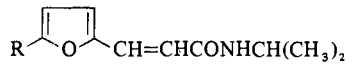
Table I



Compd no.	R[a]	Mp, °C	Crystn solvents	Formula
3	CH_3	121-122	$CHCl_3$-Et_2O	$C_{11}H_{15}NO_2$
4	$COOCH_3$	164-165	C_6H_6-Et_2O	$C_{12}H_{15}NO_4$
5	COOH	201-203	MeOH-Et_2O	$C_{11}H_{13}NO_4$
6	CH_2OCOCH_3	116-118	EtOAc-ligroin	$C_{13}H_{17}NO_4$
7	CH_2OH	185-186	MeOH	$C_{11}H_{15}NO_3$
8	CHO	157-159	Me_2CO-Et_2O	$C_{11}H_{13}NO_3$
9	CH=NOH	186-191	MeOH-Et_2O	$C_{11}H_{14}N_2O_3$
10	CH=NOH	142-146	Et_2O-C_6H_6	$C_{11}H_{14}N_2O_3$
11	CN	139-140	Et_2O-ligroin	$C_{11}H_{12}N_2O_2$

[a] All compounds were analyzed for C, H, and N, and all gave values within 0.3% of the calculated figures.

(31%), mp 115-118°. Recrystallization raised the melting point to 121-122°.

N-Isopropyl-3-(5-carboxy-2-furyl)acrylamide (5). The known[7] 3-(5-carboxymethyl-2-furyl)acrylic acid was converted to the isopropyl amide 4 by the general method described above, and 4 (0.52 g, 2.2 mmol) was dissolved in MeOH (10 ml) and 1% aqueous KOH (16.5 ml) and kept at 25° for 55 min. Acidification and extraction with EtOAc gave crude product (0.48 g) which was crystallized from MeOH-Et_2O to give 5, mp 201-203° (0.406 g, 83%).

N-Isopropyl-3-(5-hydroxymethyl-2-furyl)acrylamide (7). The known[8] 3-(5-acetoxymethyl-2-furyl)acrylic acid was converted to the isopropyl amide 6 by the general method described above, and a solution of 6 (7.80 g, 0.031 mol) in 40 ml of MeOH and 21 ml of 10% aqueous KOH was kept at 25° for 30 min. The solution was acidified, and the precipitate was filtered off, washed with water, and dried *in vacuo*. Crystallization from MeOH-Et_2O gave 7 (4.75 g, 73%), mp 182-185°; second crop (1.20 g, 18%), mp 181-183°. Recrystallization from MeOH-Et_2O raised the melting point to 185-186°.

N-Isopropyl-3-(5-formyl-2-furyl)acrylamide (8). The hydroxymethyl compound 7 (1.5 g, 7.2 mmol) in Me_2CO (300 ml) was stirred with activated MnO_2 (5.0 g) at 25° for 2 hr. More MnO_2 (1.0 g) was added, and stirring was continued for 1 hr. Another portion (1.0 g) of MnO_2 was added and stirring was continued for 1.75 hr. The mixture was filtered and the filtrate was evaporated *in vacuo* to give crude aldehyde (1.38 g). Crystallization from Me_2CO-Et_2O gave 1.28 g (85%) of 8, mp 155-157°. Recrystallization raised the melting point to 157-159°.

Formation of Oximes 9 and 10 from *N*-Isopropyl-3-(5-formyl-2-furyl)acrylamide (8). A solution of the aldehyde 8 (3.41 g, 0.016 mol) in MeOH (15 ml) and H_2O (30 ml) containing hydroxylamine sulfate (2.08 g, 0.016 mol) and NaOAc (2.62 g, 0.032 mol) was heated under reflux for 1 hr. Tlc (EtOAc-ligroin, 4:1) showed formation of two products. Removal of MeOH *in vacuo* resulted in the crystallization of the less polar oxime 9 (2.25 g, 61%) which was recrystallized from MeOH-Et_2O to give 0.814 g (22%), mp 187-191°. The aqueous mother liquor was extracted with $CHCl_3$ to give 1.50 g (41%) comprising mainly the more polar oxime 10. Crystallization from Et_2O-C_6H_6 gave pure 10, mp 142-146°.

