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# Synthesis of a Gln-Phe Hydroxy-ethylene Dipeptide Isostere

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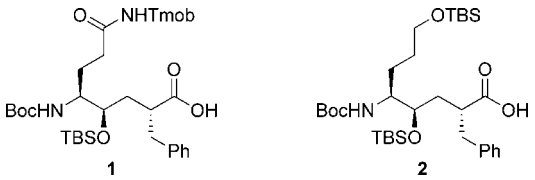
## ABSTRACT




**The protected Gln-Phe hydroxyethylene dipeptide isostere 1 was synthesized as a precursor for preparation of potential inhibitors of Botulinum neurotoxin B metalloprotease. The method allows for the synthesis of additional hydroxyethylene dipeptide isosteres such as 2 with functionalized $P_1$ side chains. The isosteres prepared were coupled with a dipeptide to produce protected pseudotetrapeptide derivatives.**

Botulinum neurotoxins (BoNTs), produced by the anaerobic bacteria *Clostridium botulinum* are among the most potent toxins known. The seven BoNT proteases (serotypes A−G) are produced as 150 kDa inactive polypeptide chains, which are further converted to 50 kDa zinc metalloproteases inside cells. The BoNT metalloproteases act by cleaving peptides in the neuroexocytosis apparatus, causing muscle paralysis and the symptoms of botulism.[1] Our target toxin, Botulinum neurotoxin B (BoNT/B) endoprotease, cleaves the human vesicle-associated membrane protein (VAMP-2) between residues Gln[76] and Phe[77] and has an extraordinary substrate specificity, with a 35-mer peptide as its minimal substrate.[2] Only a few X-ray structures of the 150 kDa holotoxins have been published,[3] and although a number of inhibitors have been reported[4] no structure of the active 50 kDa proteases with an inhibitor bound has been reported to this date. Our goal was to develop a synthesis of hydroxyethylene (HE) dipeptide isostere **1** for the scissile Gln[76]−Phe[77] bond in order to prepare transition state analogue pseudopeptide inhibitors of BoNT/B.

To date, most HE isosteres reported have unfunctionalized side chains, and most synthetic procedures for HE isosteres are focused on the assembly of the isostere backbone and chiral centers. Herein we report the synthesis of the functionalized Gln-Phe HE dipeptide isostere. In initial attempts on synthesizing HE **1**, the Horner−Wadsworth−Emmons reaction between a Gln-derived keto phosphonate[5] and 2-oxo-3-phenyl propionic acid methyl ester proved to be low yielding and difficult to reproduce. To overcome these difficulties, and to allow for a diversification of the Gln $P_1$

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(5) Compound **4** in the preceding manuscript of this journal.

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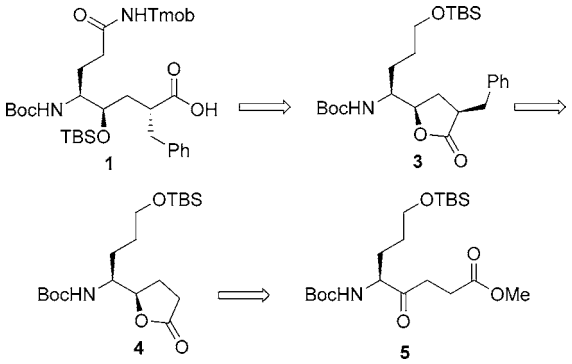
side chain of HE **1**, we envisioned introduction of the amide functionality of the target Gln-Phe isostere at a late stage in the synthesis. We chose to mask the Gln side chain as a TBS silyl ether, which could be oxidized to the carboxylic acid and functionalized to an amide at an appropriate point in the synthesis. This gave lactone **3** as a key intermediate (Scheme 1).

**Scheme 1**

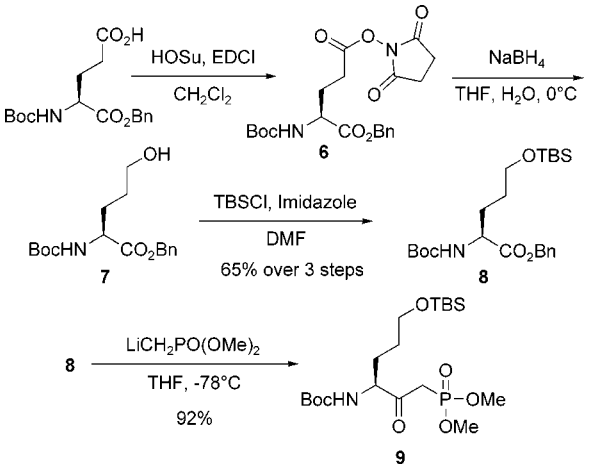



Our synthetic approach was designed to accommodate the synthesis of both the (2*R*,4*R*,5*S*)-HE **1** and the diastereoisomer with opposite chirality at the C-4. Both diastereoisomers would be valuable synthetic targets, as it is difficult to predict which isomer would bind more strongly to the BoNT/B metalloprotease. Stereoselective benzylation of either (4*R*,5*S*)-lactone **4** or the (4*S*,5*S*)-isomer would provide the backbone of the HE isosteres. The two isomers of lactone **4** could both be obtained from keto ester **5** after stereoselective reduction followed by cyclization. Herein we report the synthesis of the (2*R*,4*R*,5*S*)-HE **1**.

