Facile Synthesis of *N*-α-Boc-1,2-Dialkyl-L-Histidines: Utility in the Synthesis of Thyrotropin-Releasing Hormone (TRH) Analogs and Evaluation of the CNS Activity

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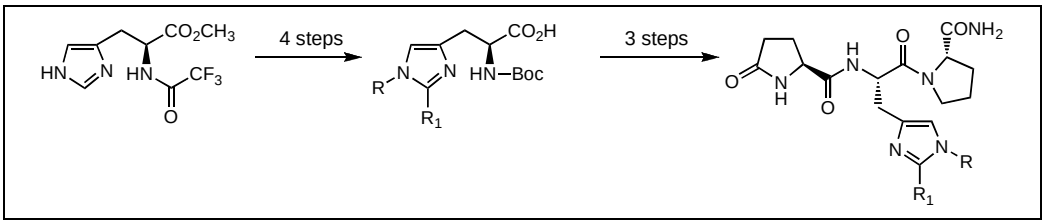

A facile synthesis of *N*-α-Boc-1,2-dialkyl-L-histidines starting from *N*-α-trifluoroacetyl-L-histidine methyl ester is reported. The key steps involve direct and regiospecific N-1(τ) ring-alkylation of the *N*-α-trifluoroacetyl-L-histidine-methyl ester by suitable alkyl iodide in the presence of NaH in DMF at -15 °C followed by homolytic free radical C-2 alkylation *via* a silver catalyzed oxidative decarboxylation of alkylcarboxylic acid in the presence of ammonium persulfate under acidic conditions. The application of newly synthesized bioimidazoles was illustrated by their incorporation into thyrotropin-releasing hormone (TRH). The synthesized TRH analogs were evaluated *in vivo* for analeptic activity. We report discovery of a TRH analog, which was found to potentiate the pentobarbital-induced sleep *in vivo*.

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INTRODUCTION

Unnatural and synthetically modified α-amino acids, utilized as building blocks, conformational constraints, molecular scaffolds or pharmacologically active products, represent a nearly infinite array of diverse structural elements for the development of new leads in peptide and non-peptide compounds [1-6]. Small-molecule combinatorial libraries containing unnatural α-amino acid residues have shown remarkable impact on drug discovery processes [7,8]. For example, it is well established that incorporation of unnatural and synthetic α-amino acids in peptide analogs results in enhanced enzymatic stability and improved pharmacological, pharmacodynamics and bioavailability properties [9-13].

The physicochemical properties of the side-chain imidazole ring, its acid-base characteristics, aromaticity, hydrogen bond donor/acceptor properties and ring tautomerism makes histidine a unique DNA encoded α-amino acid. Histidine plays a critical role in the biological activities of many enzymes, peptide hormones, neuropeptides and peptide antibiotics [14]. One such peptide, thyrotropin-releasing hormone **1** (TRH, L-pGlu-L-His-L-ProNH_2) (Fig. 1), is synthesized in the hypothalamus [15], operates in the anterior pituitary and control levels of TSH (thyroid-stimulating hormone) and prolactin [16]. The same peptide appears to be involved in a wide variety of physiological activities [17,18]. The neuroendocrine action of TRH on the anterior pituitary (PIT), where it initiates the secretion of TSH and prolactin, dominated the earlier research. However, TRH also exhibit profound effect on the central nervous system (CNS). It exhibit antidepressant, analeptic and neuroprotective effects and is considered a promising compound for the treatment of epilepsy, motor neuron diseases, spinal cord trauma, and Alzheimer's disease [19-21].

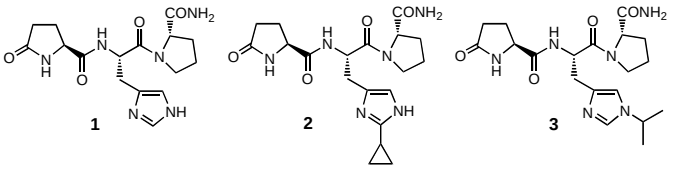


Figure 1. Structure of thyrotropin-releasing hormone (TRH, **1**) and promising analogs **2**-**3**

RESULTS AND DISCUSSION

At present, identity of two TRH receptor subtypes (TRH-R1 and TRH-R2) is known [22-26]. We have recently observed that placement of a hydrophobic alkyl group at the C-2 position of the imidazole ring of the histidine residue resulted in a highly selective agonist **2** (Fig. 1) for TRH-R2 (EC_{50} = 0.41 μM) exhibiting no activation of TRH-R1 (EC_{50} >100 μM) [27]. In continuation, we have reported the synthesis of TRH analogs in which the N-1(τ) position of the imidazole ring of the histidine residue is substituted with various alkyl groups. The most selective analog **3** (Fig. 1) was found to

activate TRH-R2 with potency (EC_{50} = 0.018 μM) but could only activate TRH-R1 at higher concentration (EC_{50} = 1.6 μM); that is, exhibited 88-fold greater selectivity for TRH-R2 [28]. These studies indicate that any change on His residue in TRH profoundly alters its receptor binding affinity and signal transducing potency.