N-Isopropyl-3-(5-cyano-2-furyl)acrylamide (11). A solution of the more polar oxime 10 (1.22 g, 5.5 mmol) in Ac_2O (1.5 ml) containing NaOAc (0.675 g) was heated under reflux for 70 min. By dilution with water and extraction with Et_2O-$CHCl_3$ (4:1), crude nitrile (1.04 g) was obtained. Crystallization from C_6H_6-ligroin gave 0.706 g (63%) of 11, still slightly impure. Dry-column chromatography on silica gel (10 g) with $CHCl_3$ as eluent gave 0.629 g (56%) of 11, which was crystallized from Et_2O-ligroin to give pure material, mp 139-140°. The nitrile 11 could also be obtained in comparable amount if the crude oxime mixture, 9 and 10, was used in the reaction.

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Nucleoside Peptides. 5.[1] Synthesis of Certain *N*-(5-Uridineacetyl)amino Acids from 5-Uridineacetic Acid

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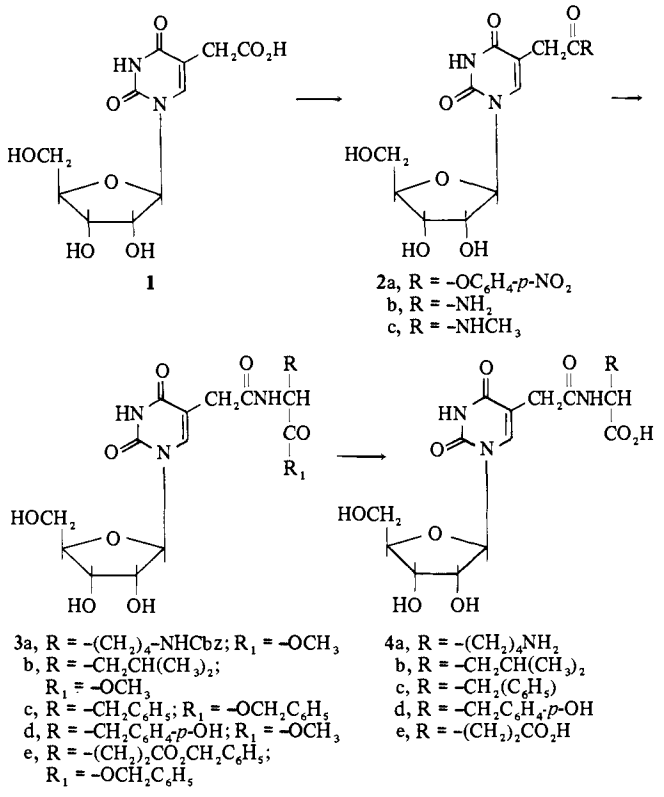
Modified nucleosides isolated from transfer ribonucleates have been proposed (for a recent review, see ref 2) to exhibit a regulatory influence on protein biosynthesis as well as influence the tertiary structure of these macromolecules. Inhibition of the growth of certain cancer cell lines by 4-thiouridine[3] and the cytokinin activity of isopentenyl adenosine[4] have been noted and suggest that other minor nucleosides and their derivatives may possess interesting biological activities.

It was, therefore, of interest to study the biological properties of the modified nucleoside 5-uridineacetic acid (1) (Scheme I) isolated from yeast transfer ribonucleates[5,6] and several of its analogs. Recently 1 was synthesized[7,8] and the synthetic product found to be identical with the product isolated from natural sources.[8] Since we have investigated the biological properties of a number of nucleoside peptide derivatives and found inhibition of protein biosynthesis[1,9] and antiviral activity[10] in cell culture, we now report the synthesis and biological properties of a number of *N*-(5-uridineacetyl)amino acids prepared from 1.

