Bioorganic & Medicinal Chemistry Letters 19 (2009) 887–890

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of novel Gn-RH analogues using Ugi-4MCR

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ARTICLE INFO

Article history:
Received 23 October 2008
Revised 26 November 2008
Accepted 26 November 2008
Available online 6 December 2008

Keywords:
Gn-RH analogues
4-MCR Ugi reaction
Triptorelin and gonadorelin analogues
Ligation

ABSTRACT

An efficient method for the synthesis of some Gn-RH analogues based on Ugi reaction has been developed. Four-component reaction of N- and C-terminus peptides, aromatic aldehydes and isocyanides affords novel Gn-RH analogues derived from triptorelin and gonadorelin. All of the products were purified using preparative HPLC and the structures were assigned according to MALDI-mass spectrometry data.

Gonadotropin-releasing hormone (Gn-RH) is secreted from the hypothalamus and its action on the pituitary gland then leads to the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Both of these hormones then act on the ovaries and are responsible for the pro-fertility effects of Gn-RH, primarily through the release of steroidal hormones. Gn-RH was first isolated from porcine hypothalamus and was shown to be a decapeptide **(I)**. Gn-RH plays a key role in the biology of reproduction. Similar peptides have been isolated and sequenced from other species such as chicken, salmon and lamprey.[1,2]

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (I)

The bulky hydrophobic amino acid residues in position 6 appear to be very important for the high potency of the analogues. In this way, substitution and addition of hydrophobicity at position 6 in Gn-RH analogues is an interesting subject. It was shown that the substitution could affect the biological activities of synthetic peptides. Results from a number of ongoing studies on prostate cancer patients using goserelin, buserelin, triptorelin and leuprolide have recently been published. Synthesis of novel Gn-RH has attracted particular interest.[3–9]

There are some methods for the coupling of peptide segments; among the techniques employed for this purpose native chemical ligation is the most useful method. The native chemical ligation (NCL) reaction[10–13] is a powerful method to join two unprotected peptides in aqueous solution. In the native chemical ligation an unprotected C-terminal peptide thioester reacts with an unprotected N-terminal cysteine residue. The requirement for the rare amino acid cysteine limits the applicability in the synthesis of naturally occurring proteins. Another method for the coupling of two peptide segments is Staudinger reaction.[14]

It was shown that the existence of more amide bonds and aryl or alkyl groups in the structure of peptides could affect the hydrophobicity of the peptide and biological activities.[15] We hoped to develop a general and practical method to synthesize novel Gn-RH analogues contained additional amide bond plus aryl or alkyl groups located in the middle position of the peptides. The best way for our design is 4-MCR Ugi reaction. MCRs applications in all areas of applied chemistry are very popular because, they offer a wealth of products, while requiring only a minimum of effort. MCR is applying for the synthesis of complex molecules in one-pot reactions with high bond-forming efficiency (BFE). Meanwhile, a wide variation among these starting materials opens up versatile opportunities for the synthesis of compound libraries. Among many types of MCRs, the most useful are isocyanide based MCRs (IMCRs).[16,17] It has been known that Ugi-4CR provides an elegant way to prepare natural products. The Ugi four-component reaction (Ugi-4CR) was used as an efficient method for the construction of peptide and cyclopeptide backbones.[18]

According to this high potential, herein, we present an efficient method for the synthesis of novel Gn-RH analogues using Ugi four-component reaction from C-terminus heptapeptides, N-terminus tripeptide, aromatic aldehydes and isocyanides (Scheme 1).

The reaction resulted new analogues of Gn-RH with additional peptide backbones between Leu-7 and Arg-8. It seems the presence of aryl and alkyl groups in Gn-RH analogues could increase hydrophobic properties of the peptides. It was shown that the existence of Arg-8 is critical for high-affinity interaction of Gn-RH analogues with receptors.[19] In all our synthetic peptides, the active sites for the interactions with receptors are remained.

