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Synthesis of a Tripeptide Derivative Containing the Phe-Arg Hydroxyethylene Dipeptide Isostere

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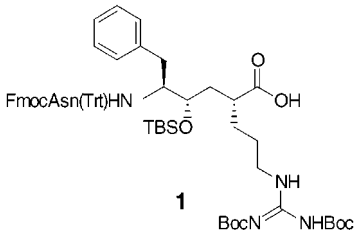
ABSTRACT



The protected hydroxyethylene dipeptide isostere of Phe-Arg and the tripeptide derivative 1 were synthesized as components of potential peptidase inhibitors. Key to the success of these syntheses is selective rhodium-catalyzed hydroboration in the presence of a readily reduced lactone. A convenient one-pot conversion of azides to diprotected guanidines was developed on the basis of the Staudinger reaction.

The botulinum family of neurotoxins (BoNT-A through G) are among the most lethal toxins known with a mouse LD_{50} = 0.1−0.5 ng/kg. Upon metabolic activation, the toxins produce zinc metalloproteases that cleave proteins involved in the release of acetylcholine at the neuromuscular junction, resulting in muscular paralysis.[1] The BoNT metallopeptidases are among the most selective peptidases yet identified as judged by their unusually large substrate size. The minimum cleavable substrate for BoNT-B is a 35-mer peptide,[2] and for BoNT-A a 17-mer peptide.[3] During the course of an investigation aimed at developing BoNT-A inhibitors, we became interested in synthesizing the Phe-Arg hydroxyethylene isostere as a possible transition-state mimetic inhibitor. The hydroxyethylene isostere, first synthesized for inhibition of renin,[4−6] has also been applied with success to the development of HIV protease[7] and β-secretase[8] inhibitors. Although the synthesis of hydroxyethylene isosteres (HE) has received considerable attention in the literature, most HE synthesized to date contain relatively unfunctionalized side chains. Herein we report the first synthesis of the fully functionalized Phe-Arg hydroxyethylene isostere as a tripeptide derivative.

The synthesis began from known lactone **2**[9] (Scheme 1) which was synthesized as described previously.[10] Alkylation of **2** with allyl bromide gave a mixture of diastereomers

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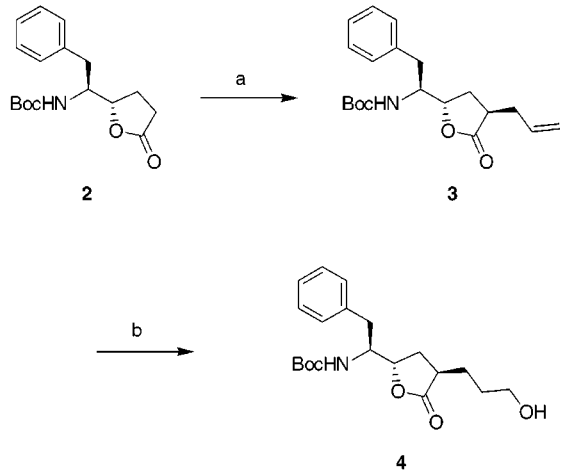
Scheme 1[a]

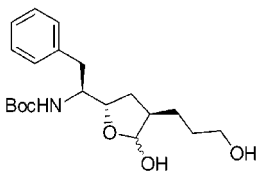


[a] (a) LDA, THF, −78 °C, 30 min; allyl bromide, DMPU, −78 °C, 15 min, then −50 °C, 1.5 h (62%); (b) $Rh(Ph_3P)_3Cl$, catecholborane, THF, 0 °C, then rt, 30 min; 30% H_2O_2, THF, EtOH, pH 7.2 buffer, rt, overnight (70%).

(5.3:1 trans to cis) that were separated by flash column chromatography to give the desired trans lactone **3** in 62% yield.

