 mmol) of Z-Tyr(Bzl)-Gly-OH dissolved in 12.5 ml of THF and 0.7 ml (5 mmol) of triethylamine were added under stirring at −10° 0.48 ml (5 mmol) of ethyl chloroformate followed by 0.73 g (5 mmol) of *tert*-butyl carbazate. After 30 min at 0° and 5 hr at room temperature the solvent was evaporated, the residue was treated with 100 ml of EtOAc, filtered, and dried, and the solvent was evaporated *in vacuo*. The residue was crystallized from C_6H_6–C_6H_{14}: yield 2.5 g (86%); mp 88–90°. *Anal.* ($C_{31}H_{36}N_4O_7$) C, H, N.

Z-Ser-Tyr-Gly-NHNHBoc. Z-Tyr(Bzl)-Gly-NHNHBoc (2.88 g, 5 mmol) in 40 ml of CH_3OH was hydrogenated in the presence of 0.20 g of 5% Pd on charcoal. After filtration and evaporation of the solvent *in vacuo*, the residue was dissolved in 10 ml of DMF and added at 0° to 1.6 g (4 mmol) of Z-Ser-ODnp[22] in 10 ml of DMF. After 3 days at 0° the solvent was evaporated *in vacuo* and the residue dissolved in $CHCl_3$–CH_3OH–C_5H_5N (100:25:1) and chromatographed on silica gel. The desired material was detected by tlc on silica gel G plates ($CHCl_3$–MeOH 4:1), R_f 0.21 with Cl-tolidine reagent. This material was collected, dissolved in CH_3OH, and precipitated with Et_2O: yield 2.12 g (79%); $[\alpha]^{25}D$ −13.1° (c 1.0, DMF). *Anal.* ($C_{27}H_{35}N_5O_9$) C, H, N.

H-Ser-Tyr-Gly-NHNHBoc (B). Z-Ser-Tyr-Gly-NHNHBoc (9.6 g, 18 mmol) in 350 ml of CH_3OH was hydrogenated in the presence of 0.90 g of 5% Pd on charcoal. After filtration and evaporation of CH_3OH *in vacuo*, the residue was crystallized from CH_3OH–Et_2O: yield 4.84 g (66%); mp 132–135°; $[\alpha]^{25}D$ −5.4° (c 1.0, DMF). *Anal.* ($C_{19}H_{29}N_5O_7$) H, N; C: calcd, 51.93; found, 51.47.

Pyr-His-Trp-Ser-Tyr-Gly-NHNH$_2$ (C). Isopentyl nitrite (2.06 ml, 15.3 mmol) was introduced under stirring at −20° into a solution of 6.5 g (14 mmol) of Pyr-His-Trp-$NHNH_2$ in 70 ml of DMF, 59 ml of DMSO, and 35 ml of 2.4 *N* HCl in THF. After 30 min at −20°, 13 ml of triethylamine was added, followed by 6.17 g (14 mmol) of H-Ser-Tyr-Gly-NHNHBoc dissolved in 60 ml of DMF. The mixture remained at 0° for 18 hr, the precipitate was removed, and the filtrate was concentrated *in vacuo*. The residue was dissolved in CH_3OH. The precipitate obtained on addition of Et_2O was collected and reprecipitated from CH_3OH with Et_2O. The white powder was stirred with 270 ml of CF_3COOH for 30 min at 0° and for 30 min at room temperature. The solution was introduced into 3 l. of Et_2O. The precipitate was collected and crystallized from CH_3OH: yield 8.1 g (65%); mp 200° (foam); $[\alpha]^{25}D$ −21.2° (c 1.0; H_2O); R_f 0.52 tlc on silica gel G plates (HOAc-BuOH-EtOAc-H_2O 1:1:1:1) detected by Cl-tolidine reagent. *Anal.* ($C_{36}H_{43}N_{11}O_9CF_3COOH$) C, H, N.

Z-Pro-Gly-NH$_2$.[30] To a stirred solution of 12.5 g (50 mmol) of Z-Pro-OH[31] and 7.26 g (52 mmol) of H-Gly-OEt HCl in 100 ml of $CHCl_3$, 7.28 ml (105 mmol) of triethylamine followed by 10.7 g (52 mmol) of DCI was added. After 16 hr at 0° the precipitate was collected and the filtrate washed with 50 ml of H_2O, 50 ml of 1 *N* HCl, and 50 ml of saturated NaCl solution, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was dissolved in 150 ml of NH_3-saturated CH_3OH and left at 0° for 48 hr. The solvent was removed *in vacuo* and the residue was crystallized from CH_3OH–Et_2O: 8.02 g (52.5%); mp 116–120°; $[\alpha]^{25}D$ −43.8° (c 1.0, MeOH). *Anal.* ($C_{15}H_{13}N_3O_4$) C, H, N.

