

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 (11*m*,*p*-14*m*,*p*). The *N*-methyl derivative 11 was prepared by methylation of free base 6 with HCHO and HCO₂H using a known procedure⁷ 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), KHCO₃ (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 Et₂O (discarded), made basic with excess solid NaHCO₃, and extracted with Et₂O. The Et₂O extract was dried (CaSO₄) 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-NH₂¹⁰]-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-NH₂¹⁰]-LH-RH *N*-alkylamides which are, in general, potent agonists. Analogues 2-5 may be regarded as isosteric with [aza-Gly-NH₂¹⁰]-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%; semithiocarbamide (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 substitutions¹ 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-NH₂¹⁰]-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-NH₂¹⁰]-LH-RH *N*-alkylamides,² whereas the other four analogues may be regarded as isosteric with [aza-Gly-NH₂¹⁰]-LH-RH, which is equipotent with LH-RH.³ Of additional interest was the observation of Coy et al. that several [des-Gly-NH₂¹⁰]-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*-carbonylhydrazide, 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 me-

tabolized more slowly and, because of the gradation in polarity of acyl substituents in going from thiocarbonyl 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 synthesis⁶ 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 CH₃NHNH₂ and deprotection of the resulting peptide with liquid HF, as previously described,⁹ gave 1. LH-RH¹⁻⁹ 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-RH¹⁻⁹ 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 filtration¹⁰ and/or

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Table I. Biological Activities of the LH-RH Analogues for Release of LH and FSH from Male Rat Hemipituitaries in Vitro

no.	compd name	LH-release ^a potency ^b		FSH-release ^a potency ^b	
		100	100	100	100
1	LH-RH [des-Gly-NH ₂ ¹⁰]-LH-RH <i>N</i> -methylhydrazide	17.0 (12.4–23.4)		11.0 (4.5–27.0)	
2	[des-Gly-NH ₂ ¹⁰]-LH-RH thiosemicarbazide	6.5 (2.2–19.0)		4.6 (1.4–14.7)	
3	[des-Gly-NH ₂ ¹⁰]-LH-RH formylhydrazide	15.3 (6.0–39.0)		10.0 (6.3–16.0)	
4	[des-Gly-NH ₂ ¹⁰]-LH-RH acetylhydrazide	1.2 (0.2–13.0)		0.6 (0.2–1.9)	
5	[des-Gly-NH ₂ ¹⁰]-LH-RH (trifluoroacetyl)hydrazide	1.0 (0.2–7.0)		0.9 (0.3–3.1)	

^a LH and FSH values were determined by radioimmunoassay. ^b 95% confidence limits are indicated in parentheses.

partition chromatography¹¹ on Sephadex G-25.

Biological Results and Discussion

In vitro bioassay procedures for agonistic activity were described previously.¹² Usually, the analogues were assayed at two dose levels in a 2 × 2 (four point) assay design, in relation to an LH-RH standard, for their release of FSH and LH when incubated with rat hemipituitaries. Media were sampled 6 h later and assayed for LH and FSH by radioimmunoassay. The results of the bioassays are shown in Table I.

The *N*-methylhydrazide analogue 1 only had 11–17% of the activity of LH-RH. Although it was hoped that this analogue would be highly active, the presence of the increasingly basic C terminus may perhaps interfere with its binding to pituitary receptors. Thus, this analogue does not show marked improvement in potency when compared to the hydrazide, previously prepared by Coy et al.¹³

The thiosemicarbazide analogue had 4.6–6.5% of LH-RH activity. Thus, the presence of sulfur instead of oxygen dramatically reduces the biological activity of [aza-Gly-NH₂¹⁰]-LH-RH, which is equipotent with the hormone. Such a drop in biological activity might be related to the change in ionic character on the C terminus caused by the thiocarbonyl group and, hence, to the decrease in affinity.

Analogues 3–5, which are isosteric with [aza-Gly-NH₂¹⁰]-LH-RH, are not very active. In fact, 4 and 5 were more than 10 times weaker than 3, suggesting that substitution of the formyl hydrogen atom in 3 with the more lipophilic CH₃ or CF₃ groups results in decreased affinity for 4 and 5.