Based upon the tissue distribution of TRH-R1 and TRH-R2, it has been speculated that TRH-R1 is mainly responsible for the endocrine activity of the TRH; whereas, TRH-R2 is involved in its CNS-mediated effects [27]. To validate the above-mentioned observations, we have evaluated analogs **2**–**3** for their analeptic activity using pentobarbital-induced sleep model. As shown in Table 1, the sleeping time after the administration of vehicle and analogs **2**–**3** were 89.88±6.8, 23.5±2.16, and 58.22±5.71 min, respectively. Analog **2** showed a significant decrease in the sleeping time as compared to vehicle as well as TRH (52.6±4.02 min); this indicates analog **2** as a promising analeptic agent confirming the above mentioned hypothesis. While, effect of analog **3** was found to be similar to TRH.

The interesting results obtained for TRH analogs **2**–**3** prompted us to undertake synthesis and *in vivo* analeptic activity evaluation of a hybrid TRH analog **12b** (Fig. 2), in which N-1(τ) position of histidine is substituted with an isopropyl group and C-2 position with a cyclopropyl group. Therefore, we required a synthetic route to *N*-α-Boc-1,2-dialkyl-L-histidines. Previously, we have reported the synthesis of 1,2-dialkyl-L-histidines starting from commercially available L-histidine methyl ester [29]. However, the synthesis of *N*-α-Boc-1,2-dialkyl-L-histidines using this six steps strategy would involve the formation of difficulty isolated imidazolium quaternary compounds in low yields and requires costly 1,1'-carbonyldiimidazole as a reagent.

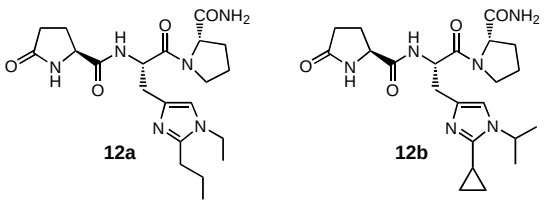
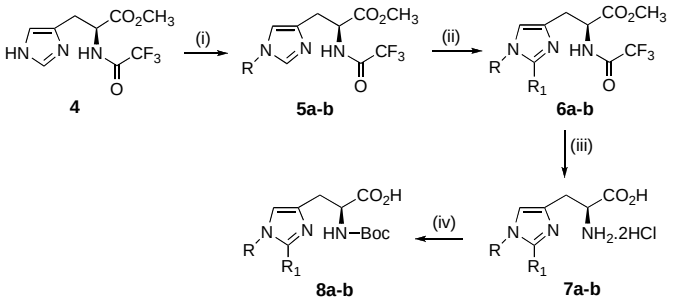


Figure 2. Structure of synthesized TRH analogs

We set our task to develop a feasible approach to these important scaffolds and report herein a facile and high yielding synthetic route to *N*-α-Boc-1,2-dialkyl-L-histidines starting from commercially available *N*-α-trifluoroacetyl-L-histidine methyl ester in four steps. The key steps of the procedure would involve direct and specific N-1(τ) ring alkylation of the *N*-α-trifluoroacetyl-L-histidine methyl ester by various alkyl iodides in the presence of NaH in DMF at -15 °C followed by a free radical initiated regiospecific C-C bond formation reaction [30-33]. The synthesized *N*-α-Boc-2-cyclopropyl-1-isopropyl-L-histidine was successfully used in the synthesis of TRH analog **12b** (Fig. 2) using solution phase peptide chemistry. The synthetic usefulness of the newly developed route to *N*-α-Boc-1,2-dialkyl-L-histidines was further established by synthesizing another TRH analog **12a** (Fig. 2).

The first step of synthesis involve N-1(τ) alkylation of the *N*-α-trifluoroacetyl-L-histidine methyl ester (**4**) by alkyl iodides in the presence of NaH in DMF at -15 °C to provide *N*-1(τ)-alkyl-*N*-α-trifluoroacetyl-L-histidine methyl esters (**5a**–**b**).

