Protocol

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# Solid-phase synthesis of C-terminal azapeptides‡

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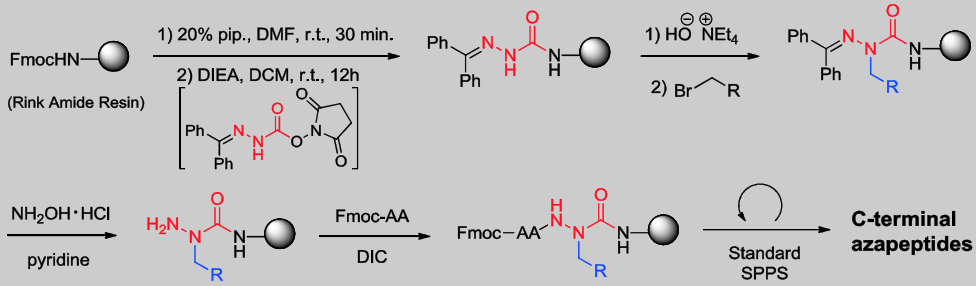
**The solid-phase synthesis of azapeptides possessing a C-terminal aza-residue has been accomplished by a protocol featuring regioselective alkylation of benzhydrylidene-aza-glycinamide and illustrated by the syntheses of [aza-Lys$^6$] growth-hormone-releasing peptide-6 analogs. Copyright © 2014 European Peptide Society and John Wiley & Sons, Ltd.**

**Keywords:** C-terminal azapeptides; regioselective alkylation; benzhydrylidene-protecting group; acylation of semicarbazide

**REACTION SCHEME**




**GENERAL OPTIMIZED PROCEDURE**

Benzophenone hydrazone was pre-activated with *N,N′*-disuccinimidyl carbonate (DSC) and reacted on the amine of Rink resin. Regioselective alkylation of supported benzhydrylidene-aza-glycinamide Rink resin was performed with aqueous tetraethylammonium hydroxide (TEAH) and various alkyl halides. The benzhydrylidene-protecting group was removed using hydroxylamine hydrochloride in pyridine. Acylation of the resulting semicarbazide using *N*-(Fmoc)amino acid symmetric anhydrides provided the *C*-terminal aza-dipeptide that was elongated, cleaved, and deprotected using standard solid-phase peptide synthesis protocols to furnish the desired *C*-terminal azapeptides.

## Scope and Comments

Azapeptides are peptide derivatives in which the Cα atom of one or more amino acids in the sequence has been replaced by a nitrogen atom [1]. In azapeptides, the use of a semicarbazide as an amino acid surrogate causes profound effects on the conformation and physical properties of the peptide because of the nature of the aza-residue. For example, spectroscopic, X-ray crystallographic and computational studies all have indicated that the semicarbazide constraint can favor a turn conformation within the azapeptide [2–4]. Moreover, the aza-residue may enhance the stability of the peptide against protease degradation [1,5]. In this regard, azapeptides offer potential for improving peptide-based drug candidates. For example, [aza-Val$^3$] angiotensin-II (bovine) [Asp-Arg-aza-Val-Tyr-Val-His-Pro-Phe] exhibited longer duration of action relative to its parent peptide, albeit with lower potency [6]. Furthermore, C-terminal aza-analogs of luteinizing hormone releasing hormone (LHRH), such as [aza-Gly$^{10}$] LHRH [7,8], retained significant biological activity and exhibited

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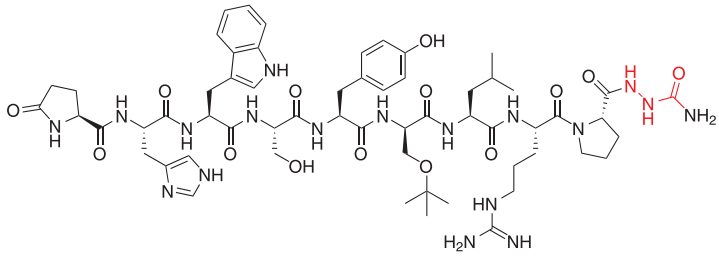

**Figure 1.** Chemical structure of Zoladex.

improved metabolic stability likely by avoiding degradation by C-terminal peptidases. Studies of C-terminal aza-analogs of LHRH led to the potent long-acting agonist pyroGlu-His-Trp-D-Ser(*t*-Bu)-Tyr-Gly-Leu-Arg-Pro-aza-Gly-$NH_2$ (goserelin acetate or Zoladex, AstraZeneca, Figure 1), which is currently used in the clinic for the treatment of prostate and breast cancer [9].