Boc-Arg(NO$_2$)-Pro-Gly-NH$_2$. Z-Pro-Gly-NH_2 (6.1 g, 20 mmol) in 80 ml of AcOH containing 20 mmol of 1 *N* HCl was hydrogenolyzed in the presence of 0.50 g of 5% Pd on charcoal. After filtration and removal of the solvent *in vacuo*, the residue was dissolved in 40 ml of DMF, basified with 3.8 ml of triethylamine, and added to a solution of 50 ml of DMF containing 6.07 g (19 mmol) of Boc-Arg(NO_2)-OH,[23] 4.37 g (38 mmol) of *N*-hydroxysuccinimide, and 3.88 g (19 mmol) of DCI. The mixture remained for 14 hr at 0° and 24 hr at room temperature. After evaporation to dryness *in vacuo* the residue was dissolved in $CHCl_3$–CH_3OH (100:15) and chromatographed on silica gel. The material is crystallized from absolute EtOH: 3.5 g (37%); mp 165°; $[\alpha]^{25}D$ −25.8° (c 1.0, DMF). *Anal.* ($C_{18}H_{32}N_8O_7$) C, H, N.

Z-Leu-Arg(NO$_2$)-Pro-Gly-NH$_2$. A solution of 8.17 g (17.6 mmol) of Boc-Arg(NO_2)-Pro-Gly-NH_2 in 50 ml of CF_3COOH was kept at 0° for 30 min. The solution was added dropwise to 500 ml of absolute Et_2O; the precipitate was filtered off and washed with Et_2O. The solid was dissolved in 60 ml of DMF containing 2.45 ml of triethylamine and stirred for 15 min at 0°. To this solution 8.6 g (19.6 mmol) of Z-Leu-OTcp[24] in 60 ml of DMF and a drop of AcOH were added. After 24 hr at 0° and 24 hr at room temperature the solvent was removed *in vacuo* and the residue dissolved in $CHCl_3$–CH_3OH (100:15) and chromatographed on silica gel. The pure fractions were dissolved in CH_3OH and precipitated with isopropyl ether: yield 8.42 g (77%); $[\alpha]^{25}D$ −38.4° (c 1.0, DMF). *Anal.* ($C_{27}H_{41}N_9O_8$) C, H, N.

H-Leu-Arg-Pro-Gly-NH$_2$ (D). Z-Leu-Arg(NO_2)-Pro-Gly-NH_2 (8.05 g, 13 mmol) dissolved in 50 ml of AcOH was hydrogenolyzed in the presence of 3 g of 5% Pd on charcoal. The reaction mixture was filtered; the solvent was concentrated *in vacuo* and added dropwise to 1 l. of Et_2O. The precipitate was collected, washed with Et_2O, and dried under high vacuum: yield 6.36 g (89%).

Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH$_2$ (LH-RH). A solution of 1.1 ml (8.3 mmol) of isopentyl nitrite in 20 ml of DMF was added at −20° to 8.4 g (9.45 mmol) of C, dissolved in 37 ml of DMF, 33 ml of DMSO, and 27.7 ml of 1.65 *N* HCl in THF. After 30 min at −20°, 7 ml of triethylamine was dropped in, followed by addition of a solution of 4.27 g (7.62 mmol) of D in 45 ml of DMF and 2.8 ml of triethylamine. The mixture was filtered, the solvents were removed *in vacuo*, and the residue was dissolved in CH_3OH. The precipitate obtained on addition of Et_2O was purified by partition chromatography on Sephadex LH-20 by elution with the lower phase of *n*-BuOH–AcOH–H_2O (8:4:40). The combined fractions were concentrated to dryness *in vacuo*; the residue was dissolved in 50 ml of CH_3OH and slowly added to 1 l. of Et_2O. The collected precipitate was dried: yield 7.5 g (78%); $[\alpha]^{25}D$ −59.8° corrected for H_2O and HCl (c 1.0, 1% AcOH); R_f 0.42 tlc on silica gel G plates (HOAc-BuOH-EtOAc-H_2O 1:1:1:1) detected by Cl-tolidine reagent. *Anal.* ($C_{55}H_{75}N_{17}O_{13}$·HCl·$2H_2O$) H, N, O, Cl; C: calcd, 52.67; found, 52.25.

[Ala^4]-LH-RH. **Boc-Ala-Tyr-Gly-OMe.** Z-Tyr(Bzl)-Gly-OMe[21] (5.9 g, 12.4 mmol) was hydrogenolyzed to H-Tyr-Gly-OMe in the usual way. This compound (5.23 g, 11.7 mmol) was coupled with 4.31 g (11.7 mmol) of Boc-Ala-$NHNH_2$ as described under LH-RH. The product was recrystallized from CH_3OH–isopropyl ether: yield 2.22 g (44%); mp 176–178°; $[\alpha]^{25}D$ −26.3° (c 1.0, DMF). *Anal.* ($C_{20}H_{20}N_4O_7$) C, H, N.

Pyr-His-Trp-Ala-Tyr-Gly-OMe. Boc-Ala-Tyr-Gly-OMe (0.42 g, 1 mmol) was treated with 3 ml of 90% CF_3COOH for 30 min at 0°. The solution was poured into 50 ml of Et_2O and the collected precipitate underwent azide coupling as described for C with 0.47 g (1 mmol) of Pyr-His-Trp-$NHNH_2$. The usual work-up provided a precipitate which was dissolved in 3 ml of CH_3OH and slowly added to 100 ml of Et_2O. The solid material was collected: yield 0.47 g (62%); mp 195–200°; $[\alpha]^{25}D$ −7.6° (c 1.0, DMF). *Anal.* ($C_{37}H_{43}N_9O_9$·$4H_2O$) C, N; H: calcd, 6.08; found, 5.43.