The active ester procedure of Bodanszky[11] provided the method of choice for peptide bond formation. Since this technique allows selective bond formation to an amino group in the presence of free hydroxyl groups,[9] it was unnecessary to block the sugar moiety of the nucleoside. A facile preparation of the active ester 5-uridineacetic acid *p*-nitrophenyl ester (2a) (Scheme I) was achieved by the action of *p*-nitrophenol and DCC on 5-uridineacetic acid (1). This useful intermediate was found to be readily susceptible to nucleophilic attack. Treatment of 2a with alcoholic ammonia or alcoholic methylamine gave 5-uridineacetamide (2b) and 5-uridine-*N*-methylacetamide (2c) in yields of 58 and 80%, respectively. Facile peptide bond formation was also achieved by treatment of 2a with appro-

Scheme I



priately blocked amino acids. N_α-(5-Uridineacetyl)-N_ϵ-benzyloxycarbonyl-L-lysine methyl ester (**3a**) was afforded in 68% yield from **2a** and *N*-benzyloxycarbonyl-L-lysine methyl ester. Similarly *N*-(5-uridineacetyl)-L-leucine methyl ester (**3b**) and *N*-(5-uridineacetyl)-L-tyrosine methyl ester (**3d**) were prepared from **2b** and L-leucine methyl ester and L-tyrosine methyl ester, respectively. L-Phenylalanine benzyl ester *p*-toluenesulfonate and L-glutamic acid dibenzyl ester *p*-toluenesulfonate reacted with **2a** to give a 64% yield of *N*-(5-uridineacetyl)-L-phenylalanine benzyl ester (**3c**) and a 55% yield of *N*-(5-uridineacetyl)-L-glutamic acid dibenzyl ester (**3e**), respectively (Table I). After samples of **2a** were stored at room temperature for 6 months, no decomposition could be detected.

Catalytic hydrogenolysis of the Cbz group followed by alkaline hydrolysis of the methyl moiety of **3a** afforded a 90% yield of N_α-(5-uridineacetyl)-L-lysine (**4a**). Removal of the methyl blocking groups from **3b** and **3d** was accomplished by alkaline hydrolysis and provided *N*-(5-uridineacetyl)-L-leucine (**4b**) and *N*-(5-uridineacetyl)-L-tyrosine (**4d**) in excellent yields of 90 and 81%, respectively. The benzyl blocking groups of **3c** and **3e** were cleaved by catalytic hydrogenolysis utilizing Pd as catalyst. The desired products *N*-(5-uridineacetyl)-L-phenylalanine (**4c**) and *N*-(5-uridineacetyl)-L-glutamic acid (**4e**) were also obtained in excellent yields (92 and 87%, respectively).

The inhibitory effects of these compounds against various bacterial strains are shown in Table II. 5-Uridineacetic acid (**1**), *N*-(5-uridineacetyl)-L-phenylalanine (**4c**), and *N*-(5-uridineacetyl)-L-glutamic acid (**4e**) showed inhibition in all strains tested, whereas *N*-(5-uridineacetyl)-L-leucine (**4b**) inhibited the growth of only *Serratia marcescens* and *Pseudomonas aeruginosa*. *N*-(5-Uridineacetyl)-L-tyrosine inhibited the above two organisms as well as *Escherichia coli*. The other compounds tested failed to inhibit growth of the bacterial strains.

It was thought that inhibition of bacterial strains might be a result of inhibition of protein biosynthesis. Therefore, these compounds were assayed for possible inhibitory effects on poly-U-directed polyphenylalanine synthesis,[12] utilizing cell-free extracts from *E. coli*. None of these compounds inhibited the synthesis of polyphenylalanine under these conditions.

Experimental Section

Physical properties of these compounds were determined with the following instruments: Thomas-Hoover apparatus (melting point, uncorrected); Cary 15 uv spectrometer (uv spectra); Perkin-Elmer Model 141 polarimeter (specific rotations); Hitachi Perkin-Elmer R20A high-resolution nmr spectrometer (nmr, Me_4Si or DSS); and Perkin-Elmer Model 257 (ir spectra, KBr). Elemental analyses were performed by Heterocyclic Chemical Corporation, Harrisonville, Mo. Where indicated by elemental analysis, hydration was verified quantitatively by nmr spectroscopy in absolute DMSO-d_6 and then by exchange with addition of D_2O and reintegration of the spectral area where the H_2O peak had occurred.

5-Uridineacetic Acid *p*-Nitrophenyl Ester (2a). A solution of 5-uridineacetic acid[8] (**1**, 3.02 g, 10 mmol) and *p*-nitrophenol (1.53 g, 11 mmol) in DMF (50 ml) was treated with DCC (2.06 g, 10 mmol) at 0°. The reaction mixture was stirred at room temperature for 4 hr and then kept at 4° for 16 hr. The precipitated *N,N'*-dicyclohexylurea was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The amorphous residue was triturated with Et_2O, collected, and crystallized to afford 4.71 g of **2a** (Table I).