The application of azapeptides in drug discovery is expected to grow with the development of effective solid-phase methods [10]. In particular, the discovery of a submonomer strategy featuring regioselective alkylation of an aza-glycine moiety on a solid support has unleashed azapeptide synthesis from the difficulties of solution-phase hydrazine chemistry and enabled broader side chain diversity to be introduced at aza-residues [11,12]. Nevertheless, the solid-phase synthesis of C-terminal azapeptides has remained a challenge. Notably, a limited number of C-terminal azapeptides have been previously synthesized using *N*-(Fmoc)-aza-amino acid and (Ddz)-aza-amino acid chlorides that were respectively prepared from *N*′-alkyl 9-fluorenylmethyl and 2-(3,5-dimethoxyphenyl)propan-2-yl carbazates on activation with phosgene prior to coupling to the amine of the Rink amide resin [13–15]. Side chain diversity is, however, limited in scope using this approach, which necessitates synthesis of hydrazine building blocks in solution [16]; moreover, the toxicity of phosgene and side products such as oxadiazole analogs from phosgene activation inhibits the versatility of this method [1,17].

In efforts to further develop the submonomer strategy, we became interested in C-terminal azapeptides and have recently communicated an approach for synthesizing growth-hormone-releasing peptide-6 (GHRP-6) analogs possessing C-terminal aza-residues [18]. Key to this approach has been the application of benzhydrylidene-aza-glycinamide Rink resin because of the ability to measure conversion to the alkylated residue by comparison of the starting and modified benzhydrylidene-aza-amino amides after cleavage of the resin with acid and analysis by LC-MS. In this protocol, details are provided for the solid-phase method to prepare C-terminal azapeptides having a broader side chain diversity, by a route featuring (i) synthesis of the benzhydrylidene-aza-glycinamide Rink resin, (ii) regioselective alkylation of the aza-glycinamide moiety, (iii) removal of the semicarbazone, and (iv) coupling of amino acids to the resulting semicarbazide employing the symmetric anhydride strategy. In addition, the utility of this method has been demonstrated by the synthesis of [aza-Lys$^6$]GHRP-6 derivatives.

Benzhydrylidene-aza-glycinamide Rink resin **1** was synthesized by activation of benzophenone hydrazone with DSC (Figure 2) [17] and coupling of the activated benzhydrylidene-carbazate to the amine of the Rink resin. Qualitative completion of the reaction was ascertained by a negative Kaiser test; moreover, formation of benzhydrylidene-aza-glycinamide was validated by cleavage of a resin aliquot with a freshly made solution of TFA/$H_2O$/triethylsilane (TES; 95/2.5/2.5), removal of the resin by filtration, and analysis of the residue by LC-MS, which indicated one single peak (retention time (R.T.) = 4.97 min, 50–90% MeOH in water containing 0.1% formic acid (FA) over 10 min) having the desired molecular ion (ESI-MS *m/z* calcd for $C_{14}H_{14}N_3O$ $[M+H]^+$ 240.1, found 240.1).

Three different side chains were initially introduced onto the aza-residue by treating aza-glycinamide resin **1** with TEAH (120 mol %, as a 40% aqueous solution) in THF followed by alkylation with the respective alkyl halide: 1-bromo-4-chlorobutane, propargyl bromide, and 4-methylbenzyl bromide. Conversion to the desired alkyl aza-glycinamide resins **2** was ascertained after cleavage of a resin aliquot with acid followed by LC-MS analysis (Figure 2). Moreover, resin **2c** could be cleaved using a freshly made solution of TFA/$H_2O$/TES (95/2.5/2.5), and purification of the residue by preparative HPLC gave aza-(4-methyl) phenylalanine derivative **3c** in 24% overall yield.

The 4-chlorobutyl and propargyl groups were selected to demonstrate further methods for adding side chain diversity at the C-terminal reside, respectively, by displacement of the chloride with different amines and copper-catalyzed addition of Mannich reagents to the alkyne (so-called $A^3$-reaction) [19,20]. In principle, these side chains may be respectively diversified using other nucleophiles to displace the chloride [18], as well as by copper-catalyzed azide-alkyne cycloaddition reactions [18,21].

