Table VIII. 2-Carboxymethylbenzenesulfonamides **8**

No.	R	Mp, °C	Yield, %	Recrystn solvent	Formula	Analyses
8a[a]	CH_3	174[b]	78	H_2O	$C_9H_{11}NO_4S$	C, H, N, S
8b	i-C_3H_7	115–117	45	H_2O	$C_{11}H_{15}NO_4S$	C, H, N, S
8c[a]	$CH_2C_6H_5$	124[c]	86	H_2O	$C_{15}H_{15}NO_4S$	C, H, N
8d	o-C_6H_4Cl	146–148	95	EtOH–H_2O	$C_{14}H_{12}ClNO_4S$	C, H, N
8e	m-C_6H_4Cl	159–163	86	H_2O	$C_{14}H_{12}ClNO_4S$	Cl, N, S
8f	p-C_6H_4Cl	128–130	94	EtOH–H_2O	$C_{14}H_{12}ClNO_4S$	Cl, N, S
8g	p-$C_6H_4SO_2NH_2$	202–204	84	H_2O	$C_{14}H_{14}N_2O_6S_2$	C, H, S

[a] See ref 9. [b] Lit.[9] 158–164°, uncrystallized. [c] Lit.[9] 107–109°.

hr, evaporation of this solution and treatment of the residue as above gave finally 0.27 g (2.8%) of the crystalline isomer **1e** (mixture melting point, tlc, and ir).

(b) **1a** (5.91 g, 0.03 mol) and PCl_5 (6.25 g, 0.03 mol) were mixed and heated at 160° for 30 min, then $POCl_3$ was removed *in vacuo*, and the residue was triturated with a little $CHCl_3$, collected, and crystallized (C_6H_6-CCl_4) to give 4.55 g (70%) of **4-chloro-1*H*-2,3-benzothiazine 2,2-dioxide (11)**, mp 172-174°. *Anal.* ($C_8H_6ClNO_2S$) C, H, Cl, N, S.

A suspension of **11** (3.23 g, 0.015 mol) in 2-PrOH (20 ml) was refluxed for 4 hr and the obtained solution let stand in the cold to complete separation of the product, that was collected, washed, dried, and recrystallized (2-PrOH) to afford 2.6 g (72%) of **10**, mp 182-183°, identical (analysis, tlc, mixture melting point, and ir) with the product described under (a).

Supplementary Material Available. A listing of the pharmacological data for weakly active or inactive compounds will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche, referring to code number JMED-73-1133.

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Synthesis of the Thyrotropin-Releasing Hormone Enantiomer and Some Diastereoisomers and *in Vitro* Studies of Their Biological Activity

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The isomers of TRH with DDD, DLL, LDL, and LLD configurations were prepared unequivocally by coupling pentachlorophenyl pyroglutamate of the required configuration with the appropriate histidylprolinamide diastereoisomer. The dipeptide amide was made by condensation of *tert*-butyloxycarbonylhistidine with prolinamide mediated by dicyclohexylcarbodiimide, removal of the blocking group in trifluoroacetic acid, and treatment of the peptide amide hydrochloride with a basic ion exchanger. Protected peptide intermediates and the final isomers were purified by chromatography on silica gel. Formation of diastereoisomers of the protected dipeptide was detected by nmr and tlc techniques. The LDL isomer was approximately 2-3% as active as TRH for TSH release in rat hemipituitaries *in vitro*, the DLL and LLD isomers were about 0.1% active, and the DDD isomer was inactive. None of these analogs inhibited TSH release *in vitro*.

The recent availability of synthetic thyrotropin-releasing hormone (TRH) and analogs has made possible for the first time studies of TRH structure–activity relationships. Burgus and collaborators[1] have demonstrated the critical importance of the N-terminal pyrrolidone group and the C-terminal amide for full biological expression of TRH, and Bowers and associates[2] have shown that of several C-terminal analogs only those containing proline were inactivated by serum. Experiments with labeled TRH suggested that the C-terminal carboxamide group hydrolysis by serum is a first metabolic step[2,3] in the inactivation

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mechanism which probably plays an important physiologic role.