5-Uridineacetamide (2b). Compound **2a** (211.5 mg, 0.5 mmol) was dissolved in anhydrous EtOH (100 ml) and the solution was saturated with NH_3 at 20°. After 15 min the solvent was evaporated *in vacuo*. The glassy residue was triturated with EtOH and the crude amide was collected and crystallized to yield 87 mg of **2b** (Table I).

5-Uridine-*N*-methylacetamide (2c). Compound **2a** (211.5 mg, 0.5 mmol) was dissolved in dry MeOH (100 ml) and the solution was saturated with $MeNH_2$ at 20°. After 15 min the solvent was evaporated *in vacuo*; the residue was triturated with MeOH-Et_2O (1:1, 3 × 6 ml) and MeOH (6 ml) and then collected (187 mg) and crystallized (Table I).

N_α-(5-Uridineacetyl)-N_ϵ-benzyloxycarbonyl-L-lysine Methyl

Table I

Compd	Yield, %	Recrystn solvent	Mp, °C	Formula[a]	$[\alpha]^{25}D$
2a	61	MeOH	172–173	$C_{17}H_{17}O_{10}N_3$	
2b	58	MeOH-H_2O	227–228	$C_{11}H_{15}O_7N_3$	−7.0 (*c* 1, DMSO)
2c	80	MeOH-H_2O	260–261	$C_{12}H_{17}O_7N_3$	−2.5 (*c* 1, DMSO)
3a	68		171–173	$C_{26}H_{34}O_{11}N_4$	12.5 (*c* 1, DMSO)
3b	52		202–203	$C_{18}H_{28}O_9N_3$	−20.2 (*c* 1, DMSO)
3c	64	MeOH	187–188	$C_{27}H_{29}O_9N_3$	14.2 (*c* 1, DMSO)
3d	27	EtOH	196–199	$C_{21}H_{25}O_{10}N_3$	−3.0 (*c* 1, DMSO)
3e	55	EtOAc-MeOH	154–157	$C_{30}H_{33}O_{11}N_3$	−14.3 (*c* 1, DMSO)
4a	90	EtOH-H_2O	78–80	$C_{17}H_{26}O_9N_4$	20.0 (*c* 1, H_2O)
4b	93		173–179	$C_{17}H_{25}O_9N_3$	−2.1 (*c* 1, H_2O)
4c	92	EtOH-H_2O	104–107	$C_{20}H_{23}O_9N_3 \cdot 2.5H_2O$	6.0 (*c* 1, DMSO)
4d	81	Amorphous solid		$C_{20}H_{23}O_{10}N_3 \cdot 3H_2O$	
4e	58	Amorphous solid		$C_{16}H_{21}O_{11}N_3 \cdot 0.5H_2O$	+18.0 (*c* 1, H_2O)

[a] Analyzed for C, H, and N.

Table II. Inhibition of Bacterial Strains in Nutrient Agar by Various 5-Uridineacetic Acid Derivatives

Compd	*S. marcescens*	*P. aeruginosa*	*Staph. aureus*	*E. coli*	*Strep. faecalis*	*B. subtilis*
1	+[a]	+	+	+	+	+
2b	–	–	–	–	–	–
2c	–	–	–	–	–	–
4a	–	–	–	–	–	–
4b	+	+	–	–	–	–
4c	+	+	+	+	+	+
4d	+	+	–	+	–	–
4e	+	+	+	+	+	+
Hexylresorcinol	+	+	++	++	++	++

[a] The symbol + indicates a circular zone of inhibition extending from 0.25 to 0.75 cm in diameter, while ++ represents a zone from 0.75 to 2.00 cm and – indicates a zone of less than 0.25 cm.