Experimental Section

Optical rotations were measured with a Rudolph polarimeter (precision ±0.01°). UV spectra were recorded in a Cary 16 recording spectrophotometer. Thin-layer electrophoresis (TLE) was performed on Eastman Chromagram thin-layer sheets with 0.1 M Py–AcOH (pH 5.6) at 500 V for 2–5 h, by means of a Desaga-Brinkman apparatus. Thin-layer chromatography (TLC) was also performed on the above thin-layer sheets. When partition solvent systems were used, the lower phase was sprayed on the plate and development was accomplished with the upper phase. Solvent systems used for column chromatography or TLC were (A) *n*-BuOH–AcOH–H₂O (4:1:5); (B) *n*-BuOH–EtOH–Py–1% AcOH (14:2:5:24); (C) *n*-BuOH–AcOH–H₂O–EtOAc (1:1:1:1); (D) *n*-BuOH–EtOH–AcOH–H₂O (8:2:1:3). For detection of peptides, Cl₂–toluidine, Ehrlich's or Pauly reactions were used. For gel permeation chromatography¹⁰ we used Sephadex G-25 (Pharmacia). Partition chromatography was accomplished on Sephadex G-25 by the method of Yamashiro.¹¹ Elution profiles were determined by the Folin–Lowry method¹⁴ or by means of a UV monitor (Uvicord, LKB Instruments). For amino acid analyses, peptides were hydrolyzed by the method of Simpson to avoid destruction of tryptophan¹⁵ and then were analyzed in a JEOL

automatic amino acid analyzer.¹⁶ For deprotection of synthetic peptides with liquid HF,⁸ an all-Teflon apparatus (Protein Research Foundation, Osaka, Japan) was used. Statistics for the biological assays were computed with the aid of an interactive graphics system terminal connected to the PROPHET computer system.¹⁷ The abbreviations employed follow the recommendation of IUPAC–IUB Commission on Biochemical Nomenclature.¹⁸ Additionally, the following abbreviations were used: Bzl, Benzyl; Boc, *tert*-butyloxycarbonyl; Tos, Tosyl; TFA, trifluoroacetic acid; TEA, triethylamine; Py, pyridine; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide.

Solid-Phase Synthesis of Peptides. Boc-Pro-resin (0.5 mmol of Pro/g), prepared as previously described,⁹ was converted to the desired protected peptide resin by the solid-phase method⁶ as modified in a previous synthesis of LH-RH.⁷ In each synthetic cycle the Boc group was removed with 25% TFA in CH₂Cl₂ and, after neutralization of the resin with TEA, coupling was performed with a 4 mol excess of the desired amino acid and DCC. For the protection of side-chain functionalities, Boc-Ser(Bzl), Boc-Tyr(Bzl), and Boc-Arg(Tos) were used. Completion of coupling was monitored by means of the Kaiser test.¹⁹

pGlu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-OMe. pGlu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-O-resin (7.1 g, 2.5 mmol based on Boc-Pro-O-Resin) was stirred in a mixture of MeOH (300 mL) and TEA (17.5 mL) at 20 °C for 4 days. The resin was filtered and washed with DMF, and the combined filtrate was evaporated under reduced pressure. The residue obtained was triturated with MeOH–EtOH and reprecipitated from MeOH, yielding 0.82 g (22%), mp 164–167 °C dec.

pGlu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-*N*-methylhydrazide. A solution of pGlu-His-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-OMe (300 mg, 0.2 mmol) in DMF (10 mL) and methylhydrazine (5 mL) was stirred at 70 °C for 3 days. The reaction mixture was evaporated under reduced pressure to an oily residue, which was triturated with Et₂O to give a crude product, 0.24 g; reprecipitation of the latter from MeOH–Et₂O gave 163 mg (55%) of product, mp 163–168 °C dec.