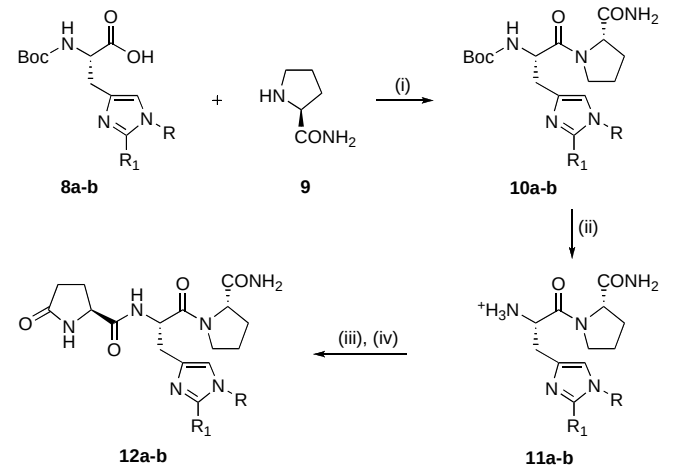
Scheme 1



Reagents and conditions: (i) NaH, RI, DMF, -15 °C to -5 °C; (ii) R_1CO_2H, $AgNO_3$, 10% H_2SO_4, $(NH_4)_2S_2O_8$, 70-80 °C; (iii) 6N HCl, reflux, 24 h; (iv) Boc_2O, 4N NaOH, 24 h, rt.

The latter compounds **5a**–**b** upon radical alkylation with suitable alkylcarboxylic acid and silver nitrate in the presence of 10% H_2SO_4 and ammonium persulfate at 70-80 °C for 15 min afforded fully protected 1,2-dialkyl-L-histidine derivatives **6a**–**b** (Scheme 1). Reaction involves nucleophilic addition of an alkyl radical (generated by the silver catalyzed oxidative decarboxylation of alkylcarboxylic acid with ammonium persulfate) to a protonated imidazole ring followed by re-aromatization leading to direct C-2 alkylation.

Scheme 2



Reagents and conditions: (i) DIC, HONB, DMF, 4 °C, 36 h; (ii) 40% TFA, rt, 20 min; (iii) 7N NH_3 in MeOH, 10 min, rt; (iv) pGlu-OTcp, DMF, 4 °C; 36 h.

A substantially lower (15% yield) was obtained for compound **6b**. This is presumably due to the decreased stability of the *in situ* generated cyclopropyl radical under the reaction conditions. Deprotection of **6a–b** by refluxing a solution of them in 6 *N* HCl produced 1,2-dialkyl-L-histidine dihydrochloride salts **7a–b**, which upon reaction with di-*tert*-butyl dicarbonate (Boc_2O) in the presence of 4 *N* NaOH solution at ambient temperature in a mixture of dioxane-water (2:1) for 24 h provided *N*-α-Boc-1,2-dialkyl-L-histidines **8a–b** (Scheme 1) in four overall steps [34].

The solution phase synthesis of the requisite TRH peptides was accomplished by first coupling L-prolinamide (**9**) with **8a–b** in the presence of DIC and HONB at 4 °C for 36 h to afford Boc-protected dipeptides **10a–b**. The latter compounds **10a–b** upon acidic hydrolysis with trifluoroacetic acid (40% solution in DCM) at ambient temperature for 20 min produced dipeptide trifluoroacetate salts **11a–b**. The free dipeptides were generated *in situ* by treatment of **11a–b** with a solution of NH_3 (7 *N* in MeOH) for 10 min. Complete removal of solvent followed by coupling of the free dipeptides with the 2,4,5-trichlorophenyl ester of pyroglutalmic acid (L-pGlu-OTcp) in DMF at 4 °C for 36 h produced requisite TRH analogs **12a–b** (Fig. 2, Scheme 2).

The synthesized TRH analogs were evaluated *in vivo* by pentobarbital-induced sleeping time model as described by Wu *et al.* [35]. Briefly, albino Swiss mice (body weight, 20-30 gm) were procured from Central Animal Facility of the Institute. They were provided food and water *ad libitum*, and were maintained at room temperature (22±2°C) with 12-hour light/dark cycle. All the experimental procedures were approved by Institutional Animal Ethics Committee prior to experimentation. Test compounds were dissolved in vehicle (saline). Vehicle or TRH analogs were injected intravenously at a dose of 10 μmol/kg (equivalent to 3.7 mg/kg of TRH). Ten minutes after treatment, each mouse received 50 mg/kg sodium pentobarbital intraperitoneally. The sleeping time was recorded as the time elapsed from the onset of loss of righting reflex until it was gained back. The sleeping time in treatment groups was compared with control group by One-Way Analysis of Variance (ANOVA) followed by *post hoc* Tukey test at $P<0.05$.

As shown in Table 1, the sleeping time after administration of vehicle and analogs **12a–b** were 89.88±6.8, 97.11±4.8, and 187.62±5.9 min, respectively. Analog **12b** resulted in significant increase in sleeping time whereas; analog **12a** did not produce any significant change. These results are in direct contrast to those obtained for parent analogs **2–3**. Increase in pentobarbital sleeping time by **12b** may be ascribed to the alternations in TRH receptor interactions connected with the specific properties of the substitution on the His residue of TRH analog. This altered interaction may potentiate GABA mediated postsynaptic inhibition through an allosteric modulation of GABA receptors or enhancement in K^+ conductance or inhibition in Ca^{2+} conductance in neurons as pentobarbital itself known to potentiate GABA activity [36-38], increase the K^+ conductance and inhibition in Ca^{2+} conductance [39-41]. TRH mediated increased cholinergic activity plays a significant role in the reduction of sodium pentobarbital induced sleeping [42,43].

