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Solid-Phase Synthesis of Azasulfurylphenylalanine[4]-GHRP-6

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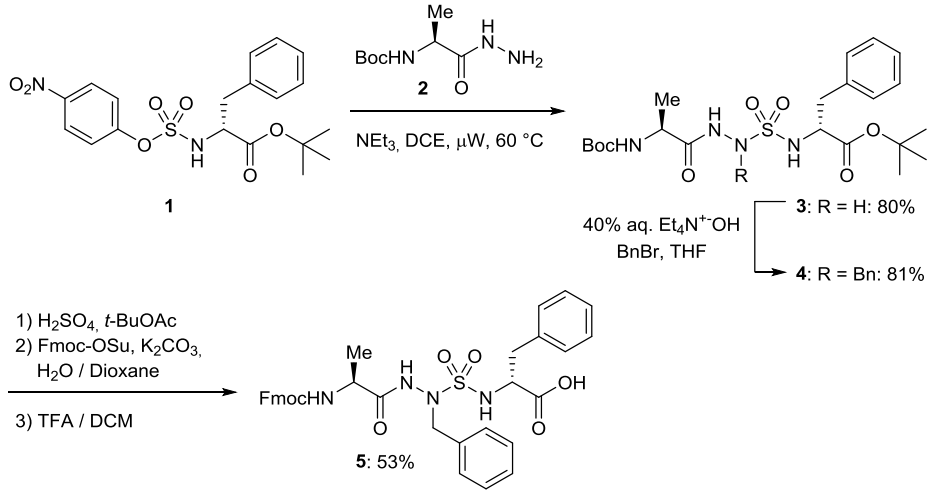
Introduction

Azasulfurylpeptides possess an amino sulfamide as an amino amide surrogate in which the $C_\alpha H$ and carbonyl components are respectively replaced by nitrogen and a sulfonyl group [1-4]. Uniting the properties of azapeptides [5] and sulfonamides [6-7], azasulfurylpeptides have served as transition state mimics of amide bond hydrolysis in a micromolar human immunodeficiency virus-1 (HIV-1) proteinase inhibitor [2]. Azasulfurylpeptides may similarly stabilize γ-turn conformations [8]. Solid-phase chemistry has however yet to be employed for the synthesis of azasulfurylpeptides. Targeting an azasulfurylpeptide analog of growth hormone releasing peptide-6 (GHRP-6, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) in the context of our program to develop modulators of the cluster of differentiation-36 [9-11], we report herein a solid-phase approach to prepare azasulfurylphenylalanine[4] [AsF[4]]-GHRP-6.

