ORGANIC LETTERS

2010
Vol. 12, No. 13
2916−2919

Copper-Catalyzed *N*-Arylation of Semicarbazones for the Synthesis of Aza-Arylglycine-Containing Aza-Peptides

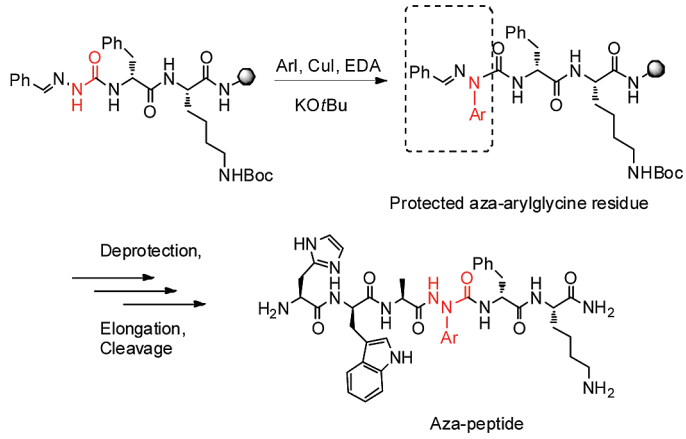
Caroline Proulx and William D. Lubell*

Département de Chimie, Université de Montréal, C.P. 6128, Succursale Centre-Ville, Montréal, Québec H3C 3J7

william.lubell@umontreal.ca

Received April 22, 2010

ABSTRACT



Parallel synthesis of 13 aza-arylglycine peptides, based on the hexapeptide sequence of Growth Hormone Releasing Peptide-6 (GHRP-6), was accomplished via selective *N*-arylation of a semicarbazone peptide building block anchored on Rink amide resin. Aza-peptides possessing aza-indolylglycine and aza-imidazoylglycine residues were obtained through use of the corresponding heteroaryl iodides, yielding, respectively, aza-Trp and aza-His peptidomimics. CD spectroscopy indicated the propensity for aza-peptides, containing aza-arylglycines at the Trp^4 position of the GHRP-6 sequence, to adopt β-turns.

Substituted hydrazines constitute important synthetic building blocks employed, namely, as precursors of a wide variety of medicinally relevant heterocycles such as pyrazolidines,[1] pyrazoles,[2] 1,3,4-triazoles,[3] as well as 1,3,4-oxa- and thiazoles.[4] Furthermore, they are key components of aza-peptides, a type of peptidomimetic in which the α-carbon of one or more amino acid residues has been substituted by a nitrogen atom.[5] In aza-peptides, the replacement of Cα by nitrogen may confer enhanced stability,[6] resistance to proteases,[7] and conformational rigidity, such that β-turn conformers have been observed by X-ray crystallography, NMR spectroscopy, and computational analysis.[8]

(1) For a selected example, see: Ahn, J. H.; Kim, J. A.; Kim, H.-M.; Kwon, H.-M.; Huh, H.-C.; Rhee, S. D.; Kim, K. R.; Yang, S.-D.; Park, S.-D.; Lee, J. M.; Kim, S. S.; Cheon, H. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1337.

(2) Deng, X.; Mani, N. S. *J. Org. Chem.* **2008**, *73*, 2412. references within.

(3) Review of synthesis and pharmacological importance: Al-Masoudi, I. A.; Al-Soud, Y. A.; Al-Salihi, N. J.; Al-Masoudi, N. A. *Chem. Heterocycl. Comp.* **2006**, *42*, 1377.

(4) (a) Taylor, E. C.; Wipf, P. *Oxazoles: Synthesis, Reactions, and Spectroscopy*; John Wiley & Sons: Hoboken, 2003. (b) Lewis, J. R. *Nat. Prod. Rep.* **1999**, *16*, 389.

(5) (a) Gante, J. *Synthesis* **1989**, 405. (b) Huang, Y.; Malcom, B. A.; Vederas, J. C. *Bioorg. Med. Chem.* **1999**, *7*, 607.

(6) (a) Gassman, J. M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1771. (b) Wipf, P.; Adeyeye, C. M.; Rusnak, J. M.; Lazo, J. S. *Bioorg. Med. Chem.* **1996**, *4*, 1585.

(7) Dugave, C.; Demange, L. *Lett. Pept. Sci.* **2003**, *10*, 1.