Table 1

A comparison of the pentotobarbital induced sleeping time in mice after the administration of vehicle and TRH analogs.[a]

Compounds	Sleeping Duration in min (Mean ± SEM)
Vehicle	89.88±6.8
TRH	52.6±4.02[b]
2	23.5±2.16[b,c]
3	58.22±5.71[b]
12a	97.11±4.8
12b	187.62±5.9[b,c]

[a]Ten min after i.v. injection of the TRH/TRH analogs, pentobarbital (50mg/kg) was injected intraperitoneally in the mice. The sleeping time was recorded as the time elapsed from the onset of loss to regain the righting reflex. Six to eight Swiss albino mice (body weight, 20-30 g) were used in each group. Values are expressed Mean ± SEM. [b]$p < 0.05$ when compared to vehicle using one way ANOVA followed by post hoc Tukey Test. [c]$p<0.05$ when compared to TRH using one way ANOVA followed by post hoc Tukey Test.

In addition to above postulated mechanisms, **12b** may also attenuate cholinergic activity resulting in potentiation of sodium pentobarbital induced sleeping. When the analog **12b** was tested at the same dose in the absence of pentobarbital, it significantly decreased open field locomotor activity (1719±243 versus vehicle 2545±203, $p< 0.05$, compared using t test) recorded using Optovarimex (Columbus Instruments, USA). Such an effect could be compared to that of diazepam, which causes anxiolytic or sedative effects [44]. These interesting pharmacological results describes the first TRH analog **12b** having CNS depressant effect as caused by potentiation of pentobarbital-induced sleeping time duration and reduction in locomotor activity. These effects may be attributed to potentiation of GABA mediated inhibition as well as K^+ conductance or attenuation of cholinergic activity.

In the present study we have developed a facile route for the synthesis of *N*-α-Boc-1,2-dialkyl-L-histidines. This synthetic procedure provides modified histidines in four steps. In addition to the synthesis of targeted 1,2-dialkyl-L-histidine analog **8b** [R = $CH(CH_3)_2$, R_1 = *c*-C_3H_5], we have also demonstrated the practical utility of this methodology by synthesizing another *N*-α-Boc-1,2-dialkyl-L-histidine **8a** [R = C_2H_5, R_1 = C_3H_7]. This synthetic route could also be easily extended to a wide variety of *N*-α-Boc-1,2-dialkyl-L-histidines. The synthe-

sized bioimidazoles were successfully incorporated in the designed of TRH analogs **12a–b**. Both TRH analogs were examined for their analeptic action using pentobarbital-induced sleep time model. We report the discovery of a TRH analog **12b**, which enhanced the pentobarbital-induced sleep time and decreased the motor activity *in vivo*.

EXPERIMENTAL

Compounds were routinely checked for their purity on pre-coated silica gel G_{254} TLC plates (Merck) and the spots were visualized under UV spectrophotometer and then by exposing them to iodine vapors. Column chromatographic purification was carried out on Merck silica gel (230–400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on 300 MHz Bruker FT–NMR (Avance DPX 300) spectrometer using tetramethylsilane (TMS) as internal standard and the chemical shifts are reported in δ units. Mass spectra were recorded on HRMS (Finnigan Mat LCQ spectrometer) (APCI mode). Elemental analyses were recorded on Elementar Vario EL spectrometer. Optical rotations were recorded on a Perkin-Elmer 241MC polarimeter. All final peptides were also checked for their homogeneity on a Shimadzu LS10AT HPLC system using a Merck Lichrospher® 100 RP-18 (10 μm) column. The samples were analyzed using a solvent system of CH_3CN–H_2O–TFA (95:5:0.05%) at a flow rate of 1 mL/min. Following abbreviations were used: pGlu, pyroglutamic acid; His, Histidine; pGlu-OTcp, 2,4,5-trichlorophenyl pyroglutamate; ProNH_2, Prolinamide; DIC, 1,3-diisopropylcarbodiimide; HONB, *endo-N*-hydroxy-5-norbornene-2,3-dicarboximide; DCM, (dichloromethane); DIEA, *N,N'*-diisopropylethylamine; TFA, trifluoroacetic acid..