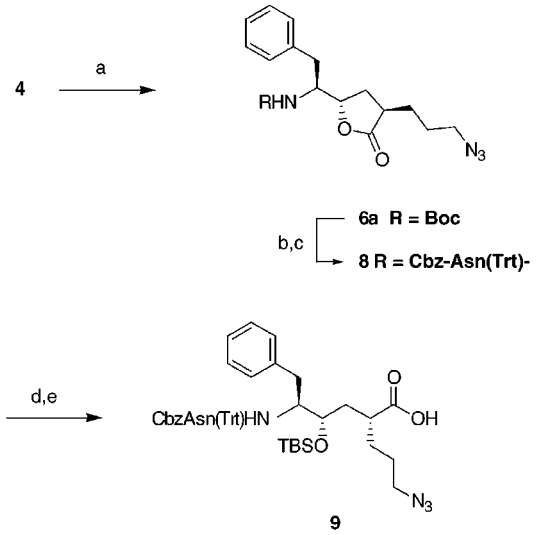
Conversion of lactone **3** to alcohol **4** by hydroboration followed by oxidation was initially problematic. Use of disiamylborane, 9-BBN, dicyclohexylborane, and (*S*)-alpineborane gave only low, variable yields of alcohol **4** due to competitive reduction of the γ-lactone to its corresponding hemiacetal **5**.[11] Attempts to suppress the formation of this side product by controlling the reaction temperature and the amount of borane used were unfruitful.

This problem was circumvented by using rhodium-catalyzed hydroboration conditions[12] followed by neutral oxidation. Under these conditions, olefin **3** was converted to alcohol **4** in 70% yield with no observable formation of **5**.



Alcohol **4** was readily converted to azide **6a** in 90% yield in a one-pot mesylation−displacement reaction (Scheme 2).[13]

Scheme 2[a]



[a] (a) MsCl, TEA, toluene, 0 °C to rt, 15 min; NaN_3, Bu_4NBr, H_2O, reflux 2 h (90%); (b) 4 N HCl, dioxane; (c) Cbz-Asn(Trt)-OH, HOBT, EDCI, DIEA, DMF, 0 °C to rt, overnight (89%, 2 steps); (d) 1 M LiOH, dioxane; (e) TBSCl, imidazole, DMF, rt, 36 h; MeOH, 4 h (74%, 2 steps).

Cleavage of the Boc group from **6a** with 4 N HCl in dioxane, followed by reprotection as the Cbz derivative, produced **6b** in 88% yield over two steps. Solvent diffusion crystallization of **6b** from hexane/ethyl acetate produced X-ray diffraction quality crystals, which allowed for the unambiguous assignment of this advanced intermediate in which all of the chiral centers have been set (Figure 1).

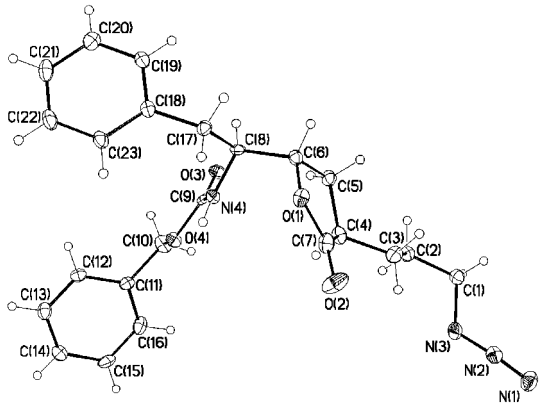


Figure 1. ORTEP plot of compound **6b**.

Compound **6b**, however, was not a viable synthetic intermediate as all attempts to convert it to the free acid via treatment with LiOH resulted in significant formation of the side product oxazolidinone **7**. Attempts to suppress oxazolidinone formation by controlling the reaction temperature and amount of hydroxide used failed.