10.1021/ol100932m
Published on Web 06/10/2010

In spite of the importance of substituted hydrazines, the inherent difficulty in distinguishing their two available nitrogen atoms has often led to the extensive use of protective groups for their regioselective assembly.[9] In exploring the combinatorial synthesis of aza-peptides on solid support, we have recently described a three-step procedure exploiting the use of a semicarbazone moiety to achieve selective alkylation and incorporation of hydrazine moieties in peptides.[10] We now report a method for selective *N*-arylation of the semicarbazone moiety in the presence of the multiple amide and carbamate nitrogens within the aza-peptide by employing copper catalysis. This novel chemistry constitutes the gateway for the rapid synthesis of aza-arylglycine moieties, whose arylglycine counterparts have long sparked interest due to their nature as nonproteogenic α-amino acids found in *β*-lactam[11] and peptide antibiotics,[12] such as ampicillin and vancomycin. Furthermore, inherent issues of racemization that plague the use of arylglycines[13] are alleviated by employing their aza-amino acid counterparts.

Our interest in aza-arylglycine peptide synthesis stems from the desire to replace tryptophan (Trp) with aza-residues bearing indolyl moieties in peptidomimetics of the hexapeptide Growth Hormone Releasing Peptide-6 (GHRP-6, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2), a lead for developing novel antiangiogenic compounds.[10] Although incorporation of an aza-Phe residue at the Trp^4 position confered selectivity for the CD36 receptor as compared to the native sequence,[10] attempts to prepare the aza-Trp peptide met with loss of the indolylmethyl moiety giving the aza-Gly counterpart after acidic cleavage with TFA (Figure 1).[14] Pursuing aza-

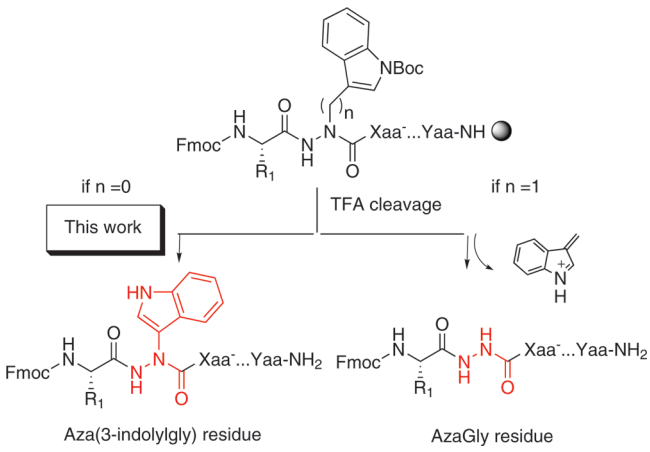
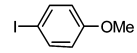
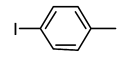
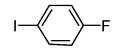
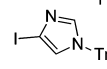


Figure 1. Synthesis of aza-peptide bearing indolyl moieties.

indolylglycine as a stable aza-Trp surrogate, we have conceived a general method for making aza-arylglycine peptide analogues, featuring *N*-arylation of a resin-bound aza-glycine peptide with a variety of aryl and heteroaryl iodides using CuI, ethylene diamine (EDA), and potassium *tert*-butoxide in dioxane.[15] To the best of our knowledge, this method constitutes the first example of selective semicarbazone monoarylation. Moreover, use of *N*-Boc-3-iodoindole[16] and *N*-trityl-4-iodoimidazole[17] in Cu-catalyzed C−N bond formation gave access to unprecedented aza-Trp and aza-His mimics. Conditions for *N*-arylation were initially screened using semicarbazone **3** anchored on Rink amide resin (Scheme 1). Among the different combinations of bases, copper salts, ligands, and solvents tested (see Supporting Information), the best results were achieved using a 5-fold excess of CuI, KO*t*Bu, EDA, and ArI in dioxane in the presence of 4 Å powdered molecular sieves for 24 h. Under these conditions, *N*-arylation of the semicarbazone at the Trp^4 position of the GHRP-6 sequence proceeded with conversions between 52 and 94% (Table 1). In all cases, LCMS

Table 1. *N*-Arylation at Residue Positions 2 and 4 of GHRP-6

entry	ArI	LCMS conversions at 214 nm Trp^4 modification	LCMS conversions at 214 nm D-Trp^2 modification
1		52	39
2		56	27
3		94	57
4		92	39
5		89	23
6		77	10
7		67	N/A

analysis indicated complete consumption of starting material with formation of byproduct. It is noteworthy that arylation using the same conditions, yet further along the peptide sequence, afforded Trp^2 analogues in lower conversions, suggesting that arylation may be impeded by the peptide adopting secondary structure on solid support. In spite of lower conversions in the arylation at the 2-position, six GHRP-6 analogues (**10a**−**f**, R = H) could be obtained in suitable yield and purity after completion of the peptide sequence, cleavage, and HPLC purification (Table 1).