In the present studies, the DDD, LLD, LDL, and DLL stereoisomers of TRH were synthesized and their biological activity was investigated in an *in vitro* system, where the variable of serum inactivation is not operative. These isomers were prepared with the expectation that they would be substantially resistant to enzymatic cleavage and that they might preserve TRH activity but with longer duration of action. Furthermore, the importance of TRH as a synthetic hormone of potential commercial interest made it desirable to ascertain whether epimers that might arise during synthesis would have inhibitory or other undesirable properties.

The TRH stereoisomers were made by a method similar to the one employed previously for a synthesis of TRH,[4] with the selection of a D- or an L-amino acid derivative as required. The desired TRH isomer was obtained by coupling pentachlorophenyl pyroglutamate of the required configuration with the appropriate His-Pro-NH_2 isomer. The free dipeptide amide was made by the condensation of N^{α}-*tert*-butyloxycarbonylhistidine[5] with prolinamide in the presence of DCC,[6] removal of the blocking group in acid, and treatment of the peptide hydrochloride with a basic ion-exchange resin. Peptide intermediates were purified by chromatography on silica gel.[4]

For the synthesis of *tert*-butyloxycarbonyl-D-histidine, *tert*-butyloxycarbonylhistidine (Boc) was refluxed in MeOH solution for 24 hr. Under these conditions, the imidazolyl-*tert*-butyloxycarbonyl group which is more susceptible to alcoholysis was cleanly removed. The *tert*-butyloxycarbonylhistidine (Boc) was made by treating D-histidine with excess *tert*-butyloxycarbonyl azide[7] in TEA, DMF, and H_2O for 24 hr. The dipeptide Boc-His-Pro-NH_2 was conveniently analyzed for optical purity by thin-layer chromatography (tlc) with 15% MeOH-$CHCl_3$ (solvent A) as the eluting solvent and Cl_2-tolidine, ninhydrin, or Pauly's spray as the detection method. Under these conditions the LL isomer and its enantiomer, the DD isomer, had an R_f of 0.30 while the DL and LD isomers had an R_f of 0.25. A combination of LL and DL isomers, for example, was usually resolved as two spots resembling a figure 8.

Similarly, in the nuclear magnetic resonance (nmr) spectrum (C_5D_5N) of this dipeptide, the chemical shift for the *tert*-butyloxycarbonyl methyl proton was identical with the LL and DD isomers (δ 1.50) and with the DL and LD isomers (δ 1.40). A combination of LL and DL isomers was easily detectable by nmr analysis. All synthetic preparations of Boc-His-Pro-NH_2 showed after column chromatography the presence of tlc fractions suggesting that diastereoisomers had formed during coupling. An nmr of such fractions revealed the CH_3-proton absorption due to the "incorrect" isomer. Appropriate pooling of clean chromatographic fractions led to optically pure dipeptide isomers according to the tlc and nmr criteria. In one purification, where 33% MeOH-$CHCl_3$ was employed to elute dipeptide material, indiscriminate elution of isomers occurred. The nmr of the "mixed" Boc-His-Pro-NH_2 revealed the presence of 5.3% of the "wrong" isomer; this value was obtained by dividing the peak height for diastereoisomer by the sum of the peak height for diastereoisomer plus the peak height for the expected isomer. One possible explanation for the formation of the "wrong" isomer is that the presence of the free imidazole group in *tert*-butyloxycarbonylhistidine leads to some epimerization of the α position of this amino acid during carbodiimide activation of the carboxyl group due to the formation of a base-sensitive acylimidazole.[8] Recently, Windridge reported a study on racemization during synthesis of histidine dipeptides and he too concluded that *tert*-butyloxycarbonylhistidine can undergo racemization to the extent of forming in one case approximately 5% of D isomer in DMF with DCC as the coupling agent.[9] Our results thus seem consistent with his findings. A reexamination of Boc-His-Pro-NH_2 (LL), previously made by the solid-phase technique,[4] revealed that 5.9% wrong diastereoisomer (DL?) was also obtained as detected by nmr analysis. The free dipeptide His-Pro-NH_2 was analyzed in the Beckman amino acid analyzer with both the short and long columns, but it was always eluted unresolved as one peak when mixtures of LL and DL dipeptide were applied; thus, this technique which is useful for dipeptides[10] could not be successfully applied to the resolution of these dipeptide amides. The final TRH isomers (DDD, LLL, LLD, LDL, and DLL) could not be differentiated by tlc in several solvent systems. The nmr spectra of these isomers were identical except for a small difference in the splitting of imidazolyl protons, which was very weak and thus could not be used to detect the presence of incorrect isomers. The high-resolution mass spectra of the TRH isomers showed the correct molecular ion as calculated for TRH (m/e 362.1702) and were consistent with the structure of the tripeptide amide. The intensity of several peaks showed subtle differences, however, which can only be ascribed to conformational effects but were of no value for the quantitative estimation of isomers. Therefore, no convenient physical method was found to check directly the optical purity of these analogs.