Typical procedure for the synthesis of *N*-α-trifluoroacetyl-1-alkyl-L-histidine methyl esters (5a–b). Sodium hydride (60% suspension, 11.7 mmol) was placed in a two-necked flask, washed with hexanes (2 × 10 mL) and solvent removed under reduced pressure. *N*-α-Trifluoroacetyl-L-histidine methyl ester (**4**, 3.9 mmol) in DMF (20 mL) was added under a nitrogen atmosphere at -15 °C. The reaction mixture was stirred for another 30 min at -15 °C, and then alkyl iodide (7.8 mmol) was added. The temperature of the reaction was raised to -5 °C, and the reaction was stirred for 4 h under N_2. The reaction was quenched by addition of methanol (5 mL) and residue was extracted with chloroform (4 × 50 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel (230–400 mesh) column chromatography eluting with 13% CH_3OH in CH_2Cl_2 to afford *N*-α-trifluoroacetyl-1-alkyl-L-histidine methyl esters **5a–b**.

***N*-α-Trifluoroacetyl-1-ethyl-L-histidine methyl ester (5a)**. Yield: 57%; oil; ^{1}H nmr ($CDCl_3$): δ 8.97 (bs, 1H, NH), 7.41 (s, 1H, Ar-H), 6.71 (s, 1H, Ar-H), 4.80 (m, 1H, α-CH), 3.93 (q, 2H, N-CH_2, *J* = 6.0 Hz), 3.70 (s, 3H, OCH_3), 3.19 (m, 1H, CH), 3.05 (m, 1H, CH), 1.43 (t, 3H, CH_3, *J* = 6.0 Hz); MS (APCI): *m/z* 294 (M+1); R_f = 0.50 [$CH_3OH/CHCl_3$ (0.6:9.4)]; $[\alpha]_D^{25}$ +5° (*c* 1.00, $CHCl_3$); *Anal.* Calcd. for $C_{11}H_{14}F_3N_3O_3$: C, 45.05; H, 4.81; N, 14.33. Found: C, 44.90; H, 4.98; N, 14.15.

***N*-α-Trifluoroacetyl-1-isopropyl-L-histidine methyl ester (5b).** Yield: 37%; oil; ^{1}H nmr ($CDCl_3$): δ 8.97 (bs, 1H, NH), 7.93 (s, 1H, Ar-H), 6.68 (s, 1H, Ar-H), 4.71 (m, 1H, α-CH), 4.20 (m, 1H, CH), 3.62 (s, 3H, OCH_3), 3.10 (m, 1H, CH), 2.97 (m, 1H, CH), 1.37 (m, 6H, 2 × CH_3); MS (APCI): *m/z* 308 (M+1); R_f = 0.75 [$CH_3OH/CHCl_3$ (0.6:9.4)]; $[\alpha]_D^{25}$ +8° (*c* 1.00, $CHCl_3$); *Anal.* Calcd. for $C_{12}H_{16}F_3N_3O_3$: C, 46.91; H, 5.25; N, 13.68. Found: C, 47.21; H, 5.66; N, 13.47.

Typical procedure for the synthesis of *N*-α-trifluoroacetyl-1,2-dialkyl-L-histidine methyl esters (6a–b). *N*-α-Trifluoroacetyl-1-alkyl-L-histidine methyl ester (**5a–b**, 1 mmol), was added to silver nitrate (0.6 mmol) and alkylcarboxylic acid (3 mmol) in a mixture of 10% H_2SO_4 (5 mL) and acetonitrile (5 mL). Reaction mixture was heated to 70-80 °C. A freshly prepared solution of ammonium persulfate (3 mmol) in water (10 mL) was added slowly during 5 min. The heating source was removed and after 10 min, the reaction was terminated by pouring it onto ice. The resulting mixture was made alkaline with 30% NH_4OH solution and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with brine (2 × 10 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford oil, which upon chromatography over silica using ethyl acetate/hexanes (4:6) as eluant gave **6a–b**.

***N*-α-Trifluoroacetyl-1-ethyl-2-propyl-L-histidine methyl ester (6a).** Yield: 30%; oil; ^{1}H nmr ($CDCl_3$): δ 9.64 (bs, 1H, NH), 6.60 (s, 1H, Ar-H), 4.75 (m, 1H, α-CH), 3.81 (q, 2H, N-CH_2, *J* = 6.0 Hz), 3.67 (s, 3H, OCH_3), 3.12 (m, 1H, CH), 2.97 (m, 1H, CH), 2.56 (t, 2H, CH_2, *J* = 6.0 Hz), 1.77 (m, 2H, CH_2) 1.43 (t, 3H, CH_3, *J* = 6.0 Hz), 0.97 (t, 3H, CH_3, *J* = 6.0 Hz); MS (APCI): *m/z* 308 (M+1); R_f = 0.35 [EtOAc/hexanes (9:1)]; $[\alpha]_D^{25}$ +14.2° (*c* 1.00, $CHCl_3$); *Anal.* Calcd. for $C_{14}H_{20}F_3N_3O_3$: C, 50.15; H, 6.01; N, 12.53. Found: C, 50.45; H, 6.32; N, 12.33.

