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Aza-1,2,3-triazole-3-alanine Synthesis via Copper-Catalyzed 1,3-Dipolar Cycloaddition on Aza-progargylglycine

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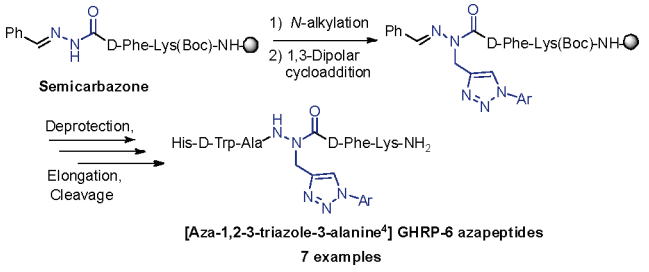

The parallel synthesis of seven aza-1,2,3-triazole-3-alanine azapeptides of the Growth Hormone Releasing Peptide-6 (GHRP-6) was accomplished via a Cu-catalyzed azide−alkyne [3+2] cycloaddition on an aza-propargylglycine residue anchored on Rink amide resin. Circular dichroism spectroscopy in water demonstrated that azapeptides which possess an aza-1,2,3-triazole-3-alanine residue at the Trp[4] position of the GHRP-6 sequence adopt β-turn conformations.

Azapeptides are peptidomimetics in which the α carbon of one or more amino acids has been replaced with a nitrogen atom.[1] The longstanding interest in aza-amino acids as peptide mimics stems from their increased stability,[2] resistance to proteases,[3] and ability to induce conformational rigidity, favoring the formation of β turns.[4] Until recently, the incorporation of side-chain diversity onto the aza-amino acid residue was limited by the solution-phase synthesis of protected *N*-alkyl hydrazine building blocks prior to their activation and incorporation onto a growing peptide chain. Toward the combinatorial synthesis of azapeptides, we described recently a three-step procedure featuring regioselective alkylation and chemoselective deprotection of an azaglycine semicarbazone to afford a variety of azapeptides directly on solid support.[5] With the idea that multiple combinatorial libraries of azapeptides could be obtained via the making of "libraries from libraries",[6] we have incorporated aza-propargylglycine moieties within a peptide to introduce a new series of aza-1,2,3-triazole-3-alanine residues (Figure 1).

Growth Hormone Releasing Peptide-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2, GHRP-6), a synthetic hexapeptide that binds to two distinct receptors,[7] was chosen as the target peptide to develop this methodology. Incorporation of aza-Phe for Trp[4] conferred selectivity for the CD36 receptor,[5] a target for the development of treatments of angiogenesis-related diseases. A combinatorial approach to incorporate novel heteroaryl alanine residues at the 4-position was deemed desirable for improving binding affinity of the lead [AzaPhe[4]]GHRP-6 azapeptide.

The Cu-catalyzed azide−alkyne [3+2] cycloaddition has proven effective for making triazole peptidomimetics.[8] Propargyl glycine, propargyl amides, and *N*-propargyl glycine peptoids all have served as substrates for triazole formation leading to macrocyclization,[9,10] carbohydrate ligation,[11] peptoid oligomer functionalization,[12] as well as amide bond and histidine isostere synthesis.[8a,13] Precluding the solution-phase synthesis of azido acids and propargyl glycine residues, and taking advantage of the propensity for aza-amino acids to adopt β-turns, we describe now the solid-phase synthesis of aza-1,2,3-triazole-3-alanine-containing azapeptides using aza-propargyl glycine residues anchored on Rink amide resin.

[Aza-1,2,3-triazole-3-alanine[4]]GHRP-6 analogues were synthesized by elaboration of the submonomer methodology for aza-propargylglycine synthesis by using propargyl bromide as electrophile[5] to alkylate a semicarbazone residue on solid phase. Subsequent 1,3-dipolar cycloaddition was accomplished with aryl iodides, sodium azide, and copper iodide in a tandem aryl azide formation/cycloaddition reaction cascade (Table 1, Figure 2).[14] Seven triazoles were synthesized in

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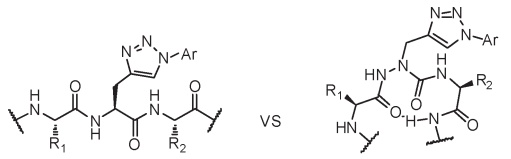
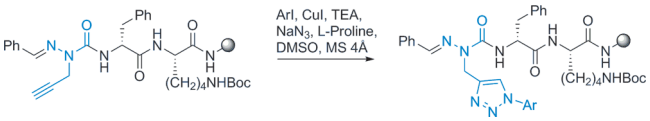


FIGURE 1. 1,2,3-Triazole-3-alanine and aza-1,2,3-triazole-3-alanine residues.