The thyrotropin (TSH)-releasing activity of the four TRH analogs was investigated *in vitro* with rat hemipituitaries. TSH release was determined by a specific radioimmunoassay for rat TSH.[11] The observed potency of "LDL" TRH was 2-3% of native TRH activity. In contrast, the LLD and DLL isomers possessed only approximately 0.1% of native TRH activity and the DDD isomer was inactive. None of the four analogs inhibited TSH release by TRH.

It is of interest that the biological potencies of these analogs have corresponded quantitatively with their binding affinities for a specific anterior pituitary receptor.[12]

Experimental Section‡

D-Prolinamide Hydrochloride. To a suspension of D-proline (5.75 g, 50 mmol) in MeOH (50 ml) was added $SOCl_2$ (6.5 ml) dropwise with the temperature being kept at 0° with external cooling. The reaction mixture was stirred at room temperature for 2 hr and then heated at reflux for 2 hr. The resulting solution was evaporated *in vacuo* to an oil which was dissolved in MeOH and the solution evaporated again to an oil, the process being repeated three times. The final oil was dried over P_2O_5 and KOH pel-

‡A Thomas-Hoover apparatus was used for melting point determinations in capillary tubes. Optical rotations were measured in 1-dm polarimeter tubes with Hilger-Watts polarimeter reading with a precision of ±0.01°. Where analyses are indicated only by symbols of the elements, analytical results were obtained for the elements within ±0.4% of the theoretical value. For tlc or electrophoretic analysis several samples ranging between 20 and 100 μg of peptide were usually employed to reveal impurities. Where tlc was used to determine purity of intermediates and products, silica gel G plates were used and were developed with one of the following solvent combinations: A, 15% MeOH-$CHCl_3$; B, MeOH-$CHCl_3$ (1:2); C, MeOH-$CHCl_3$ (2:1); with Pauly and/or Cl_2-tolidine color sprays. Where the ninhydrin color reaction was the detection method of choice, the Boc intermediate was exposed to HCl vapors in a chromatographic chamber for 10 min prior to spraying. For electrophoresis, Brinkmann precoated thin-layer cellulose sheets were employed with Py-AcOH buffer, pH 6.3 (25-45 min). The nmr spectrum of the dipeptides was obtained at 100 MHz using a Varian Associates HA-100 spectrometer. Chemical shifts are reported in parts per million from internal TMS. Mass spectra were recorded on an AEI MS-902 mass spectrometer. The following abbreviations were used throughout the text: DCC = dicyclohexylcarbodiimide; DCU = dicyclohexylurea; TFA = trifluoroacetic acid; TEA = triethylamine; Py = pyridine.

