

Synthesis of the Phosphinic Analogue of Thyrotropin Releasing Hormone

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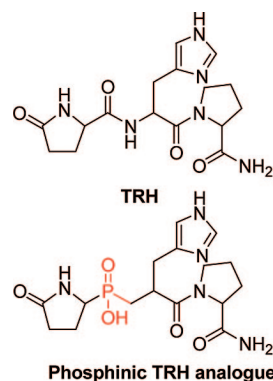
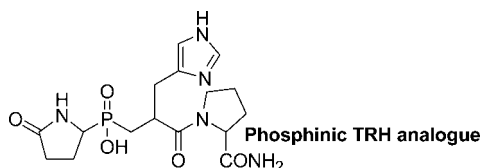


FIGURE 1. TRH and phosphinic TRH analogue.

The synthesis of the phosphinic analogue of thyrotropin releasing hormone (TRH) $\text{Glp}\Psi[\text{P}(\text{O})(\text{OH})]\text{HisProNH}_2$, where the scissile peptide bond of TRH has been replaced by the hydrolytically stable phosphinic bond, has been achieved by a multistep synthetic strategy, providing thus one of the most potent synthetic inhibitors of pyroglutamyl peptidase II (PPII) reported to date (170 nM). The key synthetic step, an Ugi-type condensation reaction, produced directly the suitably protected for solid-phase peptide synthesis pseudodipeptidic block $\text{FmocGlu}(\text{OMe})\Psi[\text{P}(\text{O})(\text{OH})]\text{His}(\text{Tr})\text{OH}$. Formation of the pyroglutamic ring was performed on solid phase, providing thus a general method for synthesizing pyroglutamyl phosphinic peptides on solid phase. Using this strategy, the phosphinic analogue of TRH has been synthesized for the first time.

During the past decade much effort has been expended to produce TRH-related analogues.¹ TRH acts as neuromodulator and neurotransmitter in the central nervous system (CNS) and appears to be involved in a wide variety of physiological activities.^{2–4} TRH is inactivated by pyroglutamyl peptidase II (PPII), or thyrotropin releasing hormone-degrading ectonzyme (TRH-DE) (EC 3.4.19.6), a Zn-peptidase with very high degree of substrate specificity, and

conversely, TRH is the only known physiological substrate of this enzyme. PPII inhibitors may be used to enhance the therapeutic actions of TRH, as well as for the investigation of the biological roles of both TRH and PPII in the CNS. Given the remarkable specificity of PPII for TRH, inhibition of PPII should exclusively affect TRH, and consequently it may offer significant investigative and therapeutic advantages. TRH (Figure 1) is hydrolyzed and thus inactivated by PPII,^{5,6} by cleavage of the peptide bond between pyroglutamic and histidine residues. A rational design of PPII inhibitors would comprise this sequence and a zinc-binding group. Nevertheless, elucidation of the mechanism of action of TRH, identification of its specific features, and separation of its multiple activities through the design of selective inhibitors of PPII have been elusive goals for years, since the design of PPII inhibitors is hampered by the narrow specificity of this enzyme. The usually used zinc-binding groups, such as hydroxamate, thiol, and phosphonamide, proved to be poor inhibitors of PPII.⁷

Phosphinic peptides are an important class of metabolically stable, Zn-metalloprotease inhibitors in which the scissile peptide bond has been replaced by the chemically stable phosphinic moiety, $-\text{P}(\text{O})(\text{OH})\text{CH}_2-$. They are transition state analogues, engaging in several types of interaction with both primed and unprimed active sites of proteases. Consequently, phosphinic peptides act as potent and selective inhibitors for a large number of metalloproteases, such as MMPs,⁸ ACE,⁹ ECE,¹⁰ enkephalin-degrading enzymes, namely NEP and APN,¹¹ neurolysin,¹² APA,¹³ astacin,¹⁴ and others.