***N*-α-Trifluoroacetyl-2-cyclopropyl-1-isopropyl-L-histidine methyl ester (6b).** Yield: 15%; oil; ^{1}H nmr ($CDCl_3$): δ 9.58 (bs, 1H, NH), 6.65 (s, 1H, Ar-H), 4.69 (m, 1H, α-CH), 4.52 (m, 1H, CH), 3.66 (s, 3H, OCH_3), 3.08 (m, 1H, CH), 2.95 (m, 1H, CH), 1.73 (m, 1H, CH), 1.41 (m, 6H, 2 × CH_3), 1.25 (m, 4H, 2 × CH_2); MS (APCI): *m/z* 348 (M+1); R_f = 0.5 [EtOAc/hexanes (9:1)]; $[\alpha]_D^{25}$ +10.5° (*c* 1.00, $CHCl_3$); *Anal.* Calcd. for $C_{15}H_{20}F_3N_3O_3$ (347.33): C, 51.87; H, 5.80; N, 12.10. Found: C, 52.08; H, 5.98; N, 11.87.

Typical procedure for the synthesis of 1,2-dialkyl-L-histidine.2HCl (7a–b). A solution of *N*-α-trifluoroacetyl-1,2-dialkyl-L-histidine methyl ester (**6a–b**, 1 mmol) in 6 *N* HCl (20 mL) was heated at 100 °C for 24 h. The dihydrochloride salts of 1,2-dialkyl-L-histidines **7a–b** were obtained directly by evaporation of the acidic hydrolysis solution.

1-Ethyl-2-propyl-L-histidine.2HCl (7a). Yield: 96%; mp. 190-190 °C (dec.); ^{1}H nmr (CD_3OD): δ 7.51 (s, 1H, Ar-H), 4.37 (m, 1H, α-CH), 4.18 (q, 2H, N-CH_2, *J* = 6.0 Hz), 3.40 (m, 2H, CH_2), 2.98 (t, 2H, CH_2, *J* = 6.0 Hz), 1.83 (m, 2H, CH_2), 1.48 (t, 3H, CH_3, *J* = 6.0 Hz), 1.06 (t, 3H, CH_3, *J* = 6.0 Hz); MS (APCI): *m/z* 226 (M+1); $[\alpha]_D^{25}$ -14.5° (*c* 1.00, H_2O); *Anal.* Calcd. for $C_{11}H_{21}Cl_2N_3O_2$ (298.21): C, 44.30; H, 7.10; N, 14.09. Found: C, 44.64; H, 6.84; N, 14.23.

2-Cyclopropyl-1-isopropyl-L-histidine.2HCl (7b). Yield: 94%; mp. 175-176 °C (dec.); ^{1}H nmr (CD_3OD): δ 7.54 (s, 1H, Ar-H), 4.93 (m, 1H, α-CH), 4.35 (m, 1H, CH), 3.34 (m, 2H, CH_2), 2.29 (m, 1H, CH), 1.55 (m, 6H, 2 × CH_3) 1.25 (m, 4H, 2 × CH_2); MS (APCI): *m/z* 238 (M+1); $[\alpha]_D^{25}$ -17.2° (*c* 1.00, H_2O); *Anal.* Calcd. for $C_{12}H_{21}Cl_2N_3O_2$ (310.22): C, 46.46; H, 6.82; N, 13.55. Found: C, 46.62; H, 6.61; N, 13.28.

Typical procedure for the synthesis of *N*-α-Boc-1,2-dialkyl-L-histidines (8a–b). 1,2-dialkyl-L-histidine.2HCl (**7a–b**, 1 mmol) was suspended in a mixture of water/dioxane (1:2, 10 mL). A solution of 4 *N* NaOH was added and pH of the

reaction mixture was adjusted to 12. After 10 min, di-*tert*-butyl dicarbonate (2 mmol) was added to the reaction mixture in one portion. Reaction mixture was stirred at ambient temperature for 24 h. The complete removal of solvent under reduced pressure followed by treatment of resulting *N*-α-Boc-His(1,2-dialkyl)-O-Na^+ with saturated aqueous solution of $KHSO_4$ to pH 3.75 generated the *N*-α-Boc-1,2-dialkyl-L-histidine. The solvent was removed under reduced pressure and the product was obtained from the resulting residue by extraction with *tert*-butyl alcohol (4 × 50 mL). Evaporation of solvent afforded *N*-α-Boc-1,2-dialkyl-L-histidines **8a–b**.