TABLE 1. LCMS Conversions of the Aryl Azide Formation/1,3-Dipolar Cycloddition Step



entry	ArI	LCMS conversions at 214 nm (%)
a	iodobenzene	61
b	4-iodoanisole	82
c	3-iodoanisole	58
d	4-iodotoluene	80
e	4-fluoroiodobenzene	69
f	3-trifluoromethyliodobenzene	79
g	1-iodonaphthalene	62

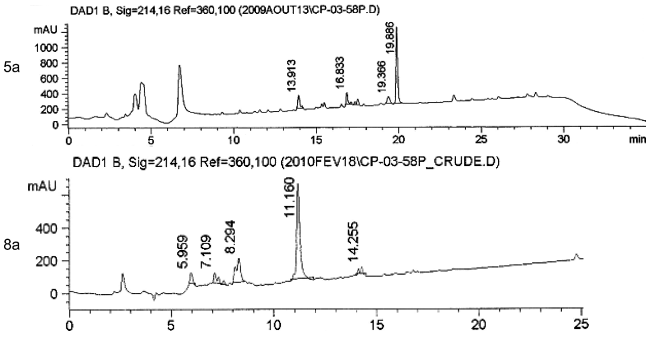
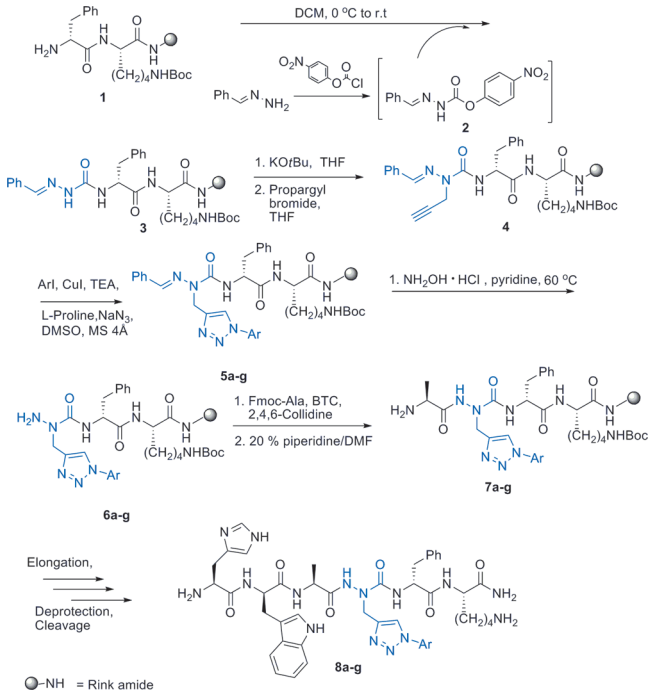


FIGURE 2. Representative LCMS traces of intermediate **5a** (upper) and peptide **8a** (lower), using MeOH/H_2O eluent (0−80% MeOH) containing 0.1% formic acid.

this way and subsequently converted to GHRP-6 analogues modified at the Trp[4] position (Scheme 1).

Addition of 4 Å powdered molecular sieves to the resin proved to be essential to afford the desired triazoles in good conversions. Both electron-rich and electron-poor aryl iodides furnished aza-1,2,3-triazole-3-alaninyl residues in good conversions (Table 1), with complete disappearance of starting material as indicated by LCMS analysis after resin cleavage. Copper scavenging was achieved by shaking the resin in a 3:1 DMF:0.1 N HCl mixture for 12 h. Selective removal of the hydrazone to reveal the aza-amino acid residue was accomplished by using hydroxylamine in pyridine under previously established conditions,[5] and azapeptide elongation was completed according to standard Fmoc protocols for solid-phase

SCHEME 1. Synthesis of [Aza-1,2,3-triazole-3-alanine]GHRP-6 Analogues



peptide synthesis.[15] Azapeptides **8a**−**g** were obtained in crude purities ranging from 52% to 69% and purified by using reverse-phase HPLC to afford a product of >99% purity in 5−11% overall yields based on initial resin loading (Table 2).