Pyr-His-Trp-Ala-Tyr-Gly-Leu-Arg-Pro-Gly-NH$_2$ ([Ala^4]-LH-RH). Pyr-His-Trp-Ala-Tyr-Gly-OMe (0.43 g, 0.56 mmol) dissolved in 60 ml of CH_3OH was treated with 0.5 ml of N_2H_4·H_2O for 40 hr at 0°. The precipitate was collected: yield 0.39 g (92%). The hydrazide (0.3 g, 0.40 mmol) underwent azide coupling with 0.22 g (0.40 mmol) of D as described for LH-RH. The isolated material is purified on Sephadex LH-20 with CH_3OH. The fractions containing the product were concentrated to 3 ml and added to 50 ml of Et_2O. The precipitate was collected: 0.19 g (49%); $[\alpha]^{25}D$ −59.6° (c 1.0, 1% AcOH). *Anal.* ($C_{55}H_{75}N_{17}O_{12}$·HCl·$3H_2O$) C, H; N: calcd, 18.96; found, 18.21.

[Ala^5]-LH-RH. **Z-Ser-Ala-Gly-OEt.** Boc-Ala-OTcp[32] (4.10 g, 11.1 mmol) was added to 1.86 g (13.3 mmol) of H-Gly-OEt·HCl dissolved in 14 ml of $CHCl_3$ containing 1.95 ml (13.9 mmol) of triethylamine. After 40 hr at room temperature and the usual work-up the material was chromatographed over silica gel with EtOAc–C_6H_6 (2:5) as eluent: yield 2.92 g (81%); nmr ($CDCl_3$) δ 1.28 (t, *J* = 7 Hz, 3 H, CH_3CH_2), 1.34 (d, *J* = 7 Hz, 3 H, CH_3–), 1.45 [s, 9 H, $(CH_3)_3C$–]. Boc-Ala-Gly-OEt (2.7 g, 9.84 mmol) was deprotected with CF_3COOH and used in the next step. The substance was dissolved in 30 ml of $CHCl_3$ and 1.4 ml (10 mmol) of triethylamine was added at 0°, followed by 3.66 g (9 mmol) of Z-Ser-ODnp. After 24 hr at 0°, the solvent was evaporated *in vacuo* and the residue chromatographed on silica gel. The material eluted with EtOAc–2% CH_3OH was recrystallized from CH_3OH–isopropyl ether: yield 2.18 g (61%); mp 152–155°. *Anal.* ($C_{18}H_{25}N_3O_7$) C, H, N.

H-Ser-Ala-Gly-OMe. Z-Ser-Ala-Gly-OEt (0.99 g, 3.8 mmol) in 75 ml of CH_3OH was hydrogenolyzed in the presence of 0.15 g of 5% Pd on charcoal. After filtration and evaporation of the solvent *in vacuo*, the residue was crystallized from MeOH–isopropyl ether. Unexpectedly, the methyl ester was isolated: yield 0.6 g (60%); mp 123–126°; nmr (DMSO-d_6) δ 1.23 (d, *J* = 7 Hz, 3 H, CH_3CH), 3.63 (s, 3 H, CH_3O), 3.87 (d, *J* = 6 Hz, 2 H, CH_2OH). *Anal.* ($C_9H_{17}N_3O_6$) C, H, N.

Pyr-His-Trp-Ser-Ala-Gly-OMe. H-Ser-Ala-Gly-OMe (0.26 g, 1 mmol) was coupled by the azide method with 0.47 g (1 mmol) of Pyr-His-Trp-$NHNH_2$, as described under C. The obtained material was dissolved in 3 ml of CH_3OH and slowly added to 100 ml of Et_2O. The precipitate was collected and reprecipitated from CH_3OH–EtOAc: yield 0.59 g (84%); mp 165–170°; $[\alpha]^{25}D$ −17.7° (*c* 1.0, AcOH). *Anal.* ($C_{31}H_{39}N_9O_9 \cdot 4H_2O$) C, N; H: calcd, 6.28; found, 5.70.

Pyr-His-Trp-Ser-Ala-Gly-Leu-Arg-Pro-Gly-NH_2 ([Ala⁵]-LH-RH). Pyr-His-Trp-Ser-Ala-Gly-OMe (0.52 g, 0.75 mmol) in 60 ml of CH_3OH was treated with 0.7 ml of $N_2H_4 \cdot H_2O$ at 0° for 48 hr. The solvent was removed *in vacuo;* the residue was dissolved in 4 ml of DMF and slowly added to 200 ml of Et_2O. The precipitate was collected and used for azide coupling with 0.41 g (0.73 mmol) of D, as described for LH-RH. The material obtained was purified on Sephadex LH-20 using CH_3OH. The pure fractions were detected by tlc on silica gel G plates (HOAc–BuOH–EtOAc–H_2O 1:1:1:1), R_f 0.25 with Cl-tolidine reagent, combined, concentrated to 3 ml, and slowly added to 50 ml of Et_2O. The precipitate was collected: yield 0.13 g (25%); $[\alpha]^{25}D$ −65.6° (*c* 1.0, 1% AcOH).