***N*-α-Boc-1-ethyl-2-propyl-L-histidine (8a).** Yield: 70%; mp. 102-103 °C (dec.); ^{1}H nmr (CD_3OD): δ 7.17 (s, 1H, Ar-H), 4.17 (m, 1H, α-CH), 4.08 (q, 2H, CH_2, *J* = 6.0 Hz), 3.08 (m, 1H, CH), 2.89 (m, 3H, CH_2, CH), 1.77 (m, 2H, CH_2), 1.42 (m, 12H, 4 × CH_3), 1.03 (t, 3H, CH_3, *J* = 6.0 Hz); MS (APCI): *m/z* = 326 (M+1); R_f = 0.35 [CH_3OH/25%NH_4OH/$CHCl_3$ (2:0.2:7.8)]; $[\alpha]_D^{25}$ +12° (*c* 1.00, CH_3OH); *Anal.* Calcd. for $C_{16}H_{27}N_3O_4$ (325.40): C, 59.06; H, 8.36; N, 12.91. Found: C, 58.88; H, 8.62; N, 12.78.

***N*-α-Boc-2-cyclopropyl-1-isopropyl-L-histidine (8b).** Yield: 75%; mp. 91-92 °C (dec.); ^{1}H nmr (CD_3OD): δ 7.20 (s, 1H, Ar-H), 4.82 (m, 1H, α-CH), 4.20 (m, 1H, CH), 3.06 (m, 1H, CH), 2.86 (m, 1H, CH), 2.13 (m, 1H, CH), 1.49 (d, 6H, 2 × CH_3, *J* = 6.0 Hz), 1.39 (s, 9H, 3 × CH_3), 1.19 (m, 2H, CH_2), 1.05 (m, 2H, CH_2); MS (APCI): *m/z* 338 (M+1); R_f = 0.4 [CH_3OH/25% NH_4OH/$CHCl_3$ (2:0.2:7.8)]; $[\alpha]_D^{25}$ +15.4° (*c* 1.00, CH_3OH); *Anal.* Calcd. for $C_{17}H_{27}N_3O_4$ (337.41): C, 60.51; H, 8.07; N, 12.45. Found: C, 60.30; H, 8.22; N, 12.65.

Typical procedure for the synthesis of *N*-α-Boc-(1,2-dialkyl)-L-His-L-ProNH$_2$ (10a–b). To a solution of *N*-α-Boc-1,2-dialkyl-L-histidine (**8a–b**, 1 mmol) in anhydrous DMF (10 mL) was added HONB (1.1 mmol). The reaction mixture was cooled to –10 °C and 1,3-diisopropylcarbodiimide (DIC, 1.1 mmol) was added in one portion. Reaction mixture was stirred for additional 5 min at -10 °C. L-ProNH$_2$ (**9**, 1 mmol) was then added to the reaction mixture and stirring continued for additional 36 h at 4 °C. Solvent was evaporated under reduced pressure and the crude product was purified by column chromatography using MeOH/$CHCl_3$ (10:90) as eluant to afford dipeptides **10a–b**.

***N*-α-Boc-(1-ethyl-2-propyl)-L-His-L-Pro-NH$_2$ (10a).** Yield: 57%; mp. 77-78 °C (dec.); ^{1}H nmr ($CDCl_3$): δ 9.28 (bs, 1H, NH), 6.69 (s, 1H, Ar-H), 4.56 (m, 2H, 2 × CH), 3.82 (q, 2H, CH_2, *J* = 6.0 Hz), 3.53 (m, 1H, CH) 3.11 (m, 1H, CH), 2.78 (m, 1H, CH), 2.51 (m, 3H, CH, CH_2), 2.17 (m, 2H, CH_2), 1.74 (m, 4H, 2 × CH_2) 1.38 (m, 12H, 4 × CH_3), 0.96 (m, 3H, CH_3); ^{13}C nmr ($CDCl_3$): δ 175.43, 172.05, 155.64, 148.24, 135.67, 116.83, 80.16, 61.14, 53.19, 47.80, 40.99, 32.82, 30.62, 29.00, 28.80, 25.14, 22.83, 16.83, 14.43; MS (APCI): *m/z* 422 (M+1); R_f = 0.5 [CH_3OH/$CHCl_3$ (1:9)]; *Anal.* Calcd. for $C_{21}H_{35}N_5O_4$ (421.53): C, 59.83; H, 8.37; N, 16.61. Found: C, 60.10; H, 8.21; N, 16.43.