Two different heptapeptides were used for the synthesis of Gn-RH analogues. In cases **5a–d**, there were Gly at the position 6 in

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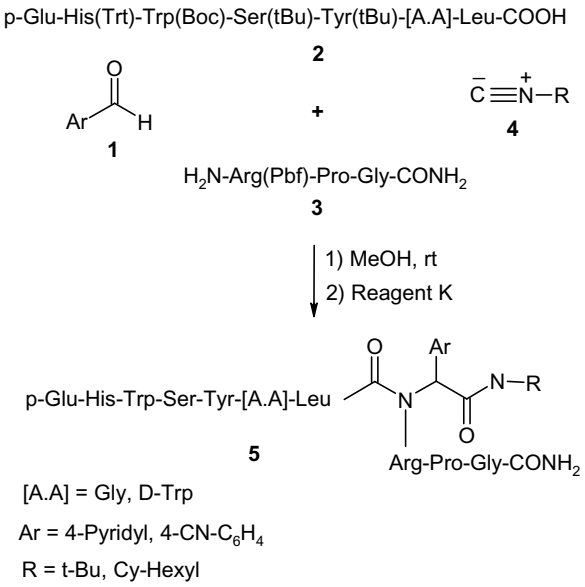
0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2008.11.111



Scheme 1.

gonadorelin analogues sequence and D-Trp in triptorelin analogues **5e–h**. Using Ugi-4MCR a new stereocenter was created in the products. In Table 1 the yields and diastereomeric ratio of products are shown. For example in compound **5f** the diastereomeric ratio was 60:40. The ratio of diastereomers was obtained according to the HPLC chromatogram (Fig. 1).

Table 1
Synthesis of novel Gn-RH analogues via Ugi-4MCR

Entry	A.A.	Ar	R	Yield[a] (%)	d.r.[b]
5a	Gly	4-CN-C_6H_4	*t*-Bu	89	30/70
5b	Gly	4-Pyridyl	*t*-Bu	75	55/45
5c	Gly	4-Pyridyl	cy-Hexyl	88	80/20
5d	Gly	4-CN-C_6H_4	cy-Hexyl	82	75/25
5e	D-Trp	4-CN-C_6H_4	*t*-Bu	80	50/50
5f	D-Trp	4-Pyridyl	*t*-Bu	69	60/40
5g	D-Trp	4-Pyridyl	cy-Hexyl	86	50/50
5h	D-Trp	4-CN-C_6H_4	cy-Hexyl	84	70/30

[a] Isolated yields.
[b] Diastereomeric ratio.

This research concerned the design and synthesis of C-terminus heptapeptides and N-terminus tripeptide as starting materials. The C-terminus heptapeptides **2** were synthesized using known Fmoc solid phase peptide synthesis strategy.[20,21]

Meanwhile, TBTU was used as the coupling reagent[22] for the synthesis of C-terminus heptapeptides and N-terminus tripeptide. The tripeptide **3** was synthesized in solution phase and with Boc/Z strategy. The details for the synthesis of N-terminus tripeptide are shown in Scheme 2.

The synthesis of tripeptide **3** started with the coupling reaction of Boc-Pro-OH with Gly-NH_2·HCl in presence of TBTU as coupling reagent and base to afford the protected dipeptide **6**. Acidification of compound **6** with Acetic acid/hydrochloric acid (1 M) is an established method for the Boc-deprotection to provide the deprotected dipeptide **7**. After removal of the Boc group, coupling reaction of dipeptide **7** with Z-Arg(Pbf)-OH provides protected tripeptide **8**, and finally Z-deprotection was done using hydrogenation in the presence of Pd/C successfully.[23]

The generally accepted mechanism of the Ugi four-component coupling (Ugi-4CC) reaction involves four elementary steps, the last of which is irreversible. In the first step, the aldehyde condenses with an amino group of N-terminus tripeptide to form an imine that could be converted and produces iminium salt in the presence of C-terminus heptapeptides. Next, the isocyanide is added to the iminium salt to produce a nitrilium ion. Then, a reactive *O*-acyl iminolate is formed via the α-addition of carboxylate anion to the nitrilium ion. The final step involves the *O*- to *N*-acyl transfer (Mumm rearrangement) to afford the novel Gn-RH analogues (Scheme 3).

The products were purified using preparative HPLC and their structures were confirmed using MALDI and ESI Mass spectroscopy methods and also amino acid analysis data.[24] In Figure 2 MALDI-Mass spectrum of compound **5f** is shown.

The prepared peptide has more amide bonds, and it seems that it could be more stable against proteases and it can be considered as a better candidate for therapeutic purposes. The presence of aryl, cyclohexyl and alkyl groups in the structure of the products mimic the peptide backbone and increase hydrophobic properties of the molecule and it seems it can affect the biological activity