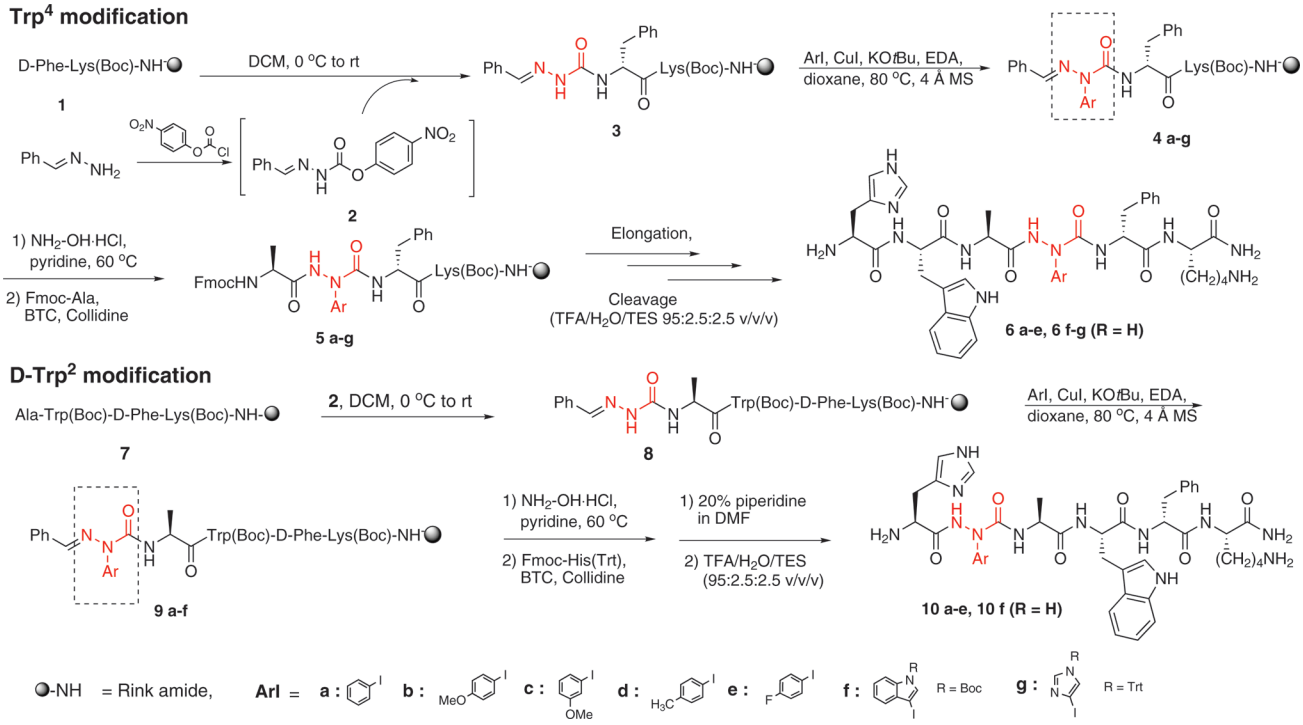
To gain additional insight into the arylation reaction, benzylidene aza-glycinyl-phenylalaninyl isopropylamide **11** (Scheme 2) was prepared and examined as a model in the

(8) (a) Reynolds, C. H.; Hormann, R. E. *J. Am. Chem. Soc.* **1996**, *118*, 9395–9401. (b) Lee, H.-J.; Song, J.-W.; Choi, Y.-S.; Park, H.-M.; Lee, K.-B. *J. Am. Chem. Soc.* **2002**, *124*, 11881–11893. (c) André, F.; Boussard, G.; Bayeul, D.; Didierjean, C.; Aubry, A.; Marraud, M. *J. Pept. Res.* **1997**, *49*, 556–562. (d) André, F.; Vicherat, A.; Boussard, G.; Aubry, A.; Marraud, M. *J. Pept. Res.* **1997**, *50*, 372–381.