***N*-α-Boc-(2-cyclopropy1-1-isopropyl)-L-His-L-ProNH$_2$ (10b).** Yield: 60%; mp. 65-66 °C (dec.); ^{1}H nmr ($CDCl_3$): δ 8.67 (bs, 1H, NH), 6.77 (s, 1H, Ar-H), 4.55 (m, 2H, 2 × CH), 3.50 (m, 1H, CH), 3.49 (m, 1H, CH), 3.25 (m, 1H, CH), 2.92 (m, 1H, CH), 2.82 (m, 1H, CH), 1.75 (m, 5H, 2 × CH_2, CH), 1.41 (s, 9H, 3 × CH_3), 1.39 (m, 6H, 2 × CH_3), 0.85 (m, 4H, 2 × CH_2); ^{13}C nmr ($CDCl_3$): δ 174.56, 172.16, 155.16, 148.03, 134.70, 13.03, 80.04, 60.59, 52.96, 47.26, 46.88, 32.49, 29.01, 28.35, 24.74, 23.42, 7.08, 6.53, 5.96; MS (APCI): *m/z* = 434 (M+1); R_f = 0.52 [CH_3OH/$CHCl_3$ (1:9)]; *Anal.* Calcd. for $C_{22}H_{35}N_5O_4$ (433.54): C, 60.95; H, 8.14; N, 16.15. Found: C, 61.20; H, 8.34; N, 16.33.

Typical procedure for the synthesis of L-pGlu-(1,2-dialkyl)-L-His-L-ProNH$_2$ (12a–b). Protected dipeptide (**10a–b**, 0.73 mmol) was treated with 40% solution of trifluoroacetic acid in DCM (15 mL) at ambient temperature for 20 min to cleave the *N*-α-Boc group. Solvent was evaporated and the dipeptide salt was neutralized with a solution of 7 *N* NH_3 in CH_3OH (10 mL) for 10 min to generate free base. A solution of free peptide in DMF (4 mL) was cooled to 4 °C, and pGlu-OTcp (0.81 mmol) was added, and the reaction mixture stirred at 4 °C for 36 h. The solvent was removed under reduced pressure and the resulting residue purified by flash column chromatography using CH_3OH/$CHCl_3$ (4:1) as eluant.

L-pGlu-(1-ethyl-2-propyl)-L-His-L-ProNH$_2$ (12a). Yield: 40%; mp. 95-96 °C (dec.); ^{1}H nmr (CD_3OD): δ 6.82 (s, 1H, Ar-H), 4.68 (m, 1H, CH), 4.29 (m, 1H, CH), 4.08 (m, 1H, CH), 3.82 (q, 2H, CH_2, *J* = 6.0 Hz), 3.67 (m, 1H, CH), 3.31 (m, 1H, CH), 2.94 (m, 1H, CH), 2.79 (m, 1H, CH), 2.55 (t, 2H, CH_2, *J* = 6.0 Hz), 2.08 (m, 8H, 4 × CH_2), 1.58 (m, 2H, CH_2), 1.26 (t, 3H, CH_3, *J* = 6.0 Hz), 0.87 (t, 3H, CH_3, *J* = 6.0 Hz). ^{13}C nmr (CD_3OD): δ 180.86, 176.39, 173.97, 172.00, 148.69, 134.68, 117.87, 61.04, 57.22, 52.55, 47.55, 47.12, 41.10, 30.10, 29.80, 28.47, 25.94, 25.21, 22.46, 15.99, 13.51; MS (APCI): *m/z* 433 (M+1); R_f = 0.5 [CH_3OH/25%NH_4OH/$CHCl_3$ (1.4:0.2:8.4)]; *Anal.* Calcd. for $C_{21}H_{32}N_6O_4$ (432.52): C, 58.32; H, 7.46; N, 19.43. Found: C, 58.54; H, 7.31; N, 19.20; HPLC: t_R = 5.66 min, purity: 98.0%.

L-pGlu-(2-cyclopropyl-1-isopropyl)-L-His-L-ProNH$_2$ (12b). Yield. 45%; mp. 81-82 °C (dec.); ^{1}H nmr (CD_3OD): δ 7.08 (s, 1H, Ar-H), 4.64 (m, 1H, CH), 4.29 (m, 1H, CH), 4.05 (m, 1H, CH), 3.63 (m, 1H, CH), 3.32 (m, 1H, CH), 3.17 (m, 1H, CH), 2.93 (m, 1H, CH), 2.78 (m, 1H, CH), 2.29 (m, 1H, CH), 2.02 (m, 8H, 4 × CH_2), 1.34 (d, 6H, 2 × CH_3, *J* = 6.0 Hz), 0.89 (m, 4H, 2 × CH_2); ^{13}C nmr ($CDCl_3$): δ 180.84, 176.30, 173.97, 170.99, 148.64, 131.86, 115.98, 60.91, 57.23, 51.86, 47.83, 47.55, 30.29, 29.81, 28.90, 26.02, 25.34, 22.53, 6.79, 6.60; MS (APCI): *m/z* 445 (M+1); R_f = 0.5 [CH_3OH/25%NH_4OH/$CHCl_3$ (1.2:0.2:8.6)]; *Anal.* Calcd. for $C_{22}H_{32}N_6O_4$ (444.53): C, 59.44; H, 7.26; N, 18.91. Found: C, 59.76; H, 7.11; N, 18.70; HPLC: t_R = 5.40 min, purity: 98.5%.

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