Ester (**3a**). A solution of N_ϵ-benzyloxycarbonyl-L-lysine methyl ester hydrochloride[13] (496 mg, 1.5 mmol), CH_2Cl_2 (10 ml), and 1.5% Na_2CO_3 (10 ml) was stirred at 4° for 15 min. The organic layer was washed with H_2O and dried (anhydrous Na_2SO_4) and the solvent was evaporated under reduced pressure. The residual oil was dissolved in dry dioxane (7 ml) and treated with **2a** (423 mg, 1.0 mmol). After 16 hr at room temperature, the solvent was evaporated *in vacuo* and the residue was applied to a silica gel column (2 × 30 cm), packed in *n*-heptane. The column was washed with a mixture of *n*-heptane and EtOAc (1:1) until no more ninhydrin-positive material was detected in the effluent and then with an EtOAc–MeOH–CH_2Cl_2 (16:3:1) solvent system, which eluted the product. The uv-absorbing fractions were collected, combined, and concentrated to a small volume. After 2 days at 4° a colorless crystalline **3a** deposited (393 mg, Table I).

N-(**5-Uridineacetyl**)-L-leucine **Methyl Ester** (**3b**). L-Leucine methyl ester hydrochloride[14] (273 mg, 1.5 mmol), CH_2Cl_2 (10 ml), and 0.5 *N* NaOH (4 ml) was stirred at 0° for 30 min. The organic phase was separated and dried (anhydrous Na_2SO_4), the drying agent was removed by filtration, and the filtrate was treated with compound **2a** (423 mg, 1.0 mmol). The solvent was removed *in vacuo* and the residue was dissolved in a mixture of 3 ml of dioxane and 3 ml of THF. After 18 hr at room temperature, the solvent was evaporated under reduced pressure. The residual glass was applied to a column of silica gel (2 × 30 cm, packed in *n*-heptane). The column was washed first with *n*-heptane–EtOAc (1:1) to remove the *p*-nitrophenol and then *n*-heptane–EtOAc (1:4) to elute the excess amino acid ester. The product was eluted with EtOAc–MeOH–CH_2Cl_2 (7:2:1) and the uv-absorbing fractions were collected, combined, and evaporated to a small volume (2 ml). After the solution had been allowed to stand at 4° for several hours, colorless **3b** deposited (277 mg).

N-(**5-Uridineacetyl**)-L-**phenylalanine Benzyl** Ester (**3c**). L-Phenylalanine benzyl ester *p*-toluenesulfonate (640 mg, 1.5 mmol), CH_2Cl_2 (10 ml), and 0.5 *N* NaOH (4 ml) were stirred at 0° for 30 min. The organic phase was separated, dried (anhydrous Na_2SO_4), and evaporated to dryness. The residue was taken up with a mixture of 4 ml of dioxane and 4 ml of THF and then **2a** (423 mg, 1.0 mmol) was added. After 20 hr at room temperature, the solvent was removed *in vacuo* and the residue was applied to a silica gel column (2 × 30 cm, packed in *n*-heptane). The by-products and excess starting material were eluted with EtOAc–*n*-heptane (4:1) and then the desired product was eluted with EtOAc–MeOH–CH_2Cl_2 (17:3:2). The uv-absorbing fractions were combined and evaporated to dryness. The residue **3c** was crystallized (Table I) to give 351 mg.

N-(**5-Uridineacetyl**)-L-**tyrosine Methyl Ester** (**3d**). L-Tyrosine methyl ester hydrochloride[15] (231 mg, 1 mmol) was neutralized with NaOMe (54 mg, 1 mmol) in MeOH (3 ml). The precipitated salt was removed by filtration and the solvent was evaporated under reduced pressure. The residue was dissolved in dioxane (8 ml) and the solution was treated with **2a** (423 mg, 1 mmol). After 18 hr at room temperature, the solvent was evaporated *in vacuo* and the residual material was chromatographed on a column of silica gel (2 × 30 cm, packed in *n*-heptane). The by-products and unchanged starting materials were eluted with *n*-heptane–EtOAc (1:4) and the product was eluted with EtOAc–MeOH–CH_2Cl_2 (17:3:1). The uv-absorbing, homogenous fractions were collected, combined, and evaporated to dryness and the colorless material was recrystallized (Table I) to yield 128 mg of **3d**.