[Phe⁵]-LH-RH. Z-Phe-Gly-OH. To a solution of 2.5 g (6.5 mmol) of Z-Phe-Gly-OEt[33] in 22.5 ml of CH_3OH and 22.5 ml of $CH_3OCH_2CH_2OH$, 8 ml of 1 *N* aqueous NaOH was added at 0° under vigorous stirring. After 1 hr the mixture was poured on 50 ml of ice water and 10 ml of 1 *N* HCl. The precipitate was filtered, washed with cold water, and crystallized from C_2H_5OH–H_2O: yield 1.5 g (65%); mp 143–145°; $[\alpha]^{25}D$ −23.3° (*c* 1.0, DMF). *Anal.* ($C_{19}H_{20}N_2O_5$) C, H, N.

Z-Phe-Gly-NHNHBoc. Triethylamine (0.47 ml) was added to 1.18 g (3.3 mmol) of Z-Phe-Gly-OH dissolved in 25 ml of THF. At −10° 0.32 ml of ethyl chloroformate was added, followed after 10 min by 0.48 g (3.4 mmol) of *tert*-butyl carbazate. After 5 hr at room temperature, the mixture was worked up in the usual way: yield 1.05 g (68%); mp 146–149°; $[\alpha]^{25}D$ −19.4° (*c* 1.0, DMF). *Anal.* ($C_{24}H_{30}N_4O_6$) C, H, N.

Z-Ser-Phe-Gly-NHNHBoc. The benzyloxycarbonyl group of Z-Phe-Gly-NHNHBoc was quantitatively removed by hydrogenolysis and 2.13 g (6.35 mmol) of H-Phe-Gly-NHNHBoc was coupled with 2.57 g (6.35 mmol) of Z-Ser-ODnp as described. After the usual work-up the substance was crystallized from CH_3OH–diisopropyl ether: yield 2.9 g (82%); mp 160–163°; $[\alpha]^{25}D$ −16.0° (*c* 1.0, DMF). *Anal.* ($C_{27}H_{35}N_5O_8$) H, N; C: calcd, 58.16; found, 57.75.

Pyr-His-Trp-Ser-Phe-Gly-NHNHBoc. The benzyloxycarbonyl group of Z-Ser-Phe-Gly-NHNHBoc was quantitatively removed by hydrogenolysis and 0.50 g (1.18 mmol) of H-Ser-Phe-Gly-NHNHBoc was coupled by the azide method with 0.55 g (1.18 mmol) of Pyr-His-Trp-$NHNH_2$ and the mixture was treated in the usual way. The solid was chromatographed on silica gel and eluted with CH_3OH–$CHCl_3$–C_5H_5N (33:66:1). The substance was crystallized from CH_3OH: yield 0.3 g (30%); mp 175–180°; $[\alpha]^{25}D$ −23.4° (*c* 1.0, DMF). *Anal.* ($C_{41}H_{51}N_{11}O_{11} \cdot 2.5H_2O$) C, H, N.

Pyr-His-Trp-Ser-Phe-Gly-Leu-Arg-Pro-Gly-NH_2 ([Phe⁵]-LH-RH). Pyr-His-Trp-Ser-Phe-Gly-NHNHBoc (0.33 g, 0.384 mmol) was treated with 15 ml of CF_3COOH for 90 min. The solution was added to 100 ml of Et_2O, and the precipitate was collected and crystallized from CH_3OH: yield 0.1 g (30%); mp 168–171°; $[\alpha]^{25}D$ −30.9° (*c* 1.0, H_2O). The hydrazide salt (0.25 g, 0.29 mmol) obtained was coupled by the azide method to 0.16 g (0.29 mmol) of D as described for LH-RH. The material, isolated after the usual work-up, was purified by partition chromatography on Sephadex LH-20 using the lower phase of *n*-BuOH–AcOH–H_2O (8:4:40). The isolated material was concentrated to an oil *in vacuo* and dissolved in 5 ml of CH_3OH and added to 60 ml of Et_2O. The precipitate was collected by filtration and dried: yield 0.12 g (30%); $[\alpha]^{25}D$ −53.1° (*c* 1.0, 1% AcOH). *Anal.* ($C_{55}H_{75}N_{17}O_{12} \cdot 2HCl \cdot H_2O$) C, H, N.

[Ser⁷]-LH-RH. Z-Ser-Arg(NO_2)-Pro-Gly-NH_2. H-Arg(NO_2)-Pro-Gly-NH_2 (0.95 g, 2 mmol) and 0.81 g (2 mmol) of Z-Ser-ODnp were dissolved in 5 ml of DMF and cooled to 0° and 0.28 ml (2 mmol) of triethylamine and 20 mg of 1-hydroxybenzotriazole were added. After 4 hr at 0° the solvent was removed *in vacuo* and the residue chromatographed on silica gel with $CHCl_3$–CH_3OH (85:15) to afford pure Z-Ser-Arg(NO_2)-Pro-Gly-NH_2: yield 0.46 g (35%). Amino acid ratios after acid hydrolysis: Ser 0.86, Arg 0.82, Pro 1.12, Gly 1.0.