lets *in vacuo*. The dried oil (8.4 g) was dissolved in MeOH (50 ml) and saturated with NH_3. The insoluble crystals which formed (mostly NH_4Cl) were removed by filtration. The clear solution was evaporated *in vacuo* to an oil which was redissolved in MeOH and the resulting solution was evaporated to an oil (three times). The residual oil was dissolved in MeOH (50 ml) and the solution was acidified with concentrated HCl until it turned Hydrion paper to a color corresponding to a pH of 3. The solution was evaporated to an oil which was dissolved in EtOH (150 ml) and the solvent was removed *in vacuo*, the whole process being repeated three times. The resulting oil was dissolved in EtOH (75 ml) and the clear solution was allowed to cool slowly at room temperature. The crystals were collected and washed with EtOH–Et_2O (1:1) and finally with Et_2O: the first crop weighed 4.95 g, mp 169–172°; the second crop weighed 1.25 g, mp 165–168°. Both crops were combined and recrystallized from EtOH (35 ml) yielding 4.97 g: mp 178–180°; $[\alpha]^{23}D$ +69.5° (*c* 2, EtOH) [lit.[13] L isomer mp 182°; $[\alpha]^{25}D$ −70.7° (*c* 2, EtOH)]. *Anal.* ($C_5H_{11}ClN_2O$) C, H, N.

***tert*-Butyloxycarbonyl-D-histidine.** To a solution of D-histidine (20.9 g) in H_2O (75 ml) was added TEA (42 ml), *tert*-butyloxycarbonyl azide (43 ml), and DMF (150 ml). The reaction mixture was stirred overnight at room temperature. The progress of the reaction was followed by tlc with solvent C and ninhydrin color reaction. As some histidine and *tert*-butyloxycarbonylhistidine were still detectable, the reaction mixture was treated with additional *tert*-butyloxycarbonyl azide (15 ml) and TEA (14 ml). After 2 hr saturated citric acid solution was added to a pH of 7 and the reaction mixture was evaporated *in vacuo* to an oil. This oil was dissolved in a small volume of H_2O and the solution was extracted with Et_2O (three times). The aqueous phase was acidified with saturated citric acid solution to pH 2–3 and extracted with $CHCl_3$ (six times). The extracts were combined and washed twice with a small volume of H_2O and saturated NaCl solution (three times) and dried (Na_2SO_4). Evaporation of the $CHCl_3$ solution *in vacuo* led to an oil. Analysis of this oil by tlc as described above showed a slow-moving minor component with R_f 0.3 equivalent to that of *tert*-butyloxycarbonyl-L-histidine and a major fast-moving component (R_f 0.6). The crude Boc-His (Boc) was refluxed in MeOH solution (1000 ml). After 24 hr tlc analysis indicated almost complete disappearance of the fast-moving component. The reaction mixture was concentrated to an oil which was dissolved in EtOH (100 ml). The EtOH solution was concentrated to a lower volume and allowed to cool slowly. The crystals which formed were collected, washed with EtOH, and dried over P_2O_5 in a desiccator; the yield was 12.1 g; mp 197–199°; $[\alpha]^{25}D$ −14.1° (*c* 2, H_2O). *Anal.* ($C_{11}H_{17}N_3O_4$) C, H, N.

***tert*-Butyloxycarbonyl-L-histidine.** This material was made by the method described above for the D isomer. The yields and properties were similar: mp 198° dec; $[\alpha]^{26}D$ +14° (*c* 2, H_2O). A commercial sample obtained from Schwarz BioResearch, Inc., had a rotation of $[\alpha]^{25}D$ +14° (*c* 2, H_2O).