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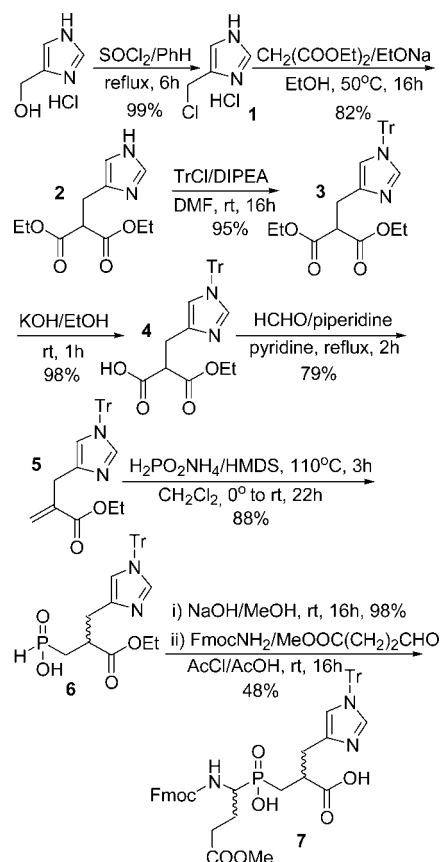
Since PPII is a Zn-metalloprotease and phosphinic peptides have proved to be efficient metalloprotease inhibitors, a suitable strategy to obtain GlpΨ[P(O)(OH)]HisXaa phosphinic pseudopeptides, mimicking not only TRH but also other related hormones such as gonadotropin releasing hormone, luteinizing hormone-releasing hormone, and others, could represent a valuable approach to evaluate their function in health and disease. Therefore, the synthesis of the phosphinic peptide analogue of TRH (Figure 1) could be a promising alternative for both potent and selective inhibition of PPII.

In a previous study concerning the synthesis of phosphinic peptide inhibitors of APA and APN, a strategy for the synthesis of phosphinic peptides containing an aspartyl or glutamyl aminophosphinic acid was developed.¹⁵ The key step of that protocol involved the indirect synthesis of aspartyl- and glutamyl-containing phosphinic peptide analogs, by oxidation of the phenyl group to carboxylic acid by use of a RuCl₃/NaIO₄ system. The primary reason for developing such a strategy was the fact that aspartyl aminophosphinic acid proved to be unreactive toward Michael-type addition to acrylate derivatives, which are precursors of the P₁' phosphinic pseudopeptide residues. The reason for this lack of reactivity has been attributed to an unexpected role played by the neighboring carboxylate group. Obviously, the use of that method is not possible, when other aromatic residues are present, just as in the present case of the phosphinic TRH analogue.

We report herein the first synthetic strategy, which leads to phosphinic TRH analogues. The key step, an Ugi-type three-component condensation reaction produced directly the suitably protected for solid-phase peptide synthesis pseudodipeptidic block FmocGlu(OMe)Ψ[P(O)(OH)]His(Tr)OH **7**. Synthesis of the acrylate precursor of the pseudohistidine residue **5** is also reported here for the first time.¹⁶ Formation of the pyroglutamyl ring was performed on the solid support, providing thus a general method for synthesizing pyroglutamyl phosphinic peptides on solid phase.

We previously described an alternative method for the synthesis of phosphinic pseudodipeptidic blocks that applies a three-component condensation reaction of FmocNH₂, aldehydes, and phosphinic acids.¹⁷ In order to apply this method in the present case of the phosphinic TRH analogue, an efficient synthetic protocol leading to the acrylate precursor **5** of the histidine residue at P₁' should be first developed (Scheme 1). In detail, 4-(hydroxymethyl)imidazole was converted to the corresponding chloride **1**,¹⁸ which was then used for the alkylation of diethylmalonate.¹⁹ The product **2** was protected at τ-N^{im} by Tr to **3** and then selectively saponified to **4**, which provided **5** by Knoevenagel-type condensation²⁰ in 60% overall yield (5 steps). Michael addition of the activated ammonium