N-(**5-Uridineacetyl**)-L-**glutamic Acid Dibenzyl** Ester (**3e**). L-Glutamic acid dibenzyl ester *p*-toluenesulfonate[16] (700 mg, 1.5 mmol), CH_2Cl_2 (20 ml), and $N(Et)_3$ (0.23 ml) were stirred at 0° for 30 min and then extracted with H_2O (5 × 30 ml). The CH_2Cl_2 layer was separated, dried (anhydrous Na_2SO_4), filtered, and evaporated to dryness. Dioxane (8 ml) and **2a** (423 mg, 1 mmol) were added and this solution was kept at room temperature for 18 hr. The solvent was evaporated *in vacuo* and the residual material was applied to a column of silica gel (2 × 40 cm, packed in ligroin). The column was washed with ligroin–Et_2O (1:1, 500 ml) and Et_2O (250 ml) to remove the by-products and excess starting material. The product was eluted with EtOAc–MeOH–CH_2Cl_2 (17:3:3). The uv-absorbing homogenous fractions were collected, combined, and evaporated to small volume (2 ml). The colorless material which separated after the solution had been cooled for several hours was recrystallized to yield **3e** (334 mg, Table I).

N_α-(**5-Uridineacetyl**)-L-lysine (**4a**). **3a** (289.3 mg, 0.5 mmol) was dissolved in 75% EtOH (30 ml) and hydrogenated over 10% Pd/C at room temperature and atmospheric pressure for 1 hr. The catalyst was removed by filtration and the solvent was evaporated *in vacuo*. The residual glass was treated with 0.5 *N* NaOH (8 ml) and kept at 4° for 2 hr; then the solution was neutralized by passing it through a column of Amberlite IRC 50 [H^+], concentrated to a small volume (2 ml) *in vacuo*, and EtOH was added until colorless crystalline **4a** deposited. This was recrystallized (Table I) to yield an analytically pure sample.

Preparation of *N*-(**5-Uridineacetyl**)-L-leucine (**4b**) and *N*-(**5-Uridineacetyl**)-L-**tyrosine** (**4d**) **by Alkaline Hydrolysis.** The respective starting material **3b** (215 mg, 0.5 mmol) or **3d** (240 mg, 0.5 mmol) was treated with 0.5 *N* NaOH (8 ml) at room temperature for 2 hr. The solution was neutralized with Amberlite IRC 50 [H^+] and lyophilized to yield pure **4b** or **4d** as amorphous solids.

Preparation of *N*-(**5-Uridineacetyl**)-L-**phenylalanine** (**4c**) **or** *N*-(**5-Uridineacetyl**)-L-**glutamic Acid** (**4e**) **by Catalytic Hydrogenolysis of Benzyl-Blocked Precursors.** Compound **3c** (269 mg, 0.5 mmol) or **3e** (305 mg, 0.5 mmol) was dissolved in 70% EtOH (40 ml). Pd on C (10%, 300 mg) was added and the mixture hydrogenated at 40° and 1 atmosphere. After 1 hr the catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to dryness. Compound **4c** was obtained in pure form by recrystallization (Table I) while compound **4e** was obtained by lyophilization of the residue in 2 ml of H_2O.

Qualitative Assay for Antibacterial Activity. The bacteria were transferred from a stock culture, suspended in 10 ml of nutrient broth (Difco), and incubated with shaking overnight at 37°. A small aliquot (*ca.* 0.02 ml) of this culture was transferred to a second tube containing 10 ml of nutrient broth and thoroughly mixed. One tube was prepared for each petri dish to be inoculated.

Freshly prepared petri dishes (10 × 100 mm) containing nutrient agar (Difco; 3 mm thickness) were inoculated by pouring the previously prepared suspension of microorganisms on the surface. After 5 min, the suspension was decanted and the petri dish was inverted and allowed to dry over an absorbent material for 45–60 min at room temperature.

A flame-cleaned microspatula was used to transfer enough compound to cover a small pin head to its given location on the petri dish after inoculation of the organism as above. Compounds were tested on three petri dishes for each organism.

The dishes were incubated at 37° for 18 hr. An active compound inhibits the growth of the organism and a circular clear area (zone of inhibition) is seen around the compound when viewed against a dark background or in an oblique light. The results of these tests are reported in Table II.

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Luteinizing Hormone Releasing Hormone. Solid-Phase Synthesis of a 5-Phenylalanine Analog Possessing High Biological Activity

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Since the initial isolation and characterization[1-3] of luteinizing hormone relasing hormone (LH-RH) of the porcine hypothalamus, the decapeptide pGlu-His-Try-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 has been synthesized by both classical[4-6] and solid-phase[5,7-10] methods. In order to establish structure-activity relationships for this peptide, with the ultimate goal of creating an inhibitor of LH release, it is essential that analogs of LH-RH be synthesized and their biological properties investigated.