Circular dichroism (CD) spectroscopy in water was used to study the conformational bias of the [aza-1,2,3-triazole-3-alanine[4]]GHRP-6 analogues and compared with the native sequence (Figure 3). The CD curve of the native GHRP-6 sequence in water is characteristic of a random coil exhibiting a negative maximum around 190 nm. On the other hand, azapeptides **8a**−**g** displayed CD signatures consistent with β-turn conformations exhibiting distinct negative maxima at 230 and 190 nm, and a distinct positive maximum at 215 nm. A similar curve shape was observed for [aza-Phe[4]]GHRP-6 indicating that such modification of the aza-residue side chain does not alter the backbone conformation of the azapeptide in water.

Seven new GHRP-6 azapeptides containing aza-1,2,3-triazole-3-alanine residues were prepared employing aza-propargylglycine in a copper-catalyzed tandem aryl azide formation/1,3-dipolar cycloaddition approach on Rink amide resin. Considering that aza-propargylglycine may be effectively introduced anywhere in a peptide sequence by submonomer solid-phase azapeptide synthesis, featuring semicarbazone propargylation,[5] this libraries-from-libraries methodology should find general application for modifying peptide structures, because solution phase synthesis of the propargyl moiety and aryl azide are avoided. The effectiveness of this reaction cascade combined with the conformational rigidity imposed by the aza-amino acid residue make this method particularly attractive for structure−activity studies of biologically active peptides.

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TABLE 2. Yields and Purities of GHRP-6 Azapeptides 8a−g

				HRMS $[M + H]^+$ or $[M + Na]^+$ ions	
peptide	crude purity[a] (%)	purity[b] (%)	yield[c] (%)	*m*/*z* (calcd)	*m*/*z* (obsd)
8a	63	>99	5	902.4532	902.4516
8b	52	>99	8	932.4638	932.4617
8c	59	>99	8	932.4638	932.4617
8d	66	>99	8	916.4689	916.4657
8e	65	>99	9	920.4438	920.4423
8f	69	>99	10	970.4406	970.4375
8g	52	>99	11	952.4689	952.4660

[a]RP-HPLC purity at 214 nm of the crude peptide in MeOH/H_2O eluent containing 0.1% formic acid (FA). [b]RP-HPLC purity at 214 nm of the purified peptide in MeOH/H_2O eluent containing 0.1% formic acid (FA). [c]Yields after purification by RP-HPLC are based on resin loading.

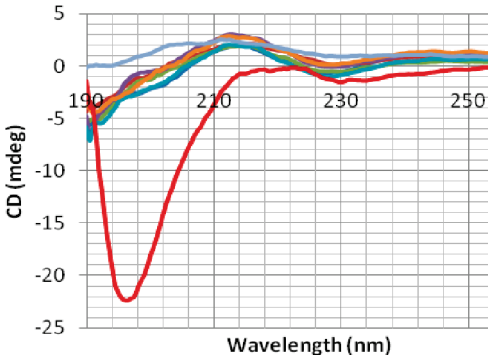

FIGURE 3. Circular dichroism in water of azapeptides **8a−g** and native GHRP-6 (red).

Experimental Section

Representative Triazole Synthesis (5a). In a 6.0 mL filtration tube, equipped with a cap and stopcock under argon, swollen semicarbazone resin **4** (200 mg, 0.128 mmol) in DMSO was treated sequentially with iodobenzene (15 μL, 5 equiv, 0.64 mmol), CuI (122 mg, 0.64 mmol, 5 equiv), L-proline (74 mg, 0.64 mmol, 5 equiv), sodium azide (42 mg, 0.64 mmol, 5 equiv), triethylamine (89 μL, 0.64 mmol, 5 equiv), and powdered 4 Å molecular sieves. The filtration tube was flushed with argon, capped, sealed with parafilm, and heated in a water bath with a sonicator at 80 °C for 24 h. The resin was filtered and washed with DMF (3 × 10 mL), DMF/0.1 N HCl (3:1, 3 × 10 mL), H_2O (3 × 10 mL), MeOH (3 × 10 mL), THF (3 × 10 mL), and DCM (3 × 10 mL). The resin was suspended in a DMF/0.1 N HCl mixture for 12 h, filtered, and subsequently washed with solvent as above. The extent of reaction conversion was shown to be 61% by LCMS analysis of the crude filtered solution, after cleavage of an aliquot of resin using TFA/TES/H_2O (95:2.5:2.5, v/v/v. Figure 2). LCMS (0−80% MeOH, 35 min) retention time (RT) = 19.89 min; LCMS (ESI) calcd for $C_{32}H_{38}N_9O_3$ $[M + H]^+$ 596.3, found *m*/*z* 596.2.

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Supporting Information Available: General Experimental Methods, compound characterization data, and copies of LCMS traces. This material is available free of charge via the Internet at http://pubs.acs.org.