D-Pyroglutamic Acid. A solution of D-glutamic acid (10 g) was refluxed in H_2O (250 ml) for 2 days. Analysis by tlc solvent C and Cl_2–tolidine color reaction shows two components, a minor component at the origin with R_f equivalent to glutamic acid and a faster component with R_f equivalent to that of L-pyroglutamic acid. The reaction mixture was evaporated to a residue which was treated with EtOH and the solvent was removed *in vacuo*. The latter treatment was repeated three times and the product obtained was treated with boiling EtOH (100 ml) and the resulting suspension filtered. The EtOH-insoluble material was mostly D-glutamic acid (1.47 g) as shown by tlc. The EtOH solution was concentrated to approximately 40 ml and allowed to crystallize overnight. The crystals formed were collected and washed with EtOH to yield a first crop of 4.50 g, mp 156–159°, $[\alpha]^{23}D$ +11.5° (*c* 2, H_2O) and from the mother liquor a second crop, 1.15 g, mp 154–157°, $[\alpha]^{23}D$ +11.5° (*c* 2, H_2O), for a combined yield of 64%, or 75% based on consumed D-glutamic acid [lit.[14] L isomer mp 160–161°; $[\alpha]^{24}D$ −11.8° (*c* 1.4, H_2O)]. *Anal.* ($C_5H_7NO_3$) C, H, N.

Pentachlorophenyl D-Pyroglutamate. This derivative was prepared essentially as described for the L isomer:[4] mp 195–198°; $[\alpha]^{23}D$ −21.2° (*c* 2, DMF) [lit.[4] L isomer mp 196–199°; $[\alpha]^{26}D$ +21° (*c* 2, DMF)]. *Anal.* ($C_{11}H_6Cl_5N_1O_3$) C, H, N.

Boc-L-His-D-Pro-NH₂. To a solution of *tert*-butyloxycarbonyl-L-histidine (2.55 g, 10 mmol) in 20 ml of DMF was added D-prolinamide hydrochloride (1.65 g, 11 mmol). The resulting clear solution was cooled to 0° and treated with TEA (1.5 ml) and DCC (2.06 g, 10 mmol). The reaction mixture was then stirred for 40 hr at room temperature, the DCU which precipitated removed by filtration, and the filtrate evaporated to an oil. For purification this product was chromatographed on silica gel with 5% MeOH–$CHCl_3$ as the eluent to remove by-products and 12% MeOH–$CHCl_3$ to elute the desired dipeptide. All effluents were monitored by tlc with solvent A (Cl_2–tolidine or ninhydrin). All desired fractions (R_f 0.25) containing none of the slightly faster moving component (R_f 0.30, corresponding probably to DD isomer) were combined. The yield of crude product was 1.91 g. A methanolic solution of this product was treated with Rexyn 201 (OH^-), filtered, and evaporated to a residue which crystallized from MeOH–EtOH. The crystals formed were collected and recrystallized from MeOH yielding the analytical sample: mp 133–137°; $[\alpha]^{23}D$ +53.2° (*c* 1, H_2O), +58.0° (*c* 1, DMF). *Anal.* ($C_{16}H_{25}N_5O_4$) C, H, N. An nmr spectrum (C_5D_5N) was consistent with the structure and the $(CH_3)_3C$-proton peak (δ 1.40) was a sharp singlet showing no evidence of the presence of DD isomer.

DL Isomer. This isomer crystallized from MeOH: mp 129–133°; $[\alpha]^{23}D$ −53.5° (*c* 1, H_2O), −57.4° (*c* 1, DMF). The nmr was identical with that of the LD isomer with the CH_3 protons showing as a singlet (δ 1.40): R_f^A 0.25. *Anal.* ($C_{16}H_{25}N_5O_4$) C, H, N.

DD Isomer. This isomer was obtained by lyophilization of chromatographically pure fractions: $[\alpha]^{23}D$ +50° (*c* 1, H_2O); $[\alpha]^{24}D$ −26.1° (*c* 1, DMF). The nmr spectrum was consistent with the structure and the CH_3 protons showed as a singlet (δ 1.50): R_f^A 0.30. *Anal.* ($C_{16}H_{25}N_5O_4$) C, H, N.

LL Isomer. This isomer was prepared as a fluffy powder obtained by lyophilization of chromatographically pure fractions: $[\alpha]^{24}D$ −49° (*c* 1, H_2O), +26.4° (*c* 1, DMF). The nmr spectrum was consistent with the structure, the CH_3 protons showing as a singlet (δ 1.50): R_f^A 0.30. *Anal.* ($C_{16}H_{25}N_5O_4$) C, H, N.