In the case of 4-chlorobutyl glycinamide **2a**, displacement of the chloride with sodium azide gave the corresponding azalysine **4** in 90% conversion as determined by LC-MS of the crude residue after TFA cleavage (Figure 3). Semicarbazone resin **4** was then converted to semicarbazide **5** by transimination employing $NH_2OH \cdot HCl$ in pyridine at 60 °C for 12 h [11]. Semicarbazide **5** was coupled to Fmoc-D-Phe-OH by way of its symmetric anhydride, which was prepared using *N*,*N*′-diisopropylcarbodiimide (DIC) [18,22]. Azapeptide **6** was elongated by standard Fmoc-based solid-phase peptide synthesis [18,20,22]. Azide **7** was reduced using tris(2-carboxy)

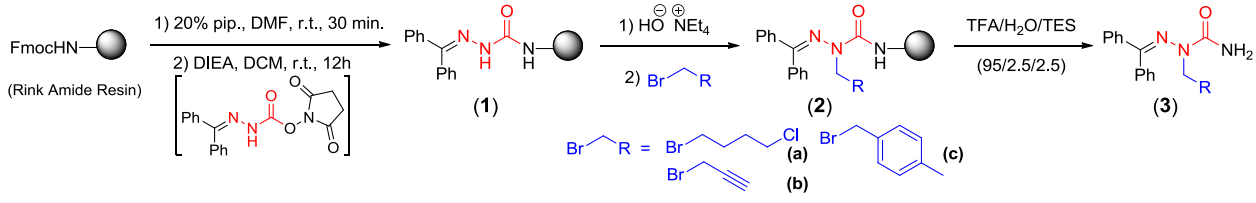


**Figure 2.** Synthesis and alkylation of a benzhydrylidene-protected aza-Gly resin.

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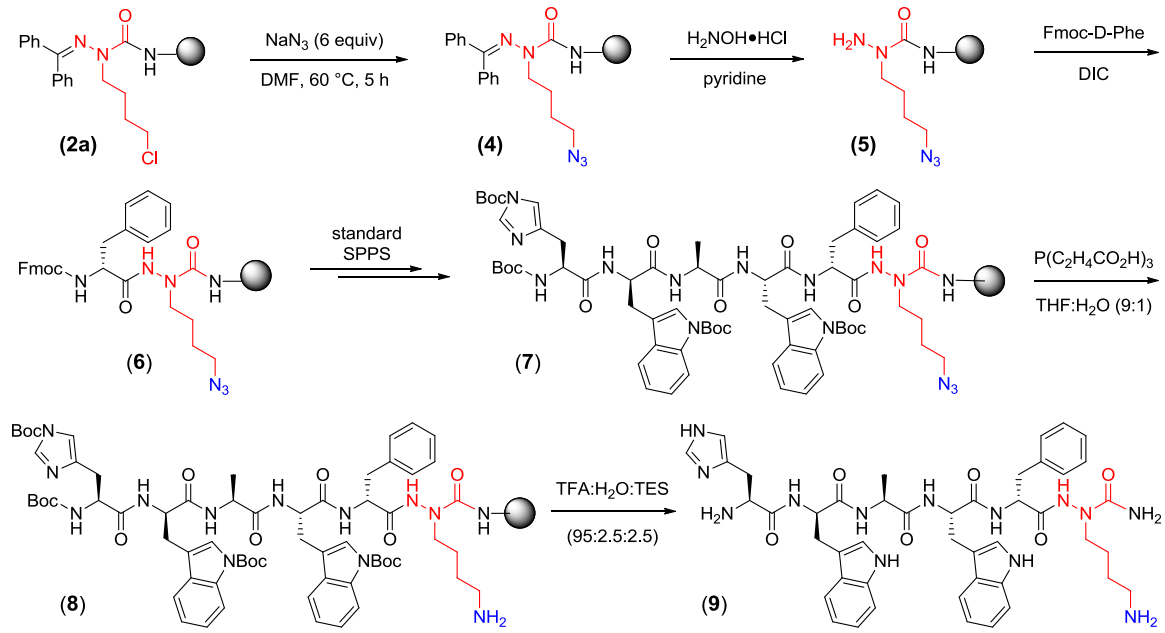


**Figure 3.** Synthesis of [aza-Lys$^6$]GHRP-6.

ethylphosphine (TCEP) in THF/$H_2O$ (9/1) for 4 h to provide peptidyl-resin **8** [18]. After deprotection and cleaving from the resin, followed by purification by preparative HPLC, azapeptide **9** was isolated in 7% overall yield and >95% purity (Figure 3).