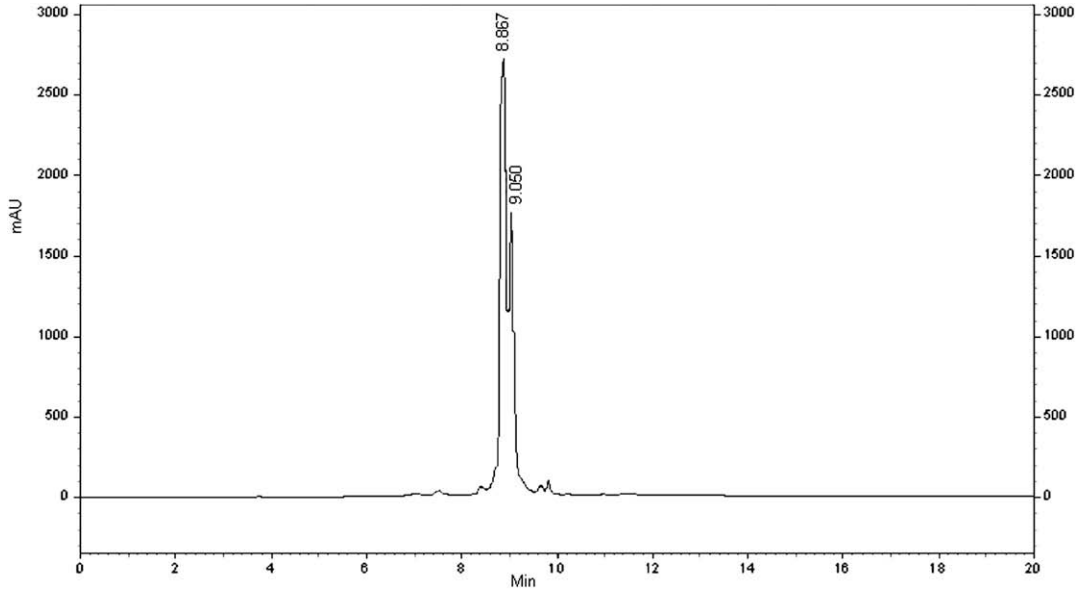
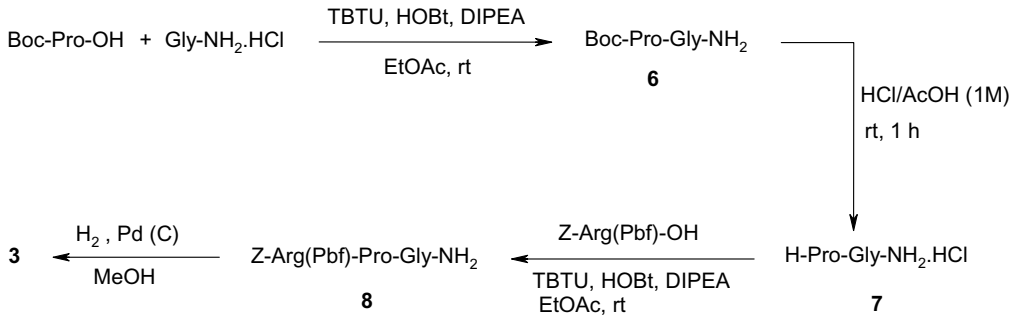
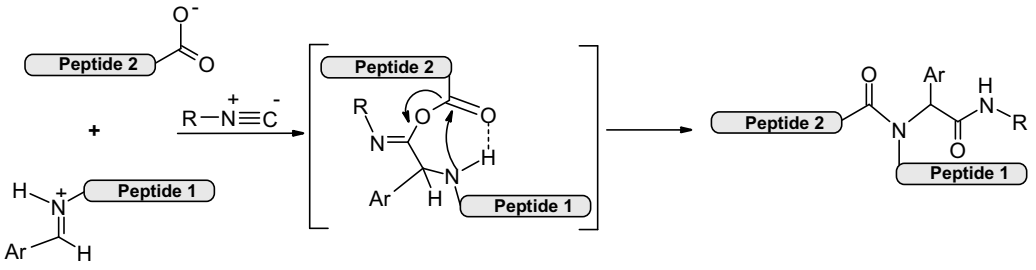


Figure 1. HPLC chromatogram of compound **5f**.



Scheme 2. Synthesis of N-terminus tripeptide.



Scheme 3. Proposed mechanism for the synthesis of Gn-RH analogues **5**.