D-<Glu-L-His-L-Pro-NH₂. A solution of Boc-L-His-L-Pro-NH₂ (LL) (703 mg) in 10 ml of TFA was allowed to stand for 15 min at room temperature. At this time tlc with solvent B revealed disappearance of the protected dipeptide amide spot (Cl_2–tolidine) and the appearance of His-Pro-NH₂ as a single spot, R_f^A 0.1 (ninhydrin +). The TFA was removed from the reaction mixture by evaporation to dryness followed by the addition of Et_2O and evaporation to dryness three additional times. The residue was dried over P_2O_5 and KOH *in vacuo* and then dissolved in MeOH, and the solution was treated with Rexyn 201 (OH^-) and filtered. The filtrate was evaporated under reduced pressure and the residue dried over P_2O_5 *in vacuo*. The dried amino dipeptide was dissolved in 2 ml of DMF and treated with pentachlorophenyl pyroglutamate (755 mg). The resulting clear solution was allowed to stand overnight at room temperature. The reaction mixture was chromatographed on silica gel employing 15% MeOH–$CHCl_3$ to elute by-products, the desired product being eluted with 33% MeOH–$CHCl_3$. Progress of the reaction and chromatographic separation was followed by tlc with solvent B (with Cl_2–tolidine and ninhydrin color reactions); fractions containing the TRH (DLL) isomer were combined and evaporated to an oil which crystallized slowly. Recrystallization from MeOH–EtOH gave 480 mg: mp 231–233°; $[\alpha]^{24}D$ −66.4° (*c* 1, H_2O). Thin-layer electrophoresis on Brinkman precoated thin-layer cellulose sheets with pyridine–AcOH buffer, pH 6.3 (25–45 min), showed this isomer to have only one component (Pauly color reaction), R_f^C 0.30. *Anal.* ($C_{16}H_{22}N_6O_4$) C, H, N.

LDL Isomer. This isomer was obtained by the method described above, except for lyophilization of the final product which yielded an amorphous powder: $[\alpha]^{24}D$ −41.2°. The nmr (C_5D_5N) was consistent with the structure. The tlc (R_f^C 0.30) and tle patterns showed one component. *Anal.* ($C_{16}H_{22}N_6O_4$) C, H, N.

LLD Isomer. This isomer was similarly obtained as an amorphous powder after lyophilization: $[\alpha]^{24}D$ +43.8° (*c* 1, H_2O). The nmr (C_5D_5N) was consistent with the structure. The tlc (R_f^C 0.30) and tle patterns showed one component. *Anal.* ($C_{16}H_{22}N_6O_4 \cdot 0.4H_2O$) C, H, N.

DDD Isomer. This isomer was obtained as an amorphous powder after lyophylization: $[\alpha]^{24}D$ +66.4° (*c* 1, H_2O) [lit. L isomer $[\alpha]^{25}D$ −65.5° *c* 1, H_2O);[4] $[\alpha]^{25}D$ −64.8° (*c* 1, H_2O);[15] also $[\alpha]^{25}D$ −50° (*c* 1.5, H_2O)[16]]. The nmr (C_5D_5N) was consistent with the structure and identical with that for the LLL isomer. The tlc (R_f^C 0.30) and tle patterns showed one component with mobility identical with TRH. *Anal.* ($C_{16}H_{22}N_6O_4 \cdot 0.7H_2O$) C, H, N.

Biologic Activity of TRH and Diastereoisomeric Analogs of TRH. Thyrotropin (TSH)—releasing activity of TRH and analogs was investigated *in vitro*. Single rat hemipituitaries were preincubated for 4 hr in 2 ml of Krebs-Ringer bicarbonate (KRB)