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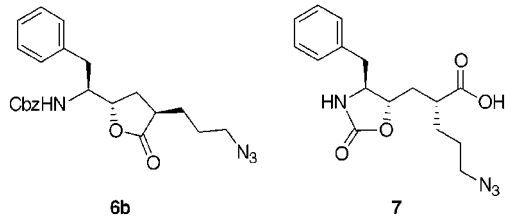


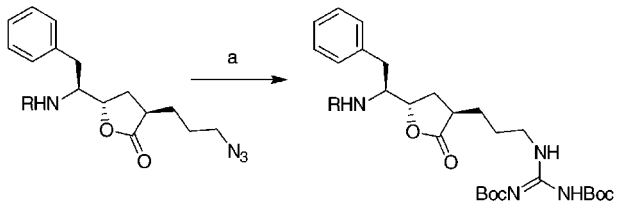
6b **7**

As our first use of the hydroxyethylene isostere will be to incorporate it into a peptide, the synthetic approach was altered to include the next N-terminal amino acid in the native sequence (Scheme 2).

Deprotection of **6a** followed by an EDCI/HOBT-mediated peptide coupling with the Z(Trt)-diprotected derivative of asparagine provided **8** in 89% yield over two steps. In this case, lactone opening with LiOH proceeded cleanly and the newly formed secondary alcohol was protected as the TBS ether **9** in 74% yield over two steps.

Attempts were first made to convert azide **6a** into the diprotected guanidine **10a** (Scheme 3). However, reduction

Scheme 3[a]

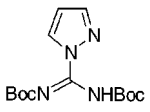


6a R = Boc
6b R = Cbz
6c R = Fmoc

10a R = Boc
10b R = Cbz
10c R = Fmoc

[a] (a) **11**, Ph_3P, H_2O, THF, rt, overnight.

of the azide to the corresponding primary amine via catalytic hydrogenation (10% or 5% Pd/C with 1 atm of H_2) or tin(II) chloride consistently gave only complex reaction mixtures and low yields of the desired amine, presumably due to an intramolecular (or intermolecular) attack of the newly formed amine on the lactone carbonyl. To minimize these possible side reactions, a one-step reduction/guanidation procedure was envisioned which would eliminate the need to isolate the free amine. The bis-Boc carboxamidine **11**[14] was chosen as the electrophilic guanidation reagent for this transformation. However, this reagent was found to be incompatible with the reduction methods thus far described.



11

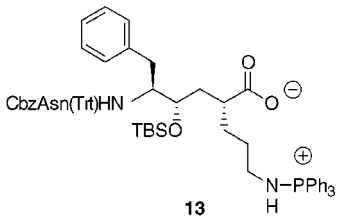
The Staudinger reaction is a mild conversion of azides to phosphazenes via treatment with phosphines.[15] While phosphazenes display a wide range of reactivities, they are readily hydrolyzed to the corresponding primary amine and phosphine oxide in the presence of water.[16] Preliminary studies indicated that reagent **11** was stable in these reduction conditions, and these conditions were chosen for the desired overall transformation.

In the event, triphenylphosphine was added to a room temperature mixture of **6a**, **11**, and H_2O in THF (Scheme 3). Evolution of nitrogen commenced immediately, and the reaction was allowed to stir overnight at room temperature. The mixture was concentrated in vacuo and the residue purified by flash column chromatography to yield 70% of the desired product **10a**.

Replacement of the N-terminal protecting group with Cbz had little effect on the reaction yield. Compound **6b** was successfully converted to its corresponding guanidine **10b** under the same reaction conditions in 71% yield.

The N-Fmoc protecting group, however, is not stable to these reaction conditions. When **6c** was used as the starting azide, the desired product **10c** was only recovered in 20% yield and significant Fmoc cleavage was apparent by TLC. This is presumed to be due to the basic nature of the intermediate phosphazene.

Surprisingly, when azide **9** was treated according to the conditions in Scheme 3, none of the desired guanidine **12** was obtained. Instead, the major product was identified as the stable betaine **13** in which the phosphazene has deprotonated the carboxylic acid. This highly polar compound was isolated by flash column chromatography (85:15 CH_2Cl_2/MeOH) in 85% yield.