In the forward sense, our synthetic route started with the conversion of Boc-Glu-OBn to keto phosphonate **9** (Scheme 2). Reduction of succinimide ester[6] **6** followed by TBS protection gave TBS silyl ether **8**, which was allowed to react with lithio dimethyl methylphosphonate.[7] Keto phosphonate **9** was utilized in a HWE reaction with freshly prepared methyl glyoxolate[8,9] to give a mixture of *cis*- and *trans*-alkenes in a 2:3 ratio (Scheme 3). Subsequent hydrogenation of the crude product mixture gave the saturated keto ester **5** in 81% yield over the two steps (Scheme 3).

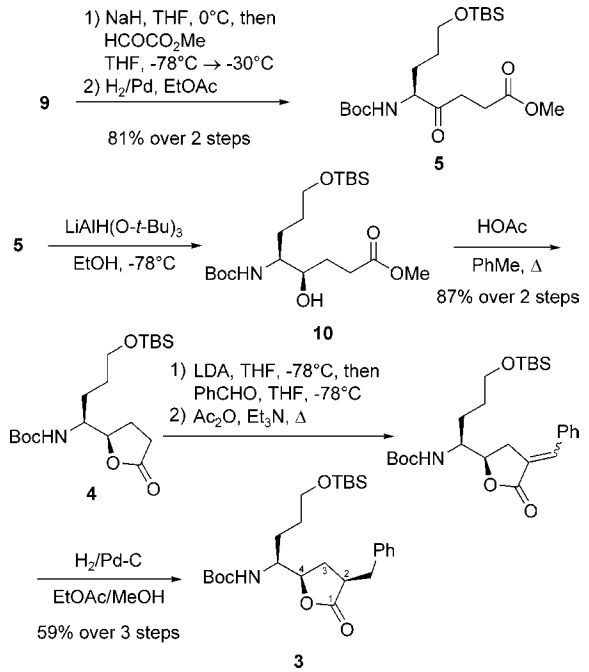
Reduction of keto ester **5** using $NaBH_4$ in methanol at −30 °C gave an inseparable mixture of the *syn*- and *anti*-amino alcohols in a 2:3 ratio, which after conversion to the corresponding diastereomeric lactones could be separated by

**Scheme 2**




column chromatography. In recent years, several methods for the stereoselective reduction of α-amino γ-keto esters have been reported.[10] In this case, using $LiAlH(O\text{-}t\text{-}Bu)_3$ as the reducing agent (Scheme 3) under chelation control

**Scheme 3**




conditions[10a] gave almost exclusively the *anti*-amino alcohol **10**. Subsequent lactonization by treatment with acetic acid in refluxing toluene[11] gave lactone **4** contaminated with only a trace of its diastereoisomer. Separation by flash chroma-

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(9) We found that to ensure full consumption of the keto phosphonate and reproducibility of the reaction, extensive drying of the crude aldehyde with anhydrous magnesium sulfate was required. See experimental part.

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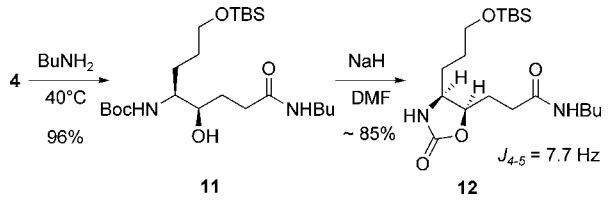
tography gave **4** as a single diastereoisomer in 87% yield over the two steps (Scheme 3). Alkylation of lactones with (4*R*,5*S*) configurations such as **4** using, e.g., LDA and alkyl halides, results in formation of the undesired (2*S*)-diastereoisomer,[12] which has the opposite chirality compared to the side chain of naturally occurring phenylalanine. However, Nadin and co-workers[13] have shown that the desired (2*R*)-diastereomer can be obtained through an Aldol-elimination−hydrogenation sequence. The hydrogenation occurs from the side opposite of the large substituent at the C-4 carbon atom of the unsaturated lactone (Scheme 3)[14] and gives rise to the desired (2*R*) stereochemistry.

Applying this methodology (Scheme 3) provided (2*R*)-lactone **3**, in 59% yield over the three steps. It should be noted that at this point all the stereocenters of target molecule HE **1** have been set. Lactone **3** showed an NOE between the H-2 and H-4 (see Scheme 3), which indicates that the desired (2*R*,4*R*) stereochemistry was obtained.