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-*N*-methylhydrazide (1). The above protected nonapeptide *N*-methylhydrazide (112 mg, 0.075 mmol) was treated with anhydrous HF (5 mL)–anisole (0.5 mL) for 60 min at 0 °C. Hydrogen fluoride was evaporated in vacuo, and the residue was dissolved in a mixture of Et₂O–H₂O. The aqueous extract was applied to a column (1.5 × 8 cm) of ion-exchange resin AG 1-X2 (AcO⁻), and the resin was eluted with H₂O. The eluent was lyophilized, and the product (80 mg) was dissolved in 1% AcOH and subjected to gel filtration on a column of Sephadex G-25 (1.5 × 94 cm). Fractions from the major peak (retention volume 140–162 mL) were lyophilized, and the product obtained was subjected to partition chromatography on a Sephadex G-25 column (2.0 × 72 cm) with solvent system A (*R*_f 0.15), yielding 35 mg of product. The latter was subjected to a second gel filtration, as described above, yielding 22.5 mg (22%) of product by lyophilization: [α]_D²⁴

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-46° (c 1, 1 M AcOH); R_f (A) 0.46, R_f (B) 0.56, R_f (C) 0.69, R_f (D) 0.29. Amino acid analysis: Gly, 1.00; His, 0.91; Tyr, 1.01; Ser, 0.92; Leu, 0.99; Pro, 1.09, Arg, 1.02; Glu, 1.08; Trp, 0.87. Anal. ($C_{54}H_{74}N_{17}O_{12} \cdot 2C_2H_4O_2 \cdot 4H_2O$) C, H, N.

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-OH. pGlu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-O-resin (2.83 g, 1 mmol based on Boc-Pro-resin) was suspended in anisole (2 mL) and treated with hydrogen fluoride (20 mL) at 0 °C for 60 min. Hydrogen fluoride was evaporated in vacuo, and the residual resin was stirred with EtOAc (50 mL), filtered, and dried over P_2O_5 -KOH in vacuo. The dried resin was stirred with H_2O (50 mL) for 5 min, filtered, and washed with H_2O (50 mL). The combined filtrate was applied to a column (1.5 × 15 cm) of ion-exchange resin AG 1-X2 (AcO^-), the resin was washed with H_2O (60 mL), and the eluent was lyophilized, yielding 0.63 g of fluffy powder. The peptide thus obtained was subjected to partition chromatography on a column of Sephadex G-25 (2.4 × 117 cm) with solvent system A. Fractions corresponding to the major peak (R_f 0.21) detected by the Folin Lowry color reaction were evaporated in vacuo and freeze-dried from 1% AcOH to yield 270 mg of product. This was further purified by gel filtration on a Sephadex G-25 column (7.4 × 117 cm) with 1% AcOH. Lyophilization of fractions from the major peak (elution volume 430–490 mL) gave nonapeptide acid, 260 mg (21%); $[\alpha]^{24}_D$ -49° (c 1, 1 M AcOH); R_f (A) 0.41, R_f (B) 0.36, R_f (C) 0.42, R_f (D) 0.23. Amino acid analysis: Glu, 1.0; His, 0.95; Ser, 0.84; Tyr, 1.03; Gly, 1.04; Leu, 0.97; Arg, 1.01; Pro, 1.00; Trp 0.85. Anal. ($C_{54}H_{70}N_{15}O_{13} \cdot 2C_2H_4O_2 \cdot H_2O$) C, H, N. Lyophilization of 1 with 1% HCl led to peptide in which AcOH was displaced by HCl. The peptide dihydrochloride monohydrate was used thereafter for coupling reactions requiring DCC.