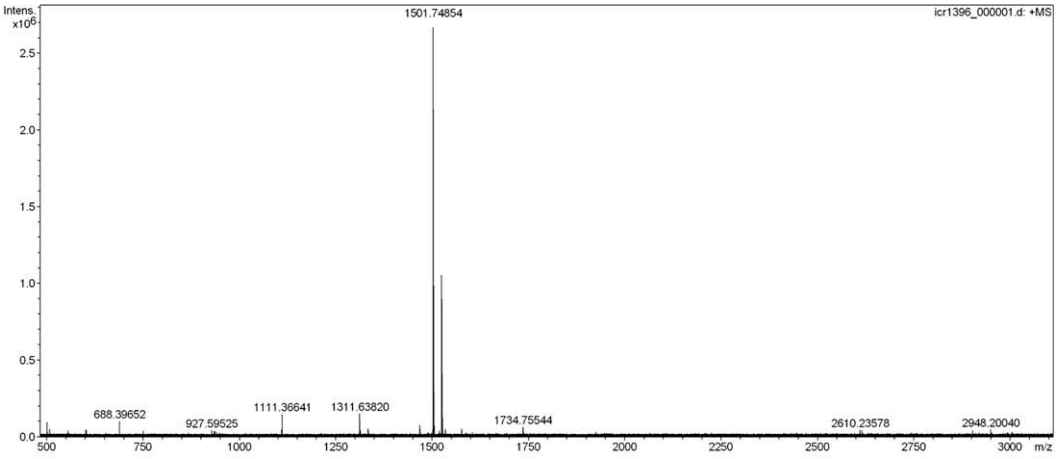


Figure 2. MALDI-MS of compound **5f**.

of the peptide. Investigations concerning biological activity are underway.

The results indicate that the current method could be used to synthesize some novel Gn-RH analogues using 4-MCR Ugi reaction. Because the current method can link two peptide segments in mild conditions via the Ugi type reaction as the key step, we named in the Ugi-ligation.

In conclusion, we created novel synthetic Gn-RH analogues via Ugi four-component reaction of C-terminus heptapeptides, N-terminus tripeptide, aromatic aldehydes, and isocyanides at room temperature. In this research, triptorelin and gonadorelin analogues were synthesized with good yields.

Acknowledgments

We thank National Research Institute for Science Policy (NRISP, Iran) for financial support. We also thank Tofigh Daru Research & Engineering Co. for kind cooperation. A part of this research work was supported by research council of K. N. Toosi University of Technology which is gratefully acknowledged.