13 **14**

The betaine structure is further supported by ^{31}P NMR in which the phosphorus of **13** displays a chemical shift consistent with know betaines[17] (Figure 2, Spectrum A). The

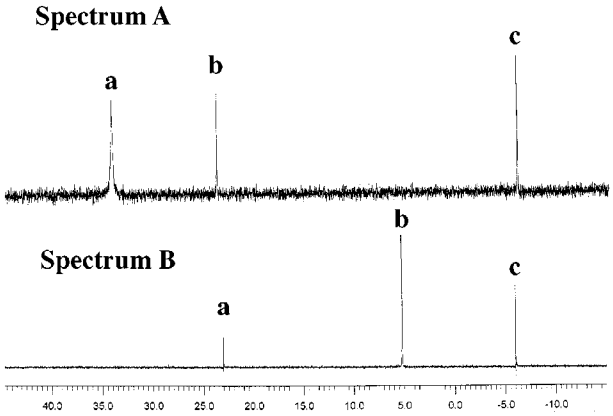
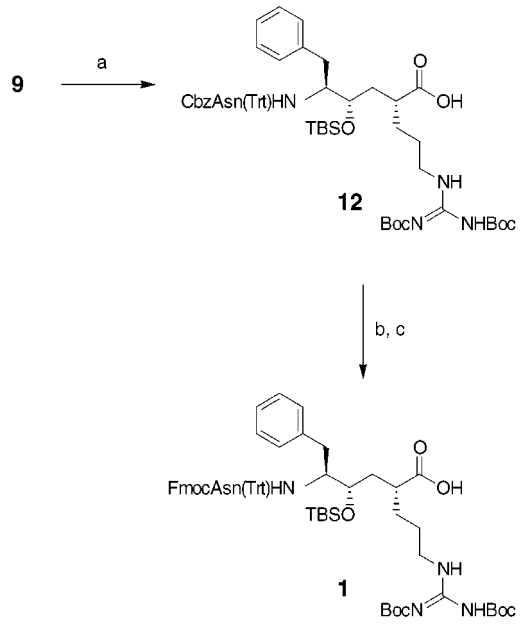


Figure 2. ^{31}P NMR spectra of betaine **13** in THF-d_8. Spectrum A peak assignments: (a) **13**, (b) Ph_3PO, (c) Ph_3P. Spectrum B peak assignments: (a) Ph_3PO, (b) **14**, (c) Ph_3P.

Scheme 4[a]



[a] (a) **11**, Ph_3P, 0.5 M LiOH, THF, rt, 48 h (78%); (b) H_2 (1 atm), 10% Pd/C, MeOH, acetic acid. (c) Fmoc-*O*-succinimide, 10% Na_2CO_3, dioxane, rt (69%, 2 steps).

^{31}P NMR spectrum of **14** is provided for comparison (Figure 2, Spectrum B) and shows the significant difference in chemical shifts between betaines and phosphazenes.

Treatment of **13** with LiOH in dioxane produced triphenylphosphine oxide and the corresponding free amine. On the basis of these findings, the reaction conditions were altered to include 1 M LiOH, and the reaction time was increased to 2 days. Under these conditions, azide **9** is converted to guanidine **12** in 62% yield after purification by flash column chromatography and crystallization. Removal of the Cbz group from **12** via hydrogenolysis, followed by protection as the Fmoc derivative, produced the desired final product **1** in 71% yield over two steps (Scheme 4).

The Phe-Arg HE (**1**) reported here is the first HE to contain a highly functionalized natural amino acid side chain and is the most complex HE synthesized to date. Several of the intermediates in this synthesis are versatile building blocks that may prove useful for the synthesis of other side chain modified HE analogues. The simple one-pot conversion of azides to diprotected guanidines takes advantage of the mild conditions associated with the Staudinger reduction, which allows azides to be reduced to free amines in the presence of guanidation reagent **11**. The protecting group scheme employed in this synthesis should render tripeptide derivative **1** amendable to solid-phase peptide synthesis.

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Supporting Information Available: Detailed experimental procedures for the synthesis and characterization of compounds **1**, **3**, **4**, **6a**, **8**, **9**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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