SCHEME 1. Synthesis of the Pseudodipeptidic Block FmocGlu(OMe)Ψ[P(O)(OH)]His(Tr)OH **7**



hypophosphorous salt to **5** led to the pseudohistidiny phosphinic acid **6**, which upon hydrolysis and Ugi-type condensation with FmocNH₂ and methyl-4-oxo-butanoate provided the four diastereoisomers of the basic pseudodipeptidic block FmocGlu(OMe)Ψ[P(O)(OH)]His(Tr)OH **7** in moderate yield.¹⁷

Coupling of **7** with the amino terminus of proline, while attached to solid support, removal of the Fmoc group under standard conditions, lactam ring formation upon basic conditions, and finally cleavage from the resin with simultaneous removal of the Trt-group led to the desired phosphinic peptide analogue of TRH **8** (Scheme 2).

In conclusion, an efficient strategy for the synthesis of pyroglutamyl phosphinic peptides is here described, as well as the synthesis of the acrylate P₁' precursor of the histidine residue. The key step is an Ugi-type three-component condensation reaction that produces directly the suitably protected for solid-phase peptide synthesis pseudodipeptidic block FmocGlu(OMe)Ψ[P(O)(OH)]His(Tr)OH. Formation of the pyroglutamic ring is performed on solid phase, providing the phosphinic analogue of TRH **8**, which exhibits an ED₅₀ value of 170 nM toward PPII.²¹ Further biological evaluation is under way.

Experimental Section

2-(1-Trityl-1H-imidazol-4-ylmethyl)-acrylic Acid Ethyl Ester (5). In a solution of **4** (25 g, 55 mmol) in pyridine (160 mL) were

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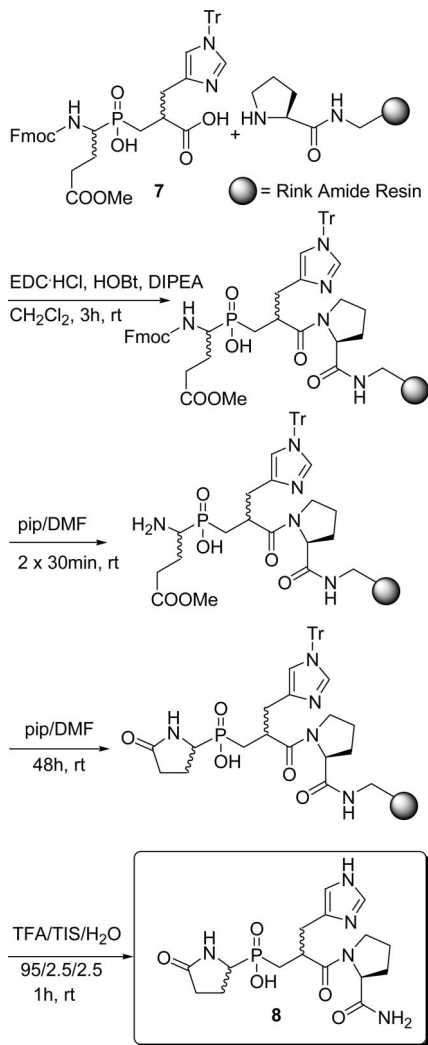
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SCHEME 2. Synthesis of the Phosphinic TRH Analogue