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NHNHCSNH₂ (2). A solution of pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-2HCl· H_2O (48.6 mg, 40 μmol), thiosemicarbazide (36.5 mg, 400 μmole and hydroxybenzotriazole hydrate (12.2 mg, 80 μmol) in DMF (0.8 ml) was treated with 1 M TEA in DMF (40 μL, 50 μmol) and 1 M DCC in CH_2Cl_2 (50 μL, 50 μmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and at 20 °C for 2 days, then it was diluted with H_2O (10 ml) and applied to a column (1.5 × 13 cm) of carboxymethylcellulose (H^+). The column was washed with H_2O (80 mL) and the peptide was eluted with 1 M AcOH (40 mL) and isolated by lyophilization. This product was subjected to partition chromatography on a column of Sephadex G-25 (1.5 × 72 cm) with solvent system A. The major peak (R_f 0.20) gave 27.5 mg of product after lyophilization. The latter was purified one more time by gel filtration on a Sephadex G-25 column (2.4 × 119 cm). Fractions corresponding to the main peak (elution volume 445–490 mL) yielded 24.9 mg of 2 after lyophilization: $[\alpha]^{24}_D$ -54° (c 1, 1 M AcOH); R_f (A) 0.52, R_f (B) 0.65, R_f (C) 0.52, R_f (D) 0.34. Amino acid analysis: Glu, 1.01; His, 0.95; Trp, 0.89; Ser, 0.99, Tyr 0.90, Gly, 0.99; Leu, 1.0; Arg, 1.01; Pro 0.92. Anal. ($C_{54}H_{73}N_{18}O_{12}S \cdot 2C_2H_4O_2 \cdot 4H_2O$) C, H, N.

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NHNHCHO (3). To a solution of the nonapeptide acid (36.5 mg, 30 mmol), formic acid hydrazide (9.0 mg, 150 μmol), and hydroxybenzotriazole hydrate (9.2 mg, 60 μmol) in DMF (0.6 mL) was added 1 M TEA in DMF (30 μL, 30 μmol) and 1 M DCC in CH_2Cl_2 (40 μL, 40 μmol) at 0 °C. After stirring for 1 h at 0 °C and 2 days at room temperature the reaction mixture was diluted with H_2O (6 mL) and applied to a column (1.5 × 8 cm) of carboxymethylcellulose (H^+). The column was washed with H_2O (60 mL), and the peptide was eluted with 1 M AcOH and isolated by lyophilization of appropriate fractions. The latter product was purified by partition chromatography in a column of Sephadex G-25 (1.5 × 72 cm) with solvent system A, which after lyophilization of the appropriate fractions (R_f 0.17) yielded 27 mg of product. A final purification of the latter product by gel filtration on a column (2.4 × 117 cm) of Sephadex G-25 (elution volume 428–472 mL) gave analogue 3, 23.5 mg (60%): $[\alpha]^{24}_D$ -59° (c 1, 1 M AcOH); R_f (A) 0.46, R_f (B) 0.63, R_f (C) 0.40, R_f (D) 0.29. Amino acid analysis: His, 0.90; Arg, 1.02; Ser, 0.82; Glu, 0.97; Pro, 0.99; Gly, 1.00; Leu 0.93; Tyr, 1.00; Trp, 0.86. Anal. ($C_{54}H_{72}N_{17}O_{13} \cdot 2C_2H_4O_2 \cdot 5H_2O$) C, H, N.

pGlu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-NHNH₂. Protected nonapeptide resin ester (2.05 g, 0.72 mmol)

was suspended in DMF and treated with anhydrous hydrazine (1 mL), and the reaction mixture was stored at 0 °C for 16 h. The suspension was filtered, the resin was washed with DMF, and the combined filtrate was evaporated to dryness under reduced pressure. The oily residue was dissolved in MeOH (5 mL) and precipitated by the addition of Et_2O , yielding 0.51 g of solid. Reprecipitation of the latter from MeOH- Et_2O yielded 0.31 g (30%) of product, mp 162–166 °C.

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NHNH₂. The above protected nonapeptide hydrazide (0.30 g, 0.2 μmol) was deprotected by treatment with HF (20 mL)-anisole (2 mL) at 0 °C for 60 min. The HF was evaporated in vacuo to an oily residue, which was dissolved in H_2O (25 mL) and Et_2O (20 mL). The aqueous layer was passed through a column (1.5 × 15 cm) of AG1-X2 (AcO^-), which was eluted with H_2O , and the eluent was lyophilized, yielding 0.26 g of product. The latter was fractionated by partition chromatography on a column of Sephadex G-25 (2.0 × 72 cm) with solvent system A. Fractions corresponding to the peak with R_f 0.22 were evaporated to dryness, and the residue was purified by gel filtration on a Sephadex G-25 column (2.4 × 117 cm), yielding 110 mg of homogeneous product: $[\alpha]^{24}_D$ -46° (c 1, 1 M AcOH); R_f (A) 0.50, R_f (B) 0.61, R_f (C) 0.57, R_f (D) 0.36. Amino acid analysis: His, 0.85; Arg, 1.00; Trp, 0.75; Ser, 1.00; Glu, 1.00; Pro, 1.07; Gly, 1.01; Leu, 0.99; Tyr, 0.98. Anal. ($C_{58}H_{72}O_{12}N_{17} \cdot 3C_2H_4O_2 \cdot H_2O$) C, H, N.