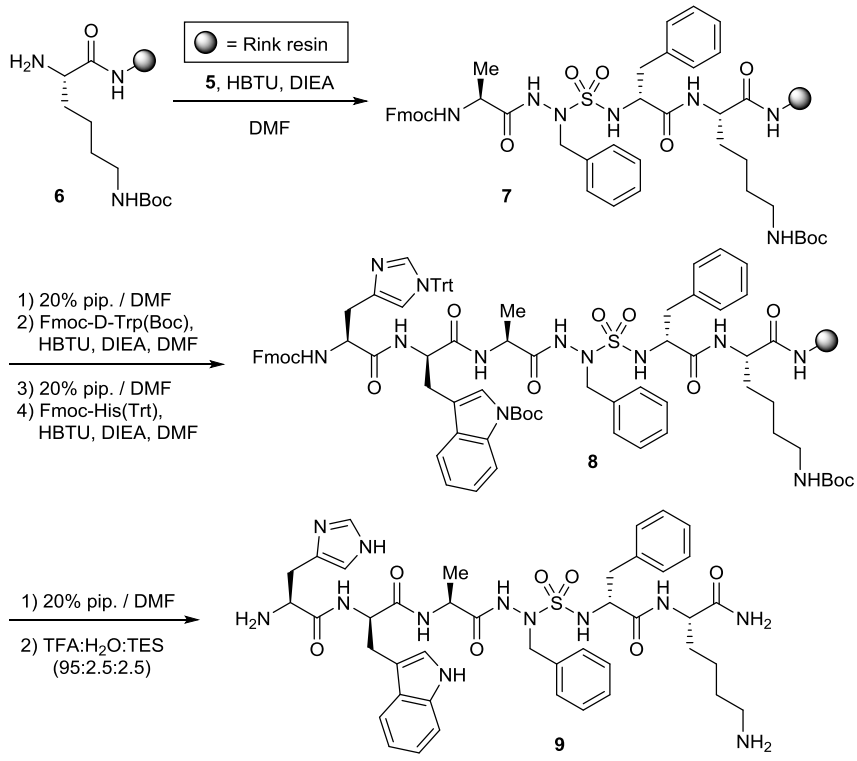
Results and Discussion

A solid-phase synthetic method was developed to make [AsF[4]]-GHRP-6 employing an AsF-tripeptide building block on Rink amide resin. The required AsF-tripeptide building block was synthesized in solution by a route featuring the construction and alkylation of azasulfurylglycine (AsG) tripeptide **3**, which was made without formation of symmetric sulfamide side product by acylation of hydrazide **2** with *p*-nitrophenyl-sulfamidate **1** (Scheme 1) [3]. Chemoselective alkylation on the AsG-tripeptide **3** using benzyl bromide and tetraethylammonium hydroxide installed the benzyl side-chain onto the *N*-aminosulfamide residue. Selective cleavage of the Boc protecting group of **4** in the presence of the *tert*-butyl ester was accomplished using 400 mol% of sulfuric acid in *tert*-butyl acetate [12]. Amine protection with the Fmoc group and carboxylate liberation by *tert*-butyl ester cleavage using TFA afforded *N*-Fmoc-alaninyl-azasulfurylphenylalaninyl-D-phenylalanine (**5**) in 53% overall yield starting from **4**.

*Scheme 1. Solution-phase syntheses of azasulfuryltripeptide N-(Fmoc)-Ala-AsF-D-Phe (**5**).*



*Scheme 2. Solid-phase peptide synthesis of [AsF[4]]-GHRP-6 (**9**).*



The AsF-tripeptide building block **5** was coupled onto the Lys(Boc) residue bound to Rink amide resin **6** using HBTU and DIEA in DMF (Scheme 2). Employing piperidine in DMF for Fmoc group removals, the peptide was elongated by couplings with Fmoc-D-Trp(Boc), followed by Fmoc-His(Trt) using HBTU and DIEA in DMF. Cleavage of the peptide from the resin using a solution of TFA:H_2O:TES (95:2.5:2.5) in a cold room (4 °C), and purification by HPLC afforded [AsF[4]]-GHRP-6 (**9**) in 12% overall yield from tripeptide **5** (Table 1).

Notably, an alternative strategy failed to provide the *N*-aminosulfamide on resin by the attempted generation of a supported sulfamidate on treatment of the peptide resin with *p*-nitrophenyl chlorosulfate, followed by coupling to hydrazide. In solution, activation of valine *iso*-propylamide with *p*-nitrophenyl chlorosulfate gave the desired sulfamidate in only 15% yield, presumably because intramolecular cyclization occurred on the *C*-terminal amide nitrogen to form a sulfahydantoin analog (not isolated) [13].

*Table 1. Yield and purity of [AsF[4]]-GHRP-6 (**9**).*

Peptide	*Crude purity[a]*	*Isolated Purity[b]*	*Isolated Yield[c]*	*HRMS*	
				m / z (cal)	*m / z (obs)*
His-D-Trp-Ala-AsF-D-Phe-Lys-NH_2 (**9**)	73%	>99%	12%	893.3851	893.3832

[a]Crude peptide purity ascertained by LC-MS analysis using 5-80% MeOH (0.1% FA) in H2O (0.1% FA) as eluent. [b]Isolated peptide purity ascertained by LC-MS analysis in two systems: MeOH (0.1% FA) in H2O (0.1% FA), and MeCN (0.1% FA) in H2O (0.1% FA). [c]Isolated yields calculated based on resin loading.

In conclusion, a solid-phase method was developed for the synthesis of [AsF^4]-GHRP-6 **(9)** featuring the solution-phase synthesis of AsF tripeptide **5** and its application on Rink amide resin. Building on this method for solid-phase synthesis, libraries of azasulfurylpeptides may be generated for studying structure-activity relationships.

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