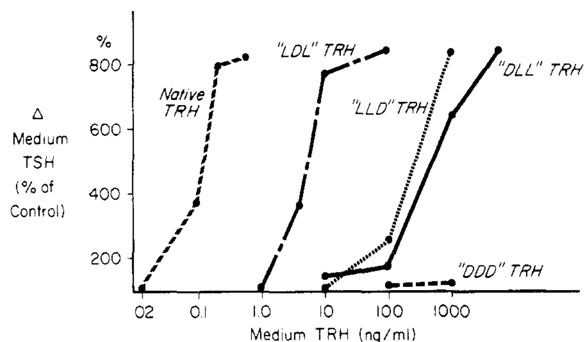

Figure 1.

medium to permit TSH release to equilibrate at a basal level. Six tissues were used per test substance. The medium was then removed and discarded and replaced by 1 ml of KRB for the succeeding 1-hr control period. Medium was then replaced again with media containing TRH or analogs at concentrations indicated in Figure 1 (abscissa). Medium TSH concentrations during control and experimental periods were determined by a specific radioimmunoassay for rat TSH.[11] Incremental changes in medium TSH are expressed as per cent of control [TSH (hr 2)/TSH (hr 1) × 100].

Native TRH yielded a sigmoidal dose-response function between 0.020 and 0.2 ml (Figure 1). LDL-TRH gave a parallel dose-response funct[illegible] shifted to the right. The observed potency of LDL-TRH was about 2-3% of native TRH activity. In contrast, the analogs LLD- and DLL-TRH possessed only about 0.1% of native TRH activity. DDD-TRH failed to exhibit any TSH-releasing activity with medium concentrations as high as 1 μg/ml.

In order to test for inhibitory properties, hemipituitaries were incubated with a submaximal TRH stimulus (100 pg/ml) and substimulating concentrations of the four analogs.

The LDL analog did not inhibit thyrotropin release at a concentration 20 times that of the hormone nor did the LLD, DLL, or DDD isomers at concentrations 1000 times that of TRH.

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Effect of Simple Amino Acid Replacements on the Biological Activity of Luteinizing Hormone-Releasing Hormone

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Six decapeptides having structures based on the LH-RH sequence, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg- Pro-Gly-NH_2, have been synthesized by modifications of the solid-phase technique *via* protected peptide intermediates. Thus, pyroglutamic acid, tryptophan, serine, glycine, proline, and glycine amide residues in the LH-RH sequence were replaced by leucine, tyrosine, threonine, isoleucine, alanine, and glycine dimethylamide, respectively. The compounds were assayed *in vivo* and found to possess the following LH-releasing potencies relative to pure, natural LH-RH: [Leu^1]-LH-RH, 0.003%; [Tyr^3]-LH-RH, 0.13%; [Thr^4]-LH-RH, 19%; [Ile^6]-LH-RH, 0.034%; [Ala^9]-LH-RH, 0.8%; and [(Gly-dimethylamide)^{10}]-LH-RH, 14%.

Recent studies[1,2] on the effects of replacement of tyrosine in position 5 of LH-RH by closely related amino acids with aromatic side chains established that the phenolic hydroxyl group does not contribute significantly toward biological activity. This paper reports methods of synthesis and biological characteristics of decapeptides containing replacements for many of the other residues.

Of the amino acids with functional side chains in LH-RH, cyclic pyroglutamic acid was replaced by leucine, tryptophan by tyrosine, and serine by the closely related threonine. While this work was in progress, data on the threonyl peptide, synthesized by fragment condensation, were reported by Fujino, *et al.*[3] The glycine residue in the sterically crowded center of the molecule and the conformationally important proline at position 9 were particularly intriguing and were replaced by isoleucine and alanine, respectively. Finally, the importance of the amide part of C-terminal glycine was examined by replacing it with a dimethylamide group.

Synthesis. All protected peptides were prepared on a resin support by versions of the Merrifield solid-phase method[4] which have been described in part previously.[1,2,5] Protected peptide amides were cleaved from the resin by treatment with ammonia in methanol or, in the case of [(glycine-dimethylamide)^{10}]-peptide, with dimethylamine.

In all the peptides the following groups were used for the protection of sensitive side chains during synthesis: arginine, N^G-tosyl; serine, threonine; and tyrosine, *O*-ben-