Organic LETTERS

Letter

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Multicomponent Diversity-Oriented Synthesis of Aza-Lysine-Peptide Mimics

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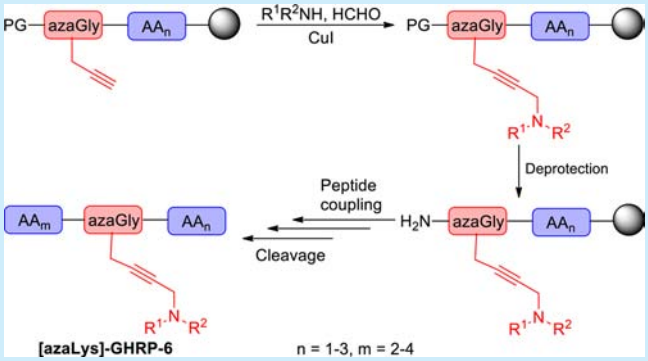
Supporting Information

ABSTRACT: Copper catalyzed coupling of Mannich reagents to aza-propargylglycine residues has been employed to synthesize constrained aza-lysine peptides. Employing growth hormone releasing peptide-6 (GHRP-6) as a model peptide and a variety of secondary amines, 18 aza-Lys analogs were synthesized by this so-called A^3-coupling reaction. This effective method for making constrained aza-Lys-peptides offers strong potential for exploring various recognition events implicating lysine residues including post-translational peptide modification.



The ε-amine of lysine (Lys) residues undergoes a variety of post-translational modifications that influence the form and function of peptides and proteins. For example, in histones, the ε-amine of lysine undergoes methylation,[1] acetylation,[2] ubiquitination,[3] and SUMOylation,[4] all of which regulate diverse biological processes such as gene transcription, DNA repair, and chromosome condensation. Modifications of Lys residues in other proteins include propionylation, butyrylation,[5] and succinylation[6] leading to different biological responses. Consequently, synthetic strategies for making *N*-ε-alkyl- and acyl-Lys residues and their mimics have been used for studying the biological impact of such modifications,[7] including the mechanism of Sir2 deacetylase,[8] folding effects of amide-π and cation-π interactions,[9] and lysine demethylase inhibition.[10]

Azapeptides are mimics characterized by an α-carbon to nitrogen replacement in one or multiple amino acids within a peptide.[11] Aza-residues in peptides can increase β-turn propensity and enhance metabolic stability.[12] Although considerable progress has been made in solution-[13] and solid-phase[14] azapeptide synthesis, few aza-Lys peptides have been reported. In a rare example, *N*-ethoxycarbonyl-D-Phe-L-Pro-aza-Lys-*p*-nitrophenyl ester served as a selective active site titrant for human and bovine thrombin.[15]

Seeking to develop a diversity-oriented approach for preparing aza-Lys-peptides, we have explored the copper-catalyzed addition of Mannich reagents to an aza-propargylglycine residue. This so-called A^3-coupling[16] reaction between acetylene, aldehyde, and secondary amine components was developed to enable modification of the ε-amine of Lys with a diverse array of alkyl and aryl substituent groups, to introduce side-chain restriction from the triple bond, and to constrain the peptide backbone, due to the urea planarity and nitrogen−nitrogen lone pair repulsion of the aza-residue. Moreover, this modification expands the utility of aza-propargylglycine residues,[17] which have already served in diversity oriented syntheses featuring CuAAC,[18] Sonogashira, and 5-*exo*-*dig* cyclization chemistry[19,20] to prepare libraries from libraries.[21] Although propargylamines have served as synthetic intermediates and medicinally relevant motifs,[22] to the best of our knowledge, the A^3-coupling reaction has never been employed for the synthesis of lysine analogs.

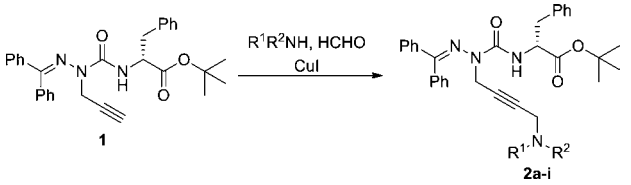
In the context of our interest in azapeptide analogs of growth hormone releasing peptide-6 (GHRP-6, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) as modulators of the Cluster of Differentiation-36 (CD36) receptor,[23] we selected this peptide to study aza-Lys residue synthesis. As a model, GHRP-6 is useful to examine the influence of potentially reactive amino acid side chains and backbone conformation on the A^3-coupling reaction. Two routes were pursued to synthesize [aza-Lys]GHRP-6 analogs: (1) solution-phase synthesis of aza-Lys dipeptide building blocks using the A^3-coupling reaction followed by incorporation into the peptide sequence and (2) A^3-coupling to resin-bound aza-propargylglycinyl residues prepared by submonomer azapeptide synthesis, followed by solid-phase peptide synthesis (SPPS).