Pyr-His-Trp-Ser-Tyr-Gly-Ser-Arg-Pro-Gly-NH_2 ([Ser⁷]-LH-RH). Z-Ser-Arg(NO_2)-Pro-Gly-NH_2 (0.39 g, 0.67 mmol) was hydrogenolyzed in the usual way. The residue was dissolved in CH_3OH and added to Et_2O. The collected material was reprecipitated. The dried material was homogeneous on electrophoresis: yield 0.23 g (82%). Pyr-His-Trp-Ser-Tyr-Gly-$NHNH_2$ (0.33 g, 0.37 mmol) and 0.21 g (0.37 mmol) of H-Ser-Arg-Pro-Gly-NH_2 were coupled by the azide method as described for LH-RH. The residue obtained was dissolved in 3 ml of CH_3OH and added to 60 ml of Et_2O. The precipitate was purified on a column of carboxymethylcellulose (Whatman CM23) by elution with 0.05 *M* NH_4OAc. The fractions containing the decapeptide were collected and rechromatographed on Sephadex LH-20 with CH_3OH. The product was dissolved in 3 ml of CH_3OH and added to 90 ml of Et_2O. The precipitate was collected and dried: yield 0.18 g (43%); $[\alpha]^{25}D$ −22.6° (*c* 1.0, AcOH). *Anal.* ($C_{52}H_{69}N_{17}O_{14} \cdot 5H_2O$) C, H; N: calcd, 19.11; found, 18.12.

[Lys⁸]-LH-RH. Z-Lys(Boc)-Pro-Gly-NH_2. To 0.83 g (4 mmol) of H-Pro-Gly-NH_2 dissolved in 10 ml of CH_3CN–DMF (1:1), 0.57 ml of triethylamine was added followed by 1.5 g (3.8 mmol) of Z-Lys(Boc)-OH in 15 ml of CH_3CN–DMF (1:1). At −15° 0.92 g (4.5 mmol) of DCI was added. After 18 hr at 0° the solid was removed, the filtrate evaporated to dryness *in vacuo*, and the residue dissolved in CH_3OH–$CHCl_3$ (1:9, containing 0.5% pyridine) and chromatographed on silica gel: yield 0.70 g (35%).

Z-Leu-Lys(Boc)-Pro-Gly-NH_2. Z-Lys(Boc)-Pro-Gly-NH_2 (0.60 g, 1.16 mmol) was hydrogenolyzed as usual to H-Lys(Boc)-Pro-Gly-NH_2 which was dissolved in 2 ml of DMF. To this solution 0.62 g (1.37 mmol) of Z-Leu-OTcp[24] in 3 ml of DMF was added at 0°. After 3 days at 0° the solvent was evaporated *in vacuo*, the residue dissolved in CH_3OH, and the product precipitated with Et_2O. Crystallization from CH_3OH–isopropyl ether gave a yield of 0.51 g (67%). *Anal.* ($C_{32}H_{50}N_6O_8$) C, H, N.

Pyr-His-Trp-Ser-Tyr-Gly-Leu-Lys-Pro-Gly-NH_2 ([Lys⁸]-LH-RH). Z-Leu-Lys(Boc)-Pro-Gly-NH_2 (0.48 g, 0.74 mmol) was hydrogenolyzed in the usual way to H-Leu-Lys(Boc)-Pro-Gly-NH_2 which was coupled by the azide method with 0.54 g (0.62 mmol) of C as described under LH-RH. The material obtained was treated with 10 ml of CF_3COOH at 0° for 30 min. The solvent was removed and the residue purified through Sephadex LH-20 with MeOH. The pure fractions were detected by tlc on silica gel G plates (HOAc–BuOH–EtOAc–H_2O 1:1:1:1), R_f 0.42 with Cl-tolidine reagent, combined, concentrated to 5 ml, and slowly added to 100 ml of Et_2O. The precipitate was collected: yield 0.34 g (48%); $[\alpha]^{25}D$ −54.3° (*c* 1.0, 1% AcOH).

[Glu⁸]-LH-RH. Z-Glu(O-*t*-Bu)-Pro-Gly-NH_2. To a stirred solution, cooled to 0°, of 1.3 g (3.86 mmol) of Z-Glu(O-*t*Bu)-OH[34,35] and 0.52 g (3.85 mmol) of 1-hydroxybenzotriazole in 10 ml of DMF was added 0.86 g (4.16 mmol) of DCI. After 1 hr at 0° and 1 hr at room temperature a solution of 0.76 g (4 mmol) of H-Pro-Gly-$NH_2 \cdot HCl$ in 12 ml of DMF and 0.65 ml of *N*-ethylmorpholine was introduced and the mixture left overnight at room temperature. After the usual work-up the substance was crystallized from EtOAc: yield 0.96 g (51%); mp 162–164°. *Anal.* ($C_{24}H_{34}N_4O_7$) H, N; C: calcd, 58.76; found, 58.34.