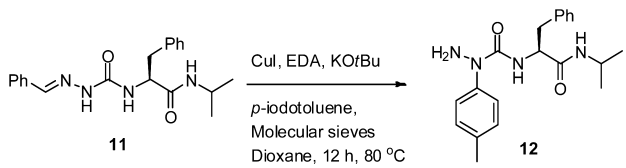
(9) For a review on the synthesis of alkyl-substituted hydrazines, see: Ragnarsson, U. *Chem. Soc. Rev.* **2001**, *30*, 205.

(10) Sabatino, D.; Proulx, C.; Klocek, S.; Bourguet, C. B.; Boeglin, D.; Ong, H.; Lubell, W. D. *Org. Lett.* **2009**, *11*, 3650.

Scheme 1. Synthesis of [Aza-Arylglycine] GHRP-6 Analogues



Scheme 2. Solution-Phase *N*-Arylation of Aza-Glycinyl-phenylalanine Isopropylamide



solution-phase *N*-arylation, which occurred with concurrent hydrazone deprotection to afford aza-toluylglycine dipeptide amide **12** as a single product, albeit in a 20% yield. Hydrazone removal may occur as a result of a nucleophilic attack by ethylene diamine. By comparison, deprotection of arylated material was also observed as a minor product peak in the LCMS analysis of material cleaved from the solid support.

On solid support, completion of the peptide sequence necessitated scavenging of copper by shaking the resin with a 3:1 DMF:0.1 N HCl mixture overnight.[18] Chemoselective deprotection of the hydrazone was achieved using $NH_2OH{\cdot}HCl$ in pyridine.[10] The remaining sequences were completed according to general methods for peptide synthesis[19] to afford [aza-arylglycine] GHRP-6 analogues with crude purities ranging from 7 to 65% (Table 2). Material of

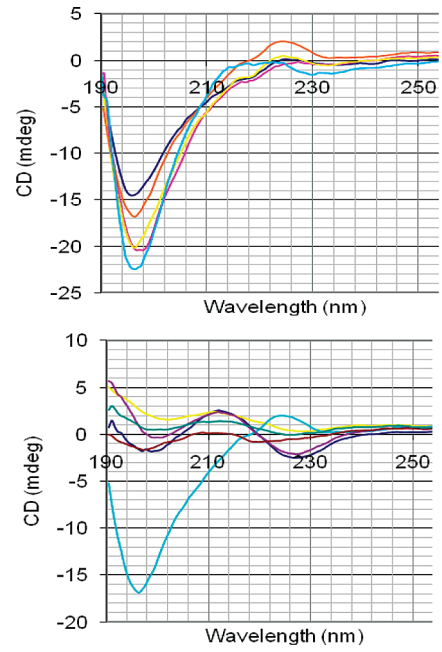


Figure 2. Circular dichroism spectra of GHRP-6 with [aza-arylgly^2]-(upper) and with [aza-arylgly^4]-GHRP-6 analogues (lower) in water.

(11) Townsend, C. A.; Brown, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 913.
(12) Williams, D. H.; Waltho, J. P. *Biochem. Pharmacol.* **1988**, *37*, 133.
(13) Smith, G. G.; Sivakua, T. *J. Org. Chem.* **1983**, *48*, 627.
(14) Boeglin, D.; Lubell, W. D. *J. Comb. Chem.* **2005**, *7*, 864.
(15) Similar conditions used for arylation of indoles on solid support in: Wu, T. Y. H.; Schultz, P. *Org. Lett.* **2002**, *4*, 4033.
(16) Wiltulski, B.; Buschmann, N.; Bergsträber, U. *Tetrahedron* **2000**, *56*, 8473.
(17) Cliff, M. D.; Pyne, S. G. *Synthesis* **1994**, 681.
(18) Xu, W.-M.; Huang, X.; Tang, E. *J. Comb. Chem.* **2005**, *7*, 726.

Table 2. Yields and Purities of Aza-Arylglycine-Containing GHRP-6 Analogues **10a**−**f** and **6a**−**g**