Preliminary inactivation studies[11] on LH-RH indicated that the hydroxyl group of the tyrosine residue was not essential for LH release. We have, therefore, made a peptide in which the hydroxyl group on the aromatic nucleus is deleted.

Synthesis. Manning and coworkers[12] have recently developed a synthesis of LH-RH in which a peptide intermediate made by solid-phase reactions is deprotected by the sodium in liquid ammonia reduction method devised by Sifferd and du Vigneaud.[13] Readily purifiable products are obtained and this route has been employed by us in the preparation of [5-Phe]-LH-RH.

The synthetic procedure is outlined in Scheme I. The protected peptide **1** was prepared by the Merrifield method[14] with modifications.[15] Functional groups were protected as follows: histidine, N^{im}-benzyl; serine, *O*-benzyl; arginine, N^G-tosyl. The N^G-nitro group has commonly been used for arginine protection in previously published solid-phase syntheses of LH-RH peptides where it is finally removed by cleavage in liquid HF. Recent reports[16,17] indicate that this reaction is accompanied by the formation of considerable amounts of hard-to-separate ornithine-containing contaminants resulting from deamidation of the arginine side chain.

Scheme I. Outline of the Solid-Phase Synthesis of the Protected Decapeptide **1** and Its Reduction to [5-Phe]-LH-RH

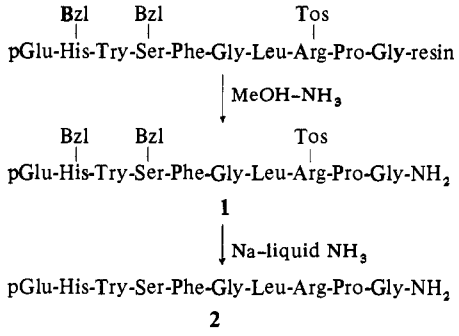

Table I. LH-RH Activity of Natural and [5-Phe]-LH-RH in Ovariectomized, Estrogen–Progesterone Treated Rats

Sample	Dose, ng/rat	Mean serum LH,[a] ng/ml ± S.E.
Saline		2.3 ± 0.9 (3)[b]
Natural LH-RH	0.5	13.6 ± 0.4 (3)
	2.5	70.0 ± 9.4 (3)
[5-Phe]-LH-RH	0.8	22.3 ± 1.1 (3)
	4.0	62.9 ± 8.7 (3)

[a] As NIH-LH-S-17. [b] Number of rats per group.

Biological Results. LH-RH activities (Table I) were determined *in vivo* by stimulation of LH release at two dose levels in ovariectomized rats pretreated with estrogen and progesterone[2,3,18] followed by radioimmunoassay for LH.[19] Serum LH levels after injection of samples are compared with those obtained after administration of saline and two doses of natural LH-RH. Using a four-point factorial assay,[20] the LH-RH activity of the [5-Phe]-peptide was calculated to be 64% of the natural hormone with 95% confidence limits of 38–108%. In a separate, but similar assay, LH-RH prepared by a similar synthetic procedure[12] possessed 120% of the activity of the natural material with 95% confidence limits of 57–305%.

The surprisingly high activity of [5-Phe]-LH-RH demonstrates conclusively that the hydroxyl group of the tyrosine residue of LH-RH is not essential for either binding to the receptor site or in the mechanism governing the release of LH.†

Experimental Section

Melting points are uncorrected. Amino acid derivatives used as starting materials were the pure L isomers and were purchased from Bachem, Inc., Marina del Rey, Calif. Microchemical analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Amino acid analyses were carried out with a Beckman Amino Acid Analyser Model 120C on samples which were hydrolyzed (18 hr) in 6 *M* HCl containing 4% thioglycolic acid[22] in sealed, evacuated ampoules at 110°. The following tlc systems were used: R_f[1],

† The FSH releasing activity of the [5-Phe]-peptide when assayed *in vitro* as described by Schally, *et al.*, was found to be 98% (50–265%) that of the natural hormone.