Z-Leu-Glu(O-*t*-Bu)-Pro-Gly-NH_2. Z-Glu(O-*t*-Bu)-Pro-Gly-NH_2 (0.75 g, 1.53 mmol) was hydrogenolyzed in the usual way and the deblocked material was coupled to 0.75 g (1.7 mmol) of Z-Leu-OTcp in the usual way. After 3 days at 0° the solvent was removed *in vacuo*, the residue dissolved in EtOAc, and the solution washed with 10% citric acid solution, 5% $NaHCO_3$ solution, and saturated NaCl solution. After drying ($MgSO_4$) and evaporation of the solvent, the oily residue was dissolved in Et_2O and precipitated with petroleum ether. After drying *in vacuo* the yield was 0.63 g (70%); nmr (DMSO-d_6) δ 0.85 (d, 6 H, CH_3), 1.40 (s, 9 H, O-*t*-Bu), 7.40 (s, 5 H, arom). *Anal.* ($C_{30}H_{45}N_5O_8 \cdot 0.5H_2O$) C, H, N.

Pyr-His-Trp-Ser-Tyr-Gly-Leu-Glu-Pro-Gly-NH_2 ([Glu⁸]-LH-RH). Z-Leu-Glu(O-*t*-Bu)-Pro-Gly-NH_2 (0.54 g, 0.89 mmol) was hydrogenolyzed in the usual way and the resulting product was coupled by the azide method to 0.44 g (0.5 mmol) of C. After the usual work-up the product was precipitated with Et_2O from CH_3OH solution. The collected precipitate was dissolved in 10 ml of CF_3COOH at 0°. After 30 min at 0° the solvent was removed *in vacuo*, the residue dissolved in CH_3OH, and the product precipitated with Et_2O. The precipitate was purified through Sephadex LH-20 with CH_3OH. The solvent was evaporated: yield 0.12 g (23%); $[\alpha]^{25}D$ −59.1° (*c* 1.0, 1% AcOH).

[Ala⁴,Phe⁵]-LH-RH. Boc-Ala-Phe-Gly-OEt. Z-Phe-Gly-OEt[36] (3 g, 7.8 mmol) was hydrogenolyzed in the usual way and the H-Phe-Gly-OEt isolated as HCl salt was dissolved in 30 ml of DMF, together with 2.88 g (7.8 mmol) of Boc-Ala-OTcp. Triethylamine (1.09 ml, 7.8 mmol) was added under ice cooling. After 3 days at

0° the solvent was removed *in vacuo* and the residue crystallized from CH_3OH–isopropyl ether: yield 2.63 g (79%); mp 145–152°. *Anal.* ($C_{21}H_{31}N_3O_6$) C, H, N.

Pyr-His-Trp-Ala-Phe-Gly-OEt. Boc-Ala-Phe-Gly-OEt (2.53 g, 6.0 mmol) was treated with 9 ml of 90% CF_3COOH at 0° for 2 hr. The solution was added dropwise to Et_2O and the precipitate of H-Ala-Phe-Gly-OEt trifluoroacetate was collected and dried. A (0.93 g, 2.0 mmol) was coupled to 0.87 g (2 mmol) of the above material by the azide method as described for C. The residue was dissolved in CH_3OH and added to 100 ml of Et_2O. The precipitate was chromatographed on silica gel using $CHCl_3$–CH_3OH (2:1). The product was crystallized from CH_3OH as a fine powder: yield 1.23 g (82%); mp 234° dec. *Anal.* ($C_{38}H_{45}N_9O_8 \cdot H_2O$) C, H, N.

Pyr-His-Trp-Ala-Phe-Gly-Leu-Arg-Pro-Gly-NH_2 ([Ala,^{4}-Phe^5]-LH-RH). Pyr-His-Trp-Ala-Phe-Gly-OEt (0.60 g, 0.79 mmol) was dissolved in 75 ml of CH_3OH and treated with 1 ml of $N_2H_4 \cdot H_2O$ at 0° for 72 hr. The precipitate of Pyr-His-Trp-Ala-Phe-Gly-$NHNH_2$ was collected and dried: yield 0.48 g (82%). This hydrazide (0.36 g, 0.48 mmol) was coupled to 0.27 g (0.48 mmol) of D by the azide method as described for LH-RH. The residue was dissolved in 5 ml of CH_3OH and slowly added to 100 ml of Et_2O. The precipitate was collected and purified on carboxymethylcellulose (Whatman CM 23) by consecutive elution with 0.005, 0.010, and 0.025 *M* aqueous NH_4OAc. The fractions containing the pure decapeptide were detected by tlc on silica gel G plates (HOAc–BuOH–EtOAc–H_2O 1:1:1:1), R_f 0.50 with Cl-tolidine reagent, combined, and concentrated to dryness. The residue was dissolved in 2 ml of CH_3OH and slowly added to 50 ml of Et_2O. The precipitate was collected and dried: yield 0.03 g (5%).