Employing benzhyldrylidene aza-propargylglycinyl resin **2b**, the A$^3$-reaction was performed in DMSO using diethylamine, aqueous formaldehyde, and CuI to give resin **10** in complete conversion, as assessed by LC-MS of the residue after resin cleavage (Figure 4) [20]. Semicarbazone **10** was converted to its semicarbazide counterpart, acylated with Fmoc-D-Phe and DIC, elongated, and cleaved as described previously to provide [*N*,*N*′-diethylamino-aza-Lys$^6$] GHRP-6 analog **12** in 13% overall yield (>99% purity) after purification by preparative HPLC.

## Experimental Procedure

### General

Polystyrene Rink amide resin (0.6 mmol/g, 75–100 mesh) was purchased from Advanced Chemtech™, and a standard Fmoc loading test was used to ascertain resin capacity. Reagents, including DSC, benzophenone hydrazone, TEAH, hydroxylamine hydrochloride, pyridine, FA, *N*,*N*-diisopropylethylamine (DIEA), diethylamine, 1-bromo-4-chlorobutane, and 4-methylbenzyl bromide, all were purchased from Aldrich and used without further purification. Protected amino acids Fmoc-L-Trp(Boc)-OH and Fmoc-D-Trp(Boc)-OH, Fmoc-D-Phe-OH, Fmoc-Ala-OH, and Boc-His(Trt)-OH were purchased from Novabiochem (EMD Bioscience Inc., San Diego, California) or GL Biochem, Ltd. (Shanghai, China). All solvents were obtained from VWR International. Analytical LC-MS analyses were performed on a 100-Å, 3-μm, 100 × 4.6-mm C18 SunFire column™ with a flow rate of 0.35 ml/min using a gradient of distilled water with 0.1% FA in acetonitrile with 0.1% FA. Peptide analogs were purified on a preparative column (250 × 21.2 mm, 5 μm, Gemini™ C18) using various gradients of distilled water with 0.1% FA in acetonitrile with 0.1% FA at a flow rate of 10.0 ml/min.

### Synthesis of Benzhydrylidene-Protected aza-Gly Resin 1

The synthesis of benzhydrylidene-protected aza-Gly resin **1** was performed using a free-amine Rink resin and benzophenone