Initially, the solution-phase approach was examined employing azapropargylglycinyl dipeptide **1**.[14] After preliminary evaluation of reaction conditions, alkyne **1** was successfully converted in >95% yields to aza-Lys-dipeptides **2a**−**f** using six different secondary amines (150 mol %), copper iodide (10 mol %), and paraformaldehyde (200 mol %) in dioxane at 80 °C (Protocol A, Table 1). In addition, aqueous formaldehyde reacted with **1** under milder conditions in DMSO at rt to give

Received: November 14, 2013
Published: December 13, 2013

dipeptides **2a–c** and **2g–i** in ≥80% yields (Protocol B, Table 1).[24]

Table 1. *tert*-Butyl Benzhydrylidene 4-(*N,N*-Disubstituted amino)but-2-ynyl-aza-glycine-D-phenylalanine Synthesis via A^3-Coupling Reaction



dipeptides	protocol[a]	secondary amine	time (min)	yield (%)
2a	A	diallylamine	40	99
	B	diallylamine	120	89
2b	A	diisopropylamine	40	99
	B	diisopropylamine	120	82
2c	A	methylbenzylamine	40	95
	B	methylbenzylamine	120	80
2d	A	diethylamine	40	99
2e	A	dicyclohexylamine	40	99
2f	A	morpholine	40	99
2g	B	dipropylamine	120	86
2h	B	dibutylamine	120	92
2i	B	piperidine	120	92

[a]Protocol A: paraformaldehyde (2.0 equiv), secondary amine (1.5 equiv), CuI (0.1 equiv), dioxane, 80 °C, 40 min; Protocol B: 37% aqueous formaldehyde (2.2 equiv), secondary amine (1.2 equiv), CuI (0.1 equiv), DMSO, rt, 120 min.

With dipeptide building blocks **2** in hand, a solid-phase protocol was used to make [aza-Lys^4]GHRP-6 analogs **8** (Scheme 1). The *tert*-butyl ester was removed with TFA in DCM, and the resulting aza-dipeptide acids **4** were coupled to lysine(ε-Boc)-Rink-amide resin **3** using HBTU and DIEA to yield semicarbazones **5**. Semicarbazides **6a–f** were liberated by treating **5** with a 1.5 M solution of hydroxylamine hydrochloride in pyridine[14] and coupled to Fmoc-Ala using BTC and collidine in THF to give tetrapeptides **7**. After Fmoc-group removal and SPPS,[25] azapeptides **8** were cleaved from the resin using a TFA/TES/H_2O (95:2.5:2.5) cocktail to furnish material esteemed to be of 64–72% purity by LC-MS. Purification by preparative reversed-phase HPLC gave [aza-Lys^4]GHRP-6 analogs **8a–f** in 4–11% yields and >99% purity, as characterized by LC-MS and HRMS (Table 2).