added HCHO (2.145 g, 71.5 mmol) and piperidine (9.37 g, 110 mmol), and the reaction mixture was refluxed until evolution of CO₂ stops (~2 h). Removal of solvent, dissolution in AcOEt, washing with 0.5 M HCl to pH 1 and brine, drying over Na₂SO₄, solvent removal, and column purification with CH₂Cl₂/MeOH = 97:3 afforded **5** (18.4 g, 79%) as a white solid. TLC *R_f* 0.61 (CHCl₃/MeOH = 95:5); ¹H NMR (200 MHz, CDCl₃) δ 1.14 (t, *J* = 7.6 Hz, 3H), 2.75 (t, *J* = 7.4 Hz, 1H), 3.00 (t, *J* = 7.4 Hz, 1H), 4.04 (q, *J* = 7.6 Hz, 2H), 6.03 (s, 1H), 6.27 (s, 1H), 6.71 (s, 1H), 6.98–7.35 (m, 15H), 7.97 (d, *J* = 5.8 Hz, 1H); ¹³C NMR (50 MHz, CDCl₃) δ 14.0, 28.6, 60.7, 77.4, 120.4, 128.4, 128.7, 129.4, 134.1, 135.4, 135.8, 140.2, 165.9; ESMS *m/z* calcd for C₂₈H₂₇N₂O₂ (*M* + 1)⁺ 423.2, found 423.3. HRMS (ES) *m/z* calcd for C₂₈H₂₇N₂O₂ (*M* + 1)⁺ 423.1994, found 423.1831.

3-Hydroxyphosphinoyl-2-(1-trityl-1*H*-imidazol-4-ylmethyl)-propionic Acid Ethyl Ester (6). A suspension of H₂P(O)(O⁻NH₄⁺) (1.18 g, 14.2 mmol) and HMDS (2.29 g, 14.2 mmol) was warmed to 110 °C under Ar for 3 h. After cooling to room temperature, anhydrous CH₂Cl₂ (20 mL) was added, and a solution of **5** (1.2 g, 2.84 mmol) in CH₂Cl₂ (7 mL) was added dropwise at 0 °C. Stirring was continued at room temperature for 22 h, and then the reaction was quenched with EtOH (5 mL). Evaporation of solvent, dissolution in AcOEt (100 mL), washing with H₂O (1 × 10 mL), drying over Na₂SO₄, and solvent removal afforded **6** (1.22 g, 88%) as a white solid. TLC *R_f* 0.58 (CHCl₃/MeOH/AcOH = 70:5:5); HPLC *t_R* 29.2; ¹H NMR (200 MHz, CDCl₃) δ 1.07 (t, *J* = 7.5 Hz, 3H),

1.75–2.17 (m, 2H), 2.99–3.38 (m, 3H), 3.93 (t, *J* = 7.5 Hz, 1H), 6.70 (s, 1H), 7.13 (d, *J* = 538.3 Hz, 1H), 6.98–7.35 (m, 15H), 8.23 (s, 1H); ¹³C NMR (50 MHz, CDCl₃) δ 14.0, 23.8, 31.1, 34.0, 34.8, 40.8, 60.1, 118.8, 127.8, 129.6, 138.3, 138.7, 142.3, 175.7; ³¹P NMR (81 MHz, CDCl₃) δ 23.8; ESMS *m/z* calcd for C₂₈H₃₀N₂O₄P (*M* + 1)⁺ 489.2, found 489.4.

4-[[2-Carboxy-3-(1-trityl-1*H*-imidazol-4-yl-propyl)-hydroxy-phosphinoyl-4-(9*H*-fluoren-9-ylmethoxycarbonylamino)-butyric Acid Methyl Ester (7). In a solution of **6** (200 mg, 0.434 mmol) in AcCl (7 mL) and AcOH (0.7 mL) were added FmocNH₂ (104 mg, 0.434 mmol) and 4-oxo-butyl acid methyl ester (55 mg, 0.477 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 16 h. Evaporation of solvents and column purification with CHCl₃/MeOH/AcOH = 90:3:3 afforded **7** (166 mg, 48%) as a white solid. TLC *R_f* 0.52 (CHCl₃/MeOH/AcOH = 70:5:5); HPLC *t_R* 41.9/42.4; ¹H NMR (600 MHz, CDCl₃) δ 1.82–2.13 (m, 4H), 2.28–2.71 (m, 4H), 3.21–3.37 (m, 1H), 3.48–3.75 (m, 4H), 4.15–4.36 (m, 4H), 5.67–5.82 (m, 1H), 6.71–6.82 (m, 1H), 7.12–7.18 (m, 6H), 7.32–7.45 (m, 14H), 7.55–7.65 (m, 2H), 7.74–7.82 (m, 2H), 8.02–8.19 (m, 1H); ¹³C NMR (50 MHz, CDCl₃) δ 24.0, 27.1, 28.9, 30.7, 30.9, 38.8, 39.3, 47.0, 49.5, 49.0, 51.2, 51.4, 66.9, 78.0, 119.8, 121.2, 127.0, 127.5, 127.5, 128.6, 128.9, 129.5, 132.6, 133.2, 135.7, 136.1, 140.0, 141.1, 143.8, 156.8, 173.7, 175.8; ³¹P NMR (81 MHz, CDCl₃) δ 37.9; ESMS *m/z* calcd for C₄₆H₄₅N₃O₈P (*M* + 1)⁺ 798.3, found 798.3. HRMS (ES) *m/z* calcd for C₄₆H₄₅N₃O₈P (*M* + 1)⁺ 798.2866, found 798.2662.