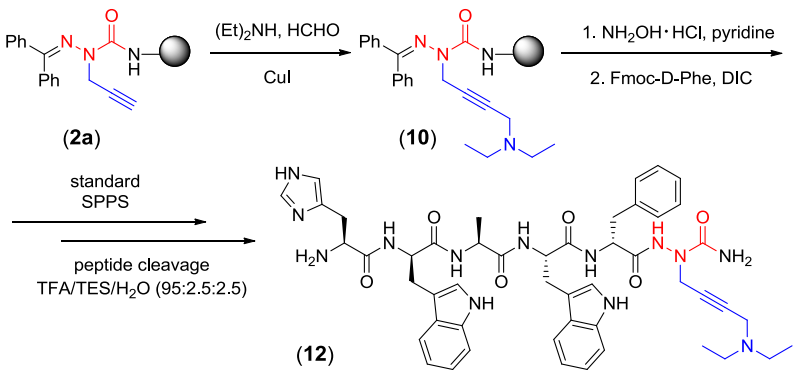


**Figure 4.** Synthesis of [*N*,*N*′-diethylamino-aza-Lys$^6$]GHRP-6 analog **12**.

hydrazone that was preactivated with DSC. In a round-bottom flask, a solution of DSC (510 mg, 2 mmol, 3.3 equiv) in DMF (8 ml) was treated dropwise with a solution of benzophenone hydrazone (353 mg, 1.8 mmol, 3 equiv), stirred for 2 h at room temperature, treated with DIEA (316 μl, 1.8 mmol, 3 equiv), and the resulting solution was quickly transferred to a plastic syringe tube equipped with a Teflon™ filter, stopper, and stopcock containing the free amine of the Rink resin (1 g, 0.6 mmol/g, 1 equiv). After agitation on an automated shaker for 16 h at room temperature, the resin was filtered, washed with DMF (3 × 10 ml), MeOH (3 × 10 ml), and DCM (3 × 10 ml), dried under vacuum, and stored in the fridge. The reaction was monitored by the Kaiser test. The residue obtained from cleavage of a resin aliquot (3 mg), after treatment with 1 ml of TFA/TES/$H_2O$ (95 : 2.5 : 2.5, v/v/v), resin filtration, and evaporation, was analyzed by LC-MS [50–90% MeOH (0.1% FA) in water (0.1% FA) over 10 min], which indicated a single peak (R.T. = 4.97 min) with a molecular ion corresponding to the desired product.

### Alkylation of Benzhydrylidene-Protected aza-Gly Resin 1

To a plastic syringe tube equipped with a Teflon™ filter, stopper, and stopcock containing a suspension of benzhydrylidene-protected aza-Gly resin **1** (200 mg, 0.6 mmol/g, 1 equiv) swollen in THF (3 ml), a solution of 40% TEAH in water (233 μl, 0.36 mmol, 3 equiv) was added, and the mixture was agitated on an automated shaker for 30 min at room temperature. Propargyl bromide (80% in toluene, 40.5 μl, 0.36 mmol, 3 equiv), 4-methylbenzyl bromide (Figure 2, bromide c; 100 μl, 0.72 mmol, 6 equiv), or 4-bromo-chlorobutane (44.3 μl, 0.38 mmol, 3.2 equiv) was respectively added to the resin mixture, which was agitated for an additional 2 h for 4-bromo-chlorobutane, or for an additional 12 h for propargyl and 4-methylbenzyl bromides. The resin was filtered; washed with DMF (3 × 10 ml), MeOH (3 × 10 ml), THF (3 × 10 ml), and DCM (3 × 10 ml); and dried under vacuum. Aliquots of resins **2a** and **2b** were respectively cleaved using a freshly made solution of TFA/$H_2O$/TES (95/2.5/2.5) for 1 h, and the residue was analyzed by LC-MS, which indicated respectively single peaks for **3a** [R.T. = 7.12 min, 20–80% MeOH (0.1% FA) in water (0.1% FA) over 14 min, ESI-MS *m/z* calcd for $C_{18}H_{20}ClN_3O$ $[M+H]^+$ 330.1, found 330.1] and for **3b** [R.T. = 5.79 min, 50–95% MeOH (0.1% FA) in water (0.1% FA) over 10 min, ESI-MS *m/z* calcd for $C_{17}H_{16}N_3O$ $[M+H]^+$ 278.1, found 278.1].

Resin **2c** (250 mg) was cleaved from the support using 5 ml of a freshly made solution of TFA/$H_2O$/TES (95/2.5/2.5) at room temperature for 2 h. The resin was filtered and rinsed twice with 2 ml of TFA. The filtrate and rinses were evaporated to obtain yellow oil, which was diluted in MeOH, and analyzed by LC-MS analysis [70–90% MeOH (0.1% FA) in water (0.1% FA) over 14 min], which demonstrated 78% conversion (R.T. = 9.47 min). The residue was purified on a Waters™ Prep LC instrument equipped with an RP Gemini™ C18 column (250 × 21.2 mm, 5 μm) using a binary solvent system consisting of 70–90% MeOH (0.1% FA) in $H_2O$ (0.1% FA) at a flow rate of 10.0 ml/min with UV detection at 254 nm over 50 min. Fractions containing >99% pure product were combined, evaporated, and dried under vacuum to give azapeptide **3c** (14 mg, 0.04 mmol, 24% yield) as yellow oil. $^1$H NMR (300 MHz, MeOD) $\delta$ ppm 2.27 (s, 3H), 3.32 (dd, $J$ = 3.3 and 1.6, 2H), 4.43 (s, 2H), 6.82 (d, $J$ = 7.0 Hz, 2H), 7.04 (d, $J$ = 7.0 Hz, 2H), 7.18 (dd, $J$ =8.0 and 1.6 Hz, 2H), 7.25–7.36 (m, 4H), and 7.36–7.57 (m, 4H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ ppm 22.0, 128.2, 129.3, 130.0, 130.6, 130.7, 131.0, 132.0, 132.3, 135.8, 138.0, 138.7, 140.7, 163.5, and 164.0. High resolution MS (HRMS) calcd *m/z* for $C_{22}H_{22}N_3O$ $[M+H]^+$ 344.1757, found 344.1772. Analysis by LC-MS [70–90% MeOH (0.1% FA) in $H_2O$ (0.1% FA) over 14 min, R.T. = 6.0 min] on a SunFire C18 analytical column (100 Å, 3.5 μm, 4.6 × 100 mm) demonstrated **3c** to be of >99% purity.