Pyr-His-Trp-Ala-Phe-Gly-Leu-Lys-Pro-Gly-NH_2 ([Ala^4,-Phe,^{5}Lys^8]-LH-RH). Pyr-His-Trp-Ala-Phe-Gly-$NHNH_2$ (1.11 g, 1.5 mmol), described under [Ala,^{4}Phe^5]-LH-RH, was coupled to 0.77 g (1.5 mmol) of H-Leu-Lys(Boc)-Pro-Gly-NH_2, described under [Lys^8]-LH-RH, by the azide method. The residue was dissolved in 3 ml of CH_3OH and added to 100 ml of Et_2O. The precipitate was collected and chromatographed on silica gel with $CHCl_3$–CH_3OH–C_5H_5N (66:33:1) to afford the Boc-protected decapeptide: yield 1.55 g (85%). For deprotection, 0.76 g (0.62 mmol) of Pyr-His-Trp-Ala-Phe-Gly-Leu-Lys(Boc)-Pro-Gly-NH_2 was treated with 10 ml of CF_3COOH for 30 min at 0°. The solvent was removed *in vacuo*; the residue was dissolved in 2 ml of CH_3OH and added to 150 ml of Et_2O. The precipitate was collected and dried and proved homogeneous on tlc: yield 0.74 g (97%); $[\alpha]^{25}D$ −50.9° (*c* 1.0, 1% AcOH).

LH-RH-NH$(CH_2)_4NH_2$. Z-Gly-NH$(CH_2)_4$NHBoc. To 1.2 g (5 mmol) of $H_2N(CH_2)_4NHBoc \cdot HCl$[29] dissolved in 12 ml of DMF and 0.8 ml of triethylamine, a solution of 1 g (5 mmol) of Z-Gly-OH in 7 ml of DMF was added at 0°, followed by 1.5 g (7.5 mmol) of DCI. After 24 hr at room temperature the mixture was worked up in the usual way. The residue was chromatographed on silica gel by eluting with EtOAc–Et_2O–C_5H_5N (100:100:1). The product was crystallized from EtOAc–Et_2O: yield 0.50 g (26%). *Anal.* ($C_{19}H_{29}N_3O_5$) C, H, N.

Boc-Arg(NO_2)-Pro-OMe. To 15.9 g (50 mmol) of Boc-Arg(NO_2)-OH and 10 g (87 mmol) of *N*-hydroxysuccinimide dissolved in 125 ml of DMF was added at 0° 10.5 g (50 mmol) of DCI. After 18 hr at 0° a solution of 8.4 g (50 mmol) of H-Pro-OMe·HCl in 100 ml of DMF and 9.5 ml of triethylamine was dropped in under stirring. After 18 hr at 0° the solids were removed and the filtrate was evaporated *in vacuo*. The residue was dissolved in EtOAc–CH_3OH (10:1) and chromatographed on silica gel: yield 10.7 g (50%); nmr ($CDCl_3$) δ 1.42 (s, 9 H, Boc), 3.67 (s, 3 H, $-COOCH_3$).

Z-Leu-Arg(NO_2)-Pro-OMe. Boc-Arg(NO_2)-Pro-OMe (10.7 g, 25 mmol) was treated with 125 ml of CF_3COOH for the removal of the *tert*-butoxycarbonyl group in the usual way. The deprotected peptide (2.3 g, 5 mmol) and 0.8 ml of triethylamine in 10 ml of DMF were added to 2.3 g (5 mmol) of Z-Leu-OTcp[24] in 10 ml of DMF. After the usual work-up and chromatography on silica gel with CH_3OH–EtOAc (1:10) the product was obtained in pure form: yield 2.0 g (69%). *Anal.* ($C_{26}H_{39}N_7O_8$) C, H.

Z-Leu-Arg(NO_2)-Pro-Gly-NH$(CH_2)_4$NHBoc. Z-Leu-Arg-(NO_2)-Pro-OMe (2 g, 3.46 mmol) dissolved in 20 ml of CH_3O-CH_2CH_2OH was treated with 7 ml of 1 *N* NaOH. The mixture remained at room temperature overnight and was then acidified with 7.5 ml of 1 *N* HCl under cooling. The solvent was evaporated *in vacuo* and the residue treated with H_2O. The precipitate of Z-Leu-Arg(NO_2)-Pro-OH was used in the next step. Z-Gly-NH$(CH_2)_4$NHBoc (0.45 g, 1.19 mmol) was hydrogenated for the removal of the benzyloxycarbonyl group. The resulting H-Gly-NH$(CH_2)_4$NHBoc and 0.65 g (1.15 mmol) of Z-Leu-Arg(NO_2)-Pro-OH were dissolved in 20 ml of EtOH and treated with 0.30 g (1.21 mmol) of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline at 0°. After 30 min at 0° and 18 hr at room temperature the solvent was evaporated *in vacuo*; the residue was dissolved in CH_3OH and precipitated with Et_2O. The precipitate was dissolved in EtOAc and the solution washed with solutions of 1 *M* citric acid, saturated NaCl, 5% $NaHCO_3$, and saturated NaCl. After drying over $MgSO_4$ and evaporation the yield was 0.54 g (57%); nmr ($CDCl_3$) δ 0.88 (d, *J* = 5 Hz, 6 H, CH_3), 1.39 [s, 9 H, $(CH_3)_3C$], 5.07 (s, 2 H, $PhCH_2$), 7.37 (s, 5 H, arom).

Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH$(CH_2)_4NH_2$. Z-Leu-Arg(NO_2)-Pro-Gly-NH$(CH_2)_4$NHBoc (0.40 g, 0.51 mmol) was hydrogenated to yield H-Leu-Arg-Pro-Gly-NH$(CH_2)_4$NHBoc. This tetrapeptide was coupled with 0.44 g (0.5 mmol) of C as described for LH-RH. After purification of the product through Sephadex LH-20 with CH_3OH, the *tert*-butoxycarbonyl group was removed by CF_3COOH. After purification through a Sephadex LH-20 column with *n*-BuOH–HOAc–H_2O (1:1:8) the desired product was obtained: yield 0.11 g (47%); $[\alpha]^{25}D$ −51.5° (*c* 1.0, 1% AcOH).

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Mercaptopyridinecarboxylic Acids, Synthesis and Hypoglycemic Activity†

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3-Mercaptopicolinic acid (1), its isomers, analogs, and derivatives were prepared and tested for hypoglycemic activity in 48-hr fasted rats. Several compounds [1, 5-mercaptopicolinic acid (**3**), 4-methyl-3-mercaptopicolinic acid (**11**), 3-phenylthiopicolinic acid (**17**), 3-benzylthiopicolinic acid (**18**), the *S*-acetyl (**20**) and *S*-benzoyl (**21**) derivatives of 1, the disulfide of 1 (**22**), and the methyl ester of 1 (**24**)] were active with 1 being the most potent. *p*-Methoxybenzyl mercaptan (MBM) was used as a novel sulfurating agent to introduce sulfur in a protected form and was used to prepare 1, 6-mercaptopicolinic acid (**4**), 5-mercaptonicotinic acid (**7**), methyl 6-chloro-3-mercaptopicolinate (**15**), (3-mercapto-2-pyridyl)methanol (**25**), 3-mercaptopicolinamide (**30**), 2-acetyl-3-mercaptopyridine (**35**), 3-acetylthiopyridine (**54**), and 3-mercaptopyridine *N*-oxide (**59**). The protecting group was usually removed in the final step by mercuric acetate in trifluoroacetic acid. The Newman-Kwart route to thiols was also utilized. The hypoglycemic activity of 1 seems highly specific, with relatively minor chemical changes causing marked changes in the ability of closely related compounds to lower glycemic levels in fasted rats.

Diabetes is a condition characterized by an insufficiency of insulin which results in a number of metabolic derangements. Among these is an enhanced rate of gluconeogenesis and an elevated blood glucose level. Current therapy focuses on trying to normalize the observed, elevated blood glucose levels.

Using drug therapy one can try to modulate glycemic levels in one of several ways: stimulate insulin secretion, potentiate insulin activity, increase the peripheral uptake and oxidation of glucose, and inhibit gluconeogenosis. Of these possible approaches we chose to see what effects inhibition of gluconeogenesis had on glycemic levels.

Our search for inhibitors of gluconeogenesis centered around the structure of quinolinic acid, a compound reported to have this property.[1] This search led to 3-mercaptopicolinic acid (**1**), a good hypoglycemic agent in fasted rats and an inhibitor of glucose synthesis from three-carbon precursors *in vitro*.[2] To further develop this finding, positional isomers, derivatives, and analogs of **1** were prepared and tested in 48-hr fasted rats.

All of the isomeric mercaptopyridinecarboxylic acids have been described in the literature[3-10] and, with the exceptions of **3** and **7**, were prepared using these procedures. In general, these acids were prepared by treating the corresponding halo acid with hydrosulfide (**2**, **4–6**, **8**, and **9**). In those instances where the isomers have the mercapto group in the 3 or 5 position of the pyridine (**1**, **3**, and **10**) sulfuration was accomplished by treating the diazonium salt of the corresponding amino acid with polysulfide.

5-Aminopicolinic acid still served as the immediate precursor of **3** but it was derived from commercially available 2-chloro-5-nitropyridine. Treatment of this reagent with the anion of diethyl malonate in DMSO gave the nitropyridyl malonate[11] which was oxidized with potassium permanganate to 5-nitropicolinic acid.[12] This acid was reduced catalytically to the desired amino acid.[12] **7** was prepared from methyl 5-bromonicotinate[13] and *p*-methoxybenzyl mercaptan, of which more will be said later.

The 4-, 5-, and 6-methyl and 5-chloro analogs of **1** (**11–14**) were prepared from the corresponding quinolinic acids. These preparations were patterned after the synthesis of **1**.[3] The required quinolinic acids were obtained by oxidation of suitably substituted quinolines.

S-Alkyl, aralkyl, and acyl derivatives of **1** (**16**, **18–21**) were obtained from **1** using Schotten–Baumann conditions. Esterification of **1** proved unexpectedly difficult. Most of the usual methods yielded the disulfide diester. However, the methyl ester **24** could be prepared by treating **1** with boron trifluoride–methanol. The anilide of **1** (**33**) was prepared by allowing **20** to react with aniline in the presence of dicyclohexylcarbodiimide to produce **34**. Subsequent acid hydrolysis yielded **33**. The known *S*-phenyl derivative of **1** (**17**) was made by treating the diazonium salt of 3-aminopicolinic acid with thiophenol.[14]

Although **1** was most conveniently prepared by the

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