[3-(2-Carbamoyl-pyrrolidin-1-yl)-2-(1*H*-imidazol-4-ylmethyl)-3-oxo-propyl]-(5-oxo-pyrrolidin-2-yl)-phosphinic Acid (8). Synthesis was carried out on pins with a Rink-type linker (SynPhase Lanterns, L-series pins, loading 15 μmol per pin, Mimotopes, France). Proline coupling was performed as previously described in detail.¹⁷ For phosphinic block coupling, a solution of **7** (1.1 equiv) is added to pins in dry CH₂Cl₂ (0.3 mL/pin) containing DIPEA (2.1 equiv), HOBt (1.1 equiv), and EDC·HCl¹⁷ (5 equiv). After 16 h at room temperature, the reaction mixture was decanted, and the pins were washed with CH₂Cl₂ (2 × 5 min, 1 mL/pin), DMF (2 × 5 min, 1 mL/pin), and CH₂Cl₂ (2 × 5 min, 1 mL/pin). The coupling step was repeated if necessary, until negative chloranil test. Removal of the Fmoc group was accomplished by 20% piperidine/DMF (1 mL/pin, 2 × 30 min, rt) and washings. Lactam formation was by addition of pip/DMF 2:1 (1 mL/pin), until negative Kaiser test (ca. 48 h) at room temperature. Washings were as generally. Cleavage from pins was by TFA/TIS/H₂O 95:2.5:2.5 (1 mL/pin). Concentration in vacuo, precipitation with dry Et₂O, and lyophilization afforded **8** (50 mg for 10 pins, 84%) as a white solid. TLC *R_f* 0.21 (CH₃CN/H₂O = 1:1); HPLC *t_R* 4.2/5.0/6.4; ¹H NMR (200 MHz, CD₃OD) δ 1.61–2.45 (m, 8H), 2.82–4.05 (m, 8H), 4.22–4.49 (m, 1H), 7.22–7.41 (m, 1H), 8.72–8.81 (m, 1H); ¹³C NMR (50 MHz, CD₃OD) δ 21.9, 22.1, 22.3, 23.1, 23.7, 23.9, 25.3, 25.6, 26.0, 29.3, 31.0, 31.3, 33.3, 38.7, 45.7, 61.2, 61.4, 62.5, 118.3, 118.9, 127.3, 129.3, 130.5, 135.0, 172.5, 177.1, 177.6, 177.8, 181.4; ³¹P NMR (81 MHz, CD₃OD) δ 38.4/40.0/40.5; ESMS *m/z* calcd for C₁₆H₂₅N₅O₅P (*M* + 1)⁺ 398.2, found 398.1. HRMS (ES) *m/z* calcd for C₁₆H₂₅N₅O₅P (*M* + 1)⁺ 398.1515, found 398.1261.

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Supporting Information Available: General considerations; full experimental procedures for compounds **1–4**; ¹H, ¹³C, and ³¹P NMR and HPLC chromatograms for compounds **6–8**; and HRMS copies for compounds **5**, **7**, and **8**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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