### $A^3$-Coupling of Benzhydrylidene-Protected aza-Propargylglycinamide Resin (2a) Using Diethylamine and Formic Aldehyde

A syringe containing resin **2b** (400 mg, 0.168 mmol) swollen in 4 ml of DMSO was treated sequentially with diethylamine (104 μl, 1.01 mmol), aqueous formaldehyde (82 μl, 1.01 mmol, 37% in $H_2O$), and CuI (6.5 mg, 0.034 mmol) and shaken on an automated shaker for 3 h. After filtration, the resin was washed sequentially with AcOH/$H_2O$/DMF (5 : 15 : 80, v/v/v, 3 × 5 ml), THF (3 × 5 ml), MeOH (3 × 5 ml), and DCM (3 × 5 ml). Examination by LC-MS of an aliquot of the residue from resin cleavage using DCM/TFA (v/v, 1 : 1) for 20 min showed the desired amide from azalysine analog **10** [R.T. = 5.24 min, 30–95% MeOH (0.1% FA) in water (0.1% FA) over 10 min, ESI-MS *m/z* calcd for $C_{22}H_{27}N_4O$ $[M+H]^+$ 363.2, found 363.2], together with benzophenone as a side product from semicarbazone hydrolysis. After cleavage of the resin (200 mg, 0.084 mmol) using DCM/TFA (v/v, 1 : 1) for 30 min, filtration of the resin, and evaporation of the filtrate and washings, the residue was purified by preparative TLC. The amide from cleavage of aza-lysine analog **10** was isolated as yellow oil (8 mg, 26%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.58–7.33 (m, 10H), 4.09 (t, $J$ = 1.9 Hz, 2H), 3.36 (t, $J$ = 1.9 Hz, 2H), 2.46 (q, $J$ = 7.2 Hz, 4H), and 1.01 (t, $J$ = 7.2 Hz, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.58, 158.85, 138.60, 135.45, 130.23, 129.84, 129.10, 128.70, 128.64, 128.15, 79.21, 78.28, 47.13, 40.67, 35.02, and 12.58. HRMS *m/z* calcd for $C_{22}H_{27}N_4O$ $[M+H]^+$ 363.2185, found 363.2179.

### Conversion of Semicarbazone 4 to Semicarbazide 5

Benzhydrylidene-4-azidobutyl-aza-glycinamide Rink resin **4** (200 mg, 0.12 mmol) in a plastic syringe tube equipped with a Teflon™ filter, stopper, and stopcock was treated with a freshly made solution of 1.5 M $NH_2OH \cdot HCl$ in pyridine (5 ml), heated in a water bath at 60 °C with sonication for 12 h, filtered, and washed with DMF (3 × 5 ml), MeOH (3 × 5 ml), and DCM (3 × 5 ml). Full conversion to the corresponding semicarbazide was determined by LC-MS analysis [20–80% MeOH (0.1% FA) in water (0.1% FA) over 14 min, R.T. = 10.5 min] of the residue obtained from cleavage of an aliquot of resin **5**.