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21. *General procedure for the synthesis of heptapeptides pGlu-His(Trt)-Trp(Boc)-Ser(tBu)-Tyr(tBu)-[A.A.]-Leu-COOH (**2**)*: Synthesis was carried out using 2-chlorotrityl chloride resin (1.0 mmol/g) following standard Fmoc strategy. Fmoc-Leu-OH (3.54 g, 10 mmol) was attached to the 2-CTC resin (5.0 g) with DIPEA (6.85 ml, 40 mmol) in anhydrous DCM: DMF (50 ml, 1:1) at room temperature for 2 h. After filtration, the remaining trityl chloride groups were capped by a solution of DCM/MeOH/DIPEA (17:2:1, 120 ml) for 30 min. The resin was filtered and washed thoroughly with DCM (1× 20 ml), DMF (4× 20 ml) and MeOH (5× 20 ml). The loading capacity was determined by weight after drying the resin under vacuum and was 1.0. The resin-bound Fmoc-amino acid was washed with DMF (3× 20 ml) and treated with 25% piperidine in DMF (65 ml) for 30 min and the resin was washed with DMF (3× 20 ml).[20] Then a solution of Fmoc-A·A-OH (7.5 mmol), TBTU (2.40 g, 7.5 mmol), DIPEA (3.0 ml, 17.5 mmol) in 30 ml DMF was added to the resin-bound free amine and shaken for 1 h at room temperature. After completion of coupling, resin was washed with DMF (4× 20 ml) and DCM (1× 20 ml). The coupling was repeated as the same methods for other amino acids of their sequences. In all cases for the presence or absence of free primary amino groups, Kaiser Test was used. Fmoc determination was done using UV spectroscopy method. After completion of couplings, resin was washed with DMF (4× 20 ml), DCM (1× 20 ml). The produced heptapeptides **2** were cleaved from resin by treatment of TFA (1%) in DCM (275 ml) and neutralization with pyridine (4%) in MeOH (85 ml). The solvent was removed under reduced pressure and precipitated in water. The yields were 90% (A.A. = Gly) and 84% (A.A. = D-Trp).
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23. Procedure for the synthesis of tripeptide H_2N-Arg(Pbf)-Pro-Gly-NH_2 (**3**)*:* To a mixture of Boc-Pro-OH (4.43 g, 20.6 mmol) and Gly-NH_2·HCl (3.18 g, 29.0 mmol) in 150 ml EtOAc were added TBTU (7.26 g, 22.6 mmol), HOBt (3.15 g, 20.6 mmol) and DIPEA (10.50 ml, 61.0 mmol) and the mixture was stirred overnight. After completion of the reaction, the solution was washed with citric acid (20%) and sodium carbonate (3%) and iso-hexane was added to the residue to afford crystals, which were collected by filtration (yield = 67%). For Boc-deprotection, dipeptide **6** (4.10 g, 15.0 mmol) was dissolved in AcOH/HCl (45 ml) and the mixture was stirred for 1 h, and then MTBE (65 ml) was added and the precipitate was collected via filtration (yield = 99%). Dipeptide H-pro-Gly-NH_2 **(7)** (3.12 g, 15 mmol) was dissolved in EtOAc (75 ml), Z-Arg(Pbf)-OH (7.2 g, 12.8 mmol), TBTU (4.37 g, 13.6 mmol), HOBt (1.96 g, 12.8 mmol) and DIPEA (6.33 ml, 37.0 mmol) was added to the solution. The solution was stirred overnight at room temperature. The work-up was done according to last procedure (yield = 62%). Z-Arg(Pbf)-Pro-Gly-NH_2 **(8)** (5.58 g, 7.8 mmol) in MeOH (40 ml) was debenzylated using H_2 over Pd/C catalyst. After removal of Pd/C and the solvent, the residue was collected (yield = 99%). The overall yield for the synthesis tripeptide **3** was 41%.
24. *General procedure for the synthesis of Gn-RH analogues (**5**):* The tripeptide **3** (0.58 g, 1.0 mmol) was added to a solution of aldehydes **1** (1.0 mmol) in methanol (6 ml) and the reaction was stirred at room temperature for 30 min. Then C-terminus heptapeptides **2** (1.0 mmol) was added and stirring was continued for 15 min, followed by addition of isocyanide derivatives **4** (1.0 mmol), the solution was stirred for 4 days at room temperature. Water (90 ml) was added and after 2 h, the precipitate was filtered and washed with saturated $NaHCO_3$ and water. The final deprotection of the protected peptides was performed by reacting with reagent K (20 ml/g peptide) for 2 h at room temperature. The final peptides **5** were dried under vacuum at 40 °C (yields: 69–88%). Further purification was done using Prep-HPLC with column (ODS-C_{18}, 120 × 20 mm) and UV detector (λ = 210 nm). The elution solvent was ACN/10 mM NaH_2PO_4 buffer. The MALDI-MS spectral data for the compound **5a**–**h** are as follows: **5a:** $C_{68}H_{89}N_{19}O_{14}$, Calcd 1396.5790, MS (MALDI), found *m/z*: $[M]^+$ = 1396.69070, $[M+H]^+$ = 1397.69403, $[M+Na]^+$ = 1419.67617; **5b:** $C_{66}H_{89}N_{19}O_{14}$, Calcd 1372.5570, MS (MALDI), found *m/z*: $[M]^+$ = 1372.69085, $[M+H]^+$ = 1373.69439, $[M+Na]^+$ = 1395.67646; **5c:** $C_{68}H_{91}N_{19}O_{14}$, Calcd 1398.5950, MS (MALDI), found *m/z*: $[M]^+$ = 1398.70666, $[M+H]^+$ = 1399.71012, $[M+Na]^+$ = 1421.69224; **5d:** $C_{70}H_{91}N_{19}O_{14}$, Calcd 1422.6170, MS (MALDI), found *m/z*: $[M]^+$ = 1422.70648, $[M+H]^+$ = 1423.70989, $[M+Na]^+$ = 1445.69214; **5e:** $C_{77}H_{96}N_{20}O_{14}$, Calcd 1525.7410, MS (MALDI), found *m/z*: $[M]^+$ = 1525.74863, $[M+H]^+$ = 1526.75211, $[M+Na]^+$ = 1548.73371; **5f:** $C_{75}H_{96}N_{20}O_{14}$, Calcd 1501.7190, MS (MALDI), found *m/z*: $[M]^+$ = 1501.74854, $[M+H]^+$ = 1502.75188, $[M+Na]^+$ = 1524.73378; **5g:** $C_{77}H_{98}N_{20}O_{14}$, Calcd 1527.7570, MS (MALDI), found *m/z*: $[M]^+$ = 1527.76467, $[M+H]^+$ = 1528.76772, $[M+Na]^+$ = 1550.74992; **5h:** $C_{79}H_{98}N_{20}O_{14}$, Calcd 1551.7790, MS (MALDI), found *m/z*: $[M]^+$ = 1551.76465, $[M+H]^+$ = 1552.76791, $[M+Na]^+$ = 1574.75116.