peptide	crude purity[a] (%)	purity[b] (%) MeOH	purity[b] (%) MeCN	yield[c] (%)	HRMS $[M + H]^+$ or $[M + Na]^+$ ions *m/z* (calcd)	HRMS $[M + H]^+$ or $[M + Na]^+$ ions *m/z* (obsd)
10a His-Aza(phenylglly)-Ala-Trp-D-Phe-Lys-NH_2	23	>99	>99	2.0	821.4205	821.4204
10b His-Aza(*m*-methoxyphenylglly)-Ala-Trp-D-Phe-Lys-NH_2	22	>99	>99	1.2	851.4311	851.4296
10c His-Aza(*p*-methoxyphenylglly)-Ala-Trp-D-Phe-Lys-NH_2	34	>99	>99	1.4	851.4311	851.4300
10d His-Aza(*p*-toluylglly)-Ala-Trp-D-Phe-Lys-NH_2	16	>99	>99	1.2	835.4362	835.4358
10e His-Aza(*p*-fluorophenylglly)-Ala-Trp-D-Phe-Lys-NH_2	7	>99	>99	1.4	839.4111	839.4105
10f His-Aza(3-indolylglly)-Ala-Trp-D-Phe-Lys-NH_2	9	91	92	0.8	882.4134	882.4121
6a His-D-Trp-Ala-Aza(phenylglly)-D-Phe-Lys-NH_2	65	>99	>99	1.0	821.4205	821.4217
6b His-D-Trp-Ala-Aza(*m*-methoxyphenylglly)-D-Phe-Lys-NH_2	22	>99	>99[d]	1.0	851.4311	851.4293
6c His-D-Trp-Ala-Aza(*p*-methoxyphenylglly)-D-Phe-Lys-NH_2	23	>99	>99	1.2	851.4311	851.4307
6d His-D-Trp-Ala-Aza(*p*-toluylglly)-D-Phe-Lys-NH_2	45	92	>99	3.4	835.4362	835.4352
6e His-D-Trp-Ala-Aza(*p*-fluorophenylglly)-D-Phe-Lys-NH_2	28	>99[d]	>99[d]	2.8	839.4111	839.4106
6f His-D-Trp-Ala-Aza(3-indolylglly)-D-Phe-Lys-NH_2	27	>99	>99	1.6	860.4314	860.4321
6g His-D-Trp-Ala-Aza(5-imidazoylglly)-D-Phe-Lys-NH_2	65	96	>99	1.6	811.4110	811.4080

[a] RP-HPLC purity at 214 nm of the crude peptide in MeOH/H_2O eluant containing 0.1% formic acid. [b] RP-HPLC purity at 214 nm of the purified peptide in MeOH/H_2O and MeCN/H_2O eluant containing 0.1% formic acid. [c] Yields after purification by RP-HPLC are based on resin loading. [d] Aza-peptide was isolated as a mixture of His epimers.

≥91% purity was subsequently isolated by reverse-phase HPLC and used in circular dichroism (CD) spectroscopic studies (Figure 2) for assessing the conformational effects of the aza-arylglycine moiety on peptide conformation. Affinity for the CD36 receptor is under investigation and will be reported in due time.

In the CD spectra in water, native GHRP-6 and aza-peptide analogues **10a**−**f** (R = H), in which D-Trp[2] was replaced by the aza-arylglycine moiety, all displayed CD signatures characteristic of a random coil, exhibiting a negative maximum around 190 nm. Conversely, CD signatures of [aza-arylgly[4]]-GHRP-6 analogues **6a**−**g** (R = H) displayed distinctive negative maxima at 230 and 190 nm and a positive maximum at 215 nm, suggestive of a β-turn conformation.

A novel solid-phase methodology featuring the copper-catalyzed *N*-arylation of a semicarbazone has furnished 13 aza-arylglycine GHRP-6 analogues. Employing heteroaryl iodides, aza-peptides bearing indole and imidazole side chains were synthesized as aza-Trp and aza-His surrogates. Replacement of Trp[4] with aza-arylglycine residues in the GHRP-6 sequence induced a β-turn conformation in water as indicated by circular dichroism spectroscopy. Their effective synthesis and the conformational bias of the aza-arylglycinyl residues make this methodology particularly attractive for studying structure−activity relationships of biologically active peptides.

Acknowledgment. This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Fonds Québecois de la Recherche sur la Nature et les Technologies (FQRNT). C.P. is grateful to NSERC and Boehringer Ingelheim for graduate student fellowships. The authors thank Dr. A. Fürtös, K. Venne, and M.-C. Tang (Université de Montréal) for assistance with mass spectrometry and Dr. David Sabatino (Université de Montréal) for help with CD spectroscopy.

Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL100932M

(19) Lubell, W. D.; Blankenship, J. W.; Fridkin, G.; Kaul, R. *Peptides. Science of Synthesis 21.11, Chemistry of Amides*; Thieme: Stuttgart, Germany, 2005; pp 713−809.