### Coupling of *N*-(Fmoc)Amino Acid to Semicarbazide Resin 5 and Azapeptide Elongation

In dry DCM (10 ml), Fmoc-D-Phe-OH (1.33 g, 3.45 mmol, 10 equiv) and DIC (270 μl, 1.73 mmol, 5 equiv) were stirred at room temperature for 30 min. The resulting suspension was concentrated *in vacuo* to a residue, which was dissolved in DMF (10 ml) and added to a plastic syringe tube equipped with a Teflon™ filter, stopper, and stopcock containing semicarbazide resin **5** (500 mg, 0.345 mmol). After agitation for 16 h at room temperature on an automatic shaker, the reaction was shown to have >90% conversion to azadipeptide **6** by LC-MS analysis [10–80% MeOH (0.1% FA) in water (0.1% FA) over 12 min, R.T. = 8.3 min] of the residue obtained from cleavage of a resin aliquot (3 mg). Subsequent Fmoc removal and couplings to complete the target sequence were performed according to conventional Fmoc-based SPPS protocols [23]. Azide **7** was reduced to peptidyl-resin **8** using TCEP as previously reported [18]. Deprotection and resin cleavage with TFA/TES/$H_2O$ (95 : 2.5 : 2.5, v/v/v) gave a crude residue, which was purified by preparative RP-HPLC using binary solvent systems consisting of 5–30% MeOH (0.1% FA) in $H_2O$ (0.1% FA) over 60 min. Pure fractions were combined, freeze-dried, and lyophilized to a white

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powder: azapeptide **9** (3.4 mg, 1% yield); LC-MS [5–30% MeOH (1% FA) in water (1% FA) over 14 min] R.T. = 7.4 min, purity >99%; LC-MS [0–50% MeCN (1% FA) in water (1% FA) over 14 min] R.T. = 5.8 min, purity >99%; HRMS *m/z*: 896.4287, $[M + Na]^+$ calcd for $C_{45}H_{55}N_{13}NaO_6$, *m/z*, 896.4291.

[Aza-Lys$^6$]GHRP-6 **12** (20.3 mg, 13% overall yield) was obtained from semicarbazone **10**, using a similar semicarbazide liberation/acylation sequence, followed by peptide elongation and resin cleavage using standard Fmoc-based SPPS protocols, and purified by preparative RP-HPLC [5–40% MeOH (1% FA) in water (1% FA)]. Product purity was ascertained by LC-MS analysis [5–60% MeOH (1% FA) in water (1% FA) over 10 min] R.T. = 7.63 min, purity >99%; [5–60% MeCN (1% FA) in water (1% FA) over 10 min] R.T. = 8.65 min, purity >99%; HRMS *m/z* calcd for $C_{49}H_{59}N_{13}NaO_6$ $[M + Na]^+$ 948.4609, found 948.4615.

## Limitations

Although the benzhydrylidene protection offers a useful means for analyzing conversion of the alkylation of the aza-glycinamide residue, attempts to isolate the corresponding benzhydrylidene-protected aza-amino amides have met with varying success because of hydrolysis and loss of benzophenone. In the alkylation step, steric hindrance from the resin and the reactivity of the alkyl halide influence conversion and reaction times, limiting use of bulky alkyl halides. The quality of the resin has influenced the performance of the described chemistry, and certain resins that have proven effective for normal SPPS have given lower yields of azapeptide for reasons that are poorly understood at this time.