Scheme 1. Solid-Phase Synthesis of [aza-Lys^4]GHRP-6 Peptides 8a–f

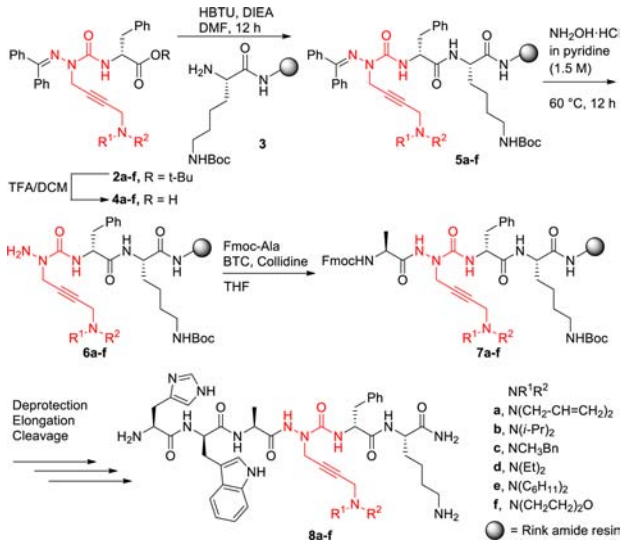


Table 2. [Aza-Lys]GHRP-6 Analogs Synthesized by A^3-Coupling Reaction

peptide	synthetic route[a]	[azaLys^n] GHRP-6 analog	crude purity (%)[b]	retention time [MeOH/MeCN] (min)[c]	yield (%)[d]
8a	I	4	69	9.41/8.32	3.8
	II	4	61	5.90/5.85	3.0
8b	I	4	64	8.87/8.13	7.9
	II	4	52	5.67/5.83	3.3
8c	I	4	69	8.19/7.79	8.0
8d	I	4	70	8.08/9.56	6.1
8e	I	4	72	9.43/8.39	11
8f	I	4	67	7.99/7.63	10
8g	II	4	53	6.29/4.33	8.4
8h	II	4	44	7.22/4.17	4.7
16a	II	5	65	5.22/6.20	6.7
16b	II	5	64	4.99/6.30	8.8
16c	II	5	72	5.24/6.58	4.0
16d	II	5	68	6.06/7.44	5.0
16e	II	5	56	5.65/7.04	5.9
17a	II	3	23	6.61/6.10	2.7
17b	II	3	31	8.11/5.90	1.2
17c	II	3	34	6.59/7.19	1.5
17d	II	3	28	7.43/6.97	1.5
17e	II	3	17	7.00/6.72	1.5

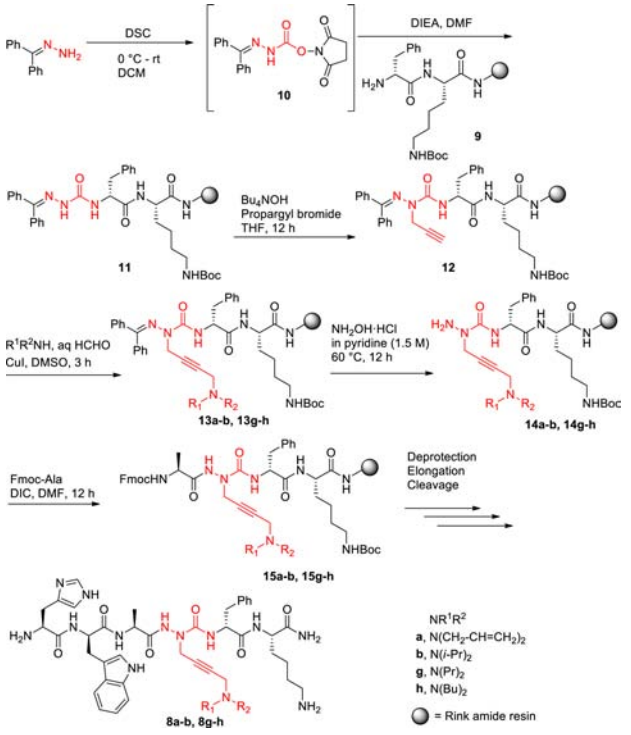
[a]Route I: A^3-coupling reaction in solution. Route II: A^3-coupling reaction on solid phase. [b]Determined by LC-MS after resin cleavage. [c]LC-MS analysis using linear gradients of MeOH or CH_3CN in water containing 0.1% formic acid: **8a** (I), **8b** (I), and **8f** (5–40% over 15 min) on a Phenomenex Gemini C18 column (particle size: 5 μm; 150 mm × 4.6 mm); **8c–8e** (5–80% over 15 min) on a Phenomenex Gemini C18 column (particle size: 5 μm; 150 mm × 4.6 mm); **8a** (II), **8b** (II), **8g–h** and **16a–17e** (5–60% over 10 min) on a Sunfire C18 column (particle size: 3.5 μm; 50 mm × 2.1 mm). [d]Yields after preparative RP-HPLC are based on resin loading.

Subsequently, A^3-coupling chemistry was examined on resin-bound aza-propargylglycinyl peptides (e.g., **12**, Scheme 2). Although ordinary propargylamines have been previously made on solid phase by A^3-coupling chemistry, they were cleaved immediately from the resin and never employed in subsequent chemistry.[26] In the submonomer azapeptide synthesis,[14] benzophenone hydrazone was acylated using *N,N′*-disuccinimidyl carbonate (DSC)[17] and resulting carbazate intermediate **10** was coupled to D-Phe-Lys(ε-Boc)-Rink-amide resin **9** to give semicarbazone **11** in good purity as assessed by LC-MS analysis of a cleaved aliquot. Propargylation was performed using tetrabutylammonium hydroxide (TBAH) and propargyl bromide to furnish aza-tripeptide **12**.[27]