pGlu-His-Trp-Ser-Tyr-Leu-Arg-Pro-NHNHCOCH₃ (4). The above nonapeptide hydrazide (41 mg, 30 μmol) was dissolved in H_2O (4 mL), and the pH of the solution was adjusted to 8 with 1 M Na_2CO_3 . To the latter solution was added a solution of 1 M acetic anhydride in dioxane (0.3 mL, 300 μmol) portionwise (100 μL each, at 5-min intervals), with vigorous stirring at 0 °C and keeping the pH of the mixture between 7.5 and 8.5 by occasional addition of 1 M Na_2CO_3 . The reaction mixture was acidified with AcOH to pH 4.5 and freeze-dried. The crude material was first purified by partition chromatography on a Sephadex G-25 column (1.5 × 72 cm) with solvent system A, and the main product, R_f 0.30, was isolated by lyophilization of appropriate fractions. The latter product was purified once more by gel filtration on a Sephadex G-25 column (1.5 × 92 cm) with 1% AcOH (elution volume 117–143 mL). Fractions from the main peak yielded, after lyophilization, 17.2 mg of 4: $[\alpha]^{24}_D$ -55° (c 1, 1 M AcOH); R_f (A) 0.54, R_f (B) 0.64, R_f (C) 0.57, R_f (D) 0.34. Amino acid analysis: His, 0.89; Arg, 1.00; Ser, 0.83; Glu, 0.98; Pro, 1.00; Gly, 1.00; Leu, 0.94; Tyr, 1.00; Trp, 0.84. Anal. ($C_{55}H_{74}N_{17}O_{13} \cdot 2C_2H_4O_2 \cdot 4H_2O$) C, H, N.

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NHNHCOCF₃ (5). Nonapeptide hydrazide triacetate monohydrate (40 mg, 30 μmole was dissolved in H_2O (5 mL), 1 N HCl (0.1 ml) was added, and the solution was lyophilized. The residue was dissolved in DMF (1 mL)-pyridine (0.1 mL) and *p*-nitrophenyl trifluoroacetate (24 mg, 100 μmol) was added. The solution was made slightly basic by the addition of *N*-methylmorpholine. After 4 h the reaction mixture was diluted with H_2O (6 mL), the resulting solution was applied to a column (1.5 × 10 cm) of ion-exchange resin AG 1-X2 (AcO^-), the resin was washed with H_2O (60 mL), and the eluent was lyophilized. The residue obtained was subjected to partition chromatography on a column of Sephadex G-25 (1.5 × 47 cm) with solvent system A. Fractions corresponding to the main peak, R_f 0.31, were pooled and lyophilized, and the residue was purified further by gel filtration on a Sephadex G-25 column (1.5 × 92 cm) with 1% AcOH. Fractions from the central portion of the peptide peak (retention volume 135–152 mL) were pooled and lyophilized, yielding 15 mg of 5 $[\alpha]^{24}_D$ -56° (c 1, 1 M AcOH); R_f (A) 0.76, R_f (B) 0.80, R_f (C) 0.62, R_f (D) = 0.47. Amino acid analysis: His, 0.84; Arg, 0.98; Ser, 0.80; Glu, 1.03; Pro, 1.07; Gly, 1.00; Leu, 0.93; Tyr, 1.00; Trp, 0.87. Anal. ($C_{55}H_{71}N_{17}O_{13}F_3 \cdot 2C_2H_4O_2 \cdot 4H_2O$) C, H, N.

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