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## References

1 Proulx C, Sabatino D, Hopewell R, Spiegel J, Garcia Ramos Y Lubell WD Azapeptides and their therapeutic potential. *Future Med. Chem.* 2011; **3**: 1139–64.
2 Proulx C, Picard E, Boeglin D, Pohankova P, Chemtob S, Ong H Lubell WD Azapeptide analogues of the growth hormone releasing peptide 6 as cluster of differentiation 36 receptor ligands with reduced affinity for the growth hormone secretagogue receptor 1a. *J. Med. Chem.* 2012; **55**: 6502–11.
3 Reynolds CH Hormann RE Theoretical Study of the Structure and Rotational Flexibility of Diacylhydrazines: Implications for the Structure of Nonsteroidal Ecdysone Agonists and Azapeptides. *J. Am. Chem. Soc.* 1996; **118**: 9395–9401.
4 Benatalah Z, Aubry A, Boussard G Marraud M Evidence for a beta-turn in an azadipeptide sequence. Synthesis and crystal structure of ButCO-Pro-AzaAla-NHPri. *Int. J. Pept. Protein Res.* 1991; **38**: 603–5.
5 Tal-Gan Y, Freeman NS, Klein S, Levitzki A Gilon C Metabolic stability of peptidomimetics: N-methyl and aza heptapeptide analogs of a PKB/Akt inhibitor. *Chem. Biol. Drug Des.* 2011; **78**: 887–92.
6 Hess H-J, Moreland WT, Laubach GD. N-[2-Isopropyl-3-(L-aspartyl-L-arginyl)-carbazoyl]-L- tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalanine,1 an Isostere of Bovine Angiotensin II. *J. Am. Chem. Soc.* 1963; **85**: 4040–4041.
7 Dutta AS, Furr BJA, Giles MB Valcaccia B Synthesis and biological activity of highly active .alpha.-aza analogs of luliberin. *J. Med. Chem.* 1978; **21**: 1018–1024.
8 Dutta AS, Furr BJ Giles MB Polypeptides. Part 15. Synthesis and biological activity of alpha-aza-analogues of luliberin modified in positions 6 and 10. *J. Chem. Soc. Perkin. 1* 1979; **2**: 379–88.
9 Bolla M, Collette L, Blank L, Warde P, Dubois JB, Mirimanoff RO, Storme G, Bernier J, Kuten A, Sternberg C, Mattelaer J, Lopez Torecilla J, Pfeffer JR, Lino Cutajar C, Zurlo A Pierart M Long-term results with immediate androgen suppression and external irradiation in patients with locally advanced prostate cancer (an EORTC study): a phase III randomised trial. *Lancet* 2002; **360**: 103–6.
10 Melendez RE Lubell WD Aza-amino acid scan for rapid identification of secondary structure based on the application of N-Boc-aza(1)-dipeptides in peptide synthesis. *J. Am. Chem. Soc.* 2004; **126**: 6759–64.
11 Sabatino D, Proulx C, Klocek S, Bourguet CB, Boeglin D, Ong H Lubell WD Exploring side-chain diversity by submonomer solid-phase aza-peptide synthesis. *Org. Lett.* 2009; **11**: 3650–3.
12 Sabatino D, Proulx C, Pohankova P, Ong H Lubell WD Structure-activity relationships of GHRP-6 azapeptide ligands of the CD36 scavenger receptor by solid-phase submonomer azapeptide synthesis. *J. Am. Chem. Soc.* 2011; **133**: 12493–506.
13 Freeman NS, Tal-Gan Y, Klein S, Levitzki A Gilon C Microwave-assisted solid-phase aza-peptide synthesis: aza scan of a PKB/Akt inhibitor using aza-arginine and aza-proline precursors. *J. Org. Chem.* 2011; **76**: 3078–85.
14 Boeglin D Lubell WD Aza-amino acid scanning of secondary structure suited for solid-phase peptide synthesis with fmoc chemistry and aza-amino acids with heteroatomic side chains. *J. Comb. Chem.* 2005; **7**: 864–78.
15 Boeglin D, Xiang Z, Sorenson NB, Wood MS, Haskell-Luevano C Lubell WD Aza-scanning of the potent melanocortin receptor agonist Ac-His-D-Phe-Arg-Trp-NH. *Chem. Biol. Drug Des.* 2006; **67**: 275–83.
16 Ragnarsson U Synthetic methodology for alkyl substituted hydrazines. *Chem. Soc. Rev.* 2001; **30**: 205–213.
17 Garcia-Ramos Y Lubell WD Synthesis and alkylation of aza-glycinyl dipeptide building blocks. *J. Pept. Sci.* 2013; **19**: 725–9.
18 Traore M, Doan ND Lubell WD Diversity-oriented synthesis of azapeptides with basic amino Acid residues: aza-lysine, aza-ornithine, and aza-arginine. *Org. Lett.* 2014; **16**: 3588–91.
19 Peshkov VA, Pereshivko OP Van der Eycken EV A walk around the A3-coupling. *Chem. Soc. Rev.* 2012; **41**: 3790–807.
20 Zhang J, Proulx C, Tomberg A, Lubell WD Multicomponent diversity-oriented synthesis of aza-lysine-peptide mimics. *Org. Lett.* 2014; **16**: 298–301.
21 Proulx C, Lubell WD Aza-1,2,3-triazole-3-alanine Synthesis via Copper-Catalyzed 1,3-Dipolar Cycloaddition on Aza-progargylglycine. *J. Org. Chem.* 2010; **75**: 5385–5387.
22 Doan ND, Hopewell R Lubell WD N-aminoimidazolidin-2-one peptidomimetics. *Org. Lett.* 2014; **16**: 2232–5.
23 Lubell WD, Blankenship JW, Fridkin G Kaul R. *Science of Synthesis 21.11, Chemistry of Amides*, Thieme: Stuttgart, Germany,2005; 713–809.

  