Protocols A and B for the A^3-coupling reaction were both examined on solid phase. Using protocol A, resin **12** was treated with CuI (10 mol %) and 300 mol % of both paraformaldehyde and piperidine at 80 °C for 1 h, when LC-MS analysis of a cleaved resin aliquot indicated only 33% conversion to the product possessing the desired molecular ion. Subsequent hydrazone deprotection led, however, to a complex

dx.doi.org/10.1021/ol403297v | Org. Lett. 2014, 16, 298–301

Scheme 2. Solid-Phase Synthesis of [aza-Lys^4]GHRP-6 Peptides 8a−b and 8g−h



mixture, which is believed to be due to issues in removing paraformaldehyde. On the other hand, conversion to semicarbazone **13a** was observed by LC-MS to be complete after exposing resin **12** to Protocol B: CuI (20 mol %), diallylamine (600 mol %), and 37% aqueous formaldehyde (600 mol %) in DMSO at rt for 3 h. A set of different amines were successfully employed for the synthesis of semicarbazones **13**, which were converted to semicarbazides **14** using hydroxylamine in pyridine without incident. Subsequently, coupling to Fmoc-Ala using DIC provided tetrapeptides **15**. Removal of the Fmoc group, elongation, and cleavage from resin as described before provided [aza-Lys^4]GHRP-6 analogs **8a−b** and **8g−h** in 44−61% purities. After purification by preparative reversed-phase HPLC, the desired products were obtained in 3−9% yields with >99% purity (Table 2). Notably, [*N*,*N*-diallylamino-aza-Lys^4]-GHRP-6 **8a** prepared by both methods exhibited the same retention time using the identical HPLC column and conditions.

Sets of [aza-Lys^5]- and [aza-Lys^3]GHRP-6 analogs **16** and **17** were subsequently prepared using an analogous solid-phase protocol (Figure 1, Table 2, Supporting Information). In the coupling of the amino acid to the resin-bound semicarbazide, conversion dropped from >95% to ∼70%, and diastereomer formation from epimerization during coupling increased (up to ∼30% in the case of certain [aza-Lys^3]GHRP-6 analogs) as the sequence grew longer, which may reflect both the nature of the Fmoc-amino acid employed and the formation of peptide secondary structure, which may hinder coupling.

In principle, the alkyne of the aza-lysine analogs offers potential for making alkene and saturated analogs in order to explore the influence of side-chain orientation. A preliminary study to selectively reduce the triple bond was performed on dipeptide **2i** employing palladium-catalyzed hydrogenation with formic acid (Scheme 3).[28] Aza-lysine **18i** was isolated in 91%

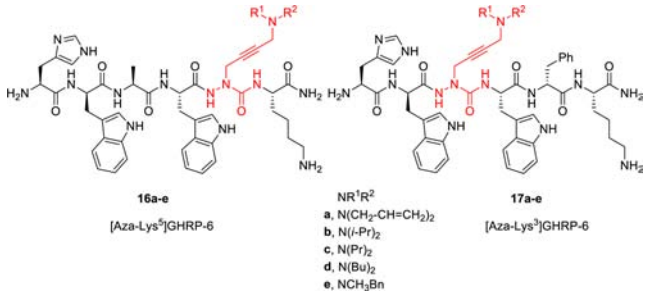
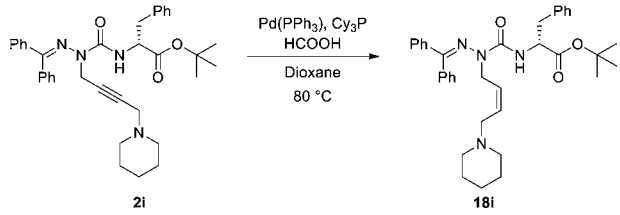


Figure 1. [aza-Lys^5]GHRP-6 and [aza-Lys^3]GHRP-6 analogs synthesized by solid-phase A^3-coupling reaction.

Scheme 3. Hydrogenation of Alkyne 2i



yield and assigned the *Z*-alkene geometry based on mechanistic considerations,[28] and the 11.7 Hz coupling constant that was observed beween the vinyl protons using a selective decoupling experiment.[29] Experiments to prepare the *E*-alkene and saturated aza-Lys analogs are now in progress.

In conclusion, an efficient method for synthesizing constrained aza-lysine-peptides has been developed using the A^3-coupling reaction, both in solution and on solid phase. By employing this approach to insert aza-Lys residues at the 3−5 positions of GHRP-6, 18 different analogs were successfully synthesized in high purity and yields suitable for biological testing. The activity of [aza-Lys]GHRP-6 analogs is currently under investigation and will be reported in due time. Considering the physiological importance of post-translationally modified lysine residues, this approach should be valuable for exploring the effects of the conformation and amine substituent in a variety of systems.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, ^{1}H and ^{13}C NMR spectrum of compounds **2a−i** and **18i**, and LC-MS analytical data for **8a−h**, **16a−e**, and **17a−e**. This material is available free of charge via the Internet at http://pubs/acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Natural Sciences and Engineering Research Council of Canada, the Ministère du développement économique de l'innovation et de l'exportation du Quebec (No. 878-2012, Traitement de la dégénerescence maculaire) and Amorchem for support.

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