The relative stereochemistry of *N*-Boc-protected amino alcohol **10** was confirmed by conversion of lactone **4** to oxazolidinone **12** (Scheme 4) following the procedure of

**Scheme 4**

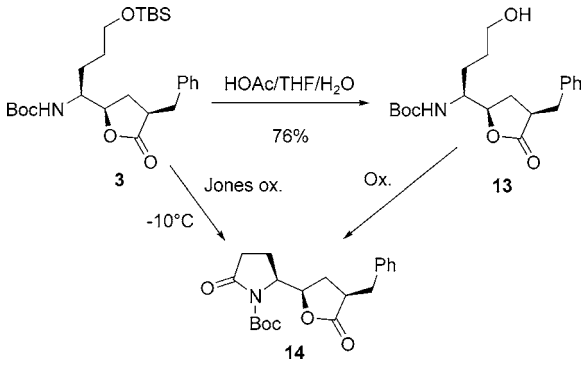



Litera et al.[11] Oxazolidinone **12** showed a coupling constant of 7.7 Hz between H-4 and H-5, which is typical for *cis*-oxazolidinones reported in the literature.[10a,11,15]

Attempted removal of the TBS group from lactone **3** using TBAF resulted in epimerization of the C-2 position to give an inseparable mixture of diastereomeric alcohols **13** in a 2:1 ratio in 85% yield. Alternatively, the TBS group could be removed with acetic acid in THF−water[16] to give alcohol **13** (Scheme 5) as a single diastereoisomer in 76% yield.

Oxidation of primary alcohol **13** to the carboxylic acid proved to be difficult, and treatment of **13** with $RuCl_3/NaIO_4$ or TEMPO/PhI(OAc)$_2$ resulted in formation of pyroglutamate **14** (Scheme 5) with only trace amounts of the desired carboxylic acid. The cyclized side product was also obtained after direct oxidation of the TBS silyl ether[17] **3** using the Jones reagent.[18] Pyroglutamate formation had previously been observed by Olsen et al.[19] upon oxidation of a *tert*-

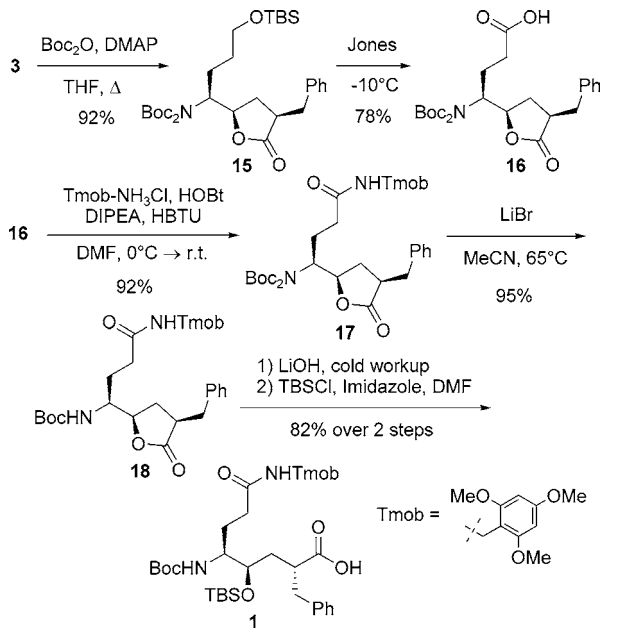
**Scheme 5**




butyl ester analogue of primary alcohol **7** with $CrO_3$-pyridine in an effort to prepare the corresponding aldehyde.[19] It was hypothesized that once the aldehyde was formed, it rapidly underwent cyclization to the aminal, which was further oxidized to yield the pyroglutamate.[19]

Pyroglutamate formation could be avoided by introduction of a second *N*-Boc protecting group on the amine (Scheme 6) before submission to the Jones oxidation conditions. In

**Scheme 6**




this case, oxidation of alcohol **15** proceeded uneventfully to provide acid **16** in good yield. The amide functionality of the Gln $P_1$ side chain was introduced through coupling with 2,4,6-trimethoxy benzylamine[20] to give **17**, which has the

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desired protection of the amide side chain. Further elaboration required the selective removal of one *N*-Boc protecting group, as attempted lactone hydrolysis of **17** with lithium hydroxide gave a complex mixture in which some Boc cleavage was noted as well as possible epimerization at the α-carbon atom.[21]

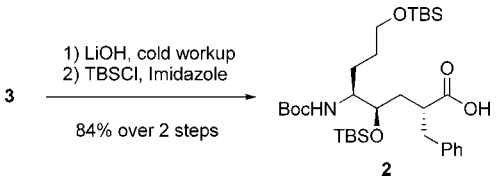
Hernandez et al.[22] recently discovered that one *N*-Boc group can be selectively cleaved from *N*,*N*-di-Boc-protected amino compounds by treatment with LiBr. Stirring lactone **17** with an excess of LiBr in MeCN at 65 °C overnight provided mono-*N*-Boc-protected lactone **18** in near quantitative yield.

Upon hydrolysis of the lactone to give the hydroxy acid, we found that relactonization occurs very readily when an acidic aqueous workup procedure was followed, much in agreement with what was initially discovered in an earlier synthesis of a Gln-Arg HE isostere.[23] We were able to obtain protected Gln-Phe HE isostere **1** without any trace of relactonization (Scheme 6) by using the same optimized cold workup under basic conditions developed for the Gln-Arg system.[23] The secondary alcohol of the free hydroxy acid obtained after treatment of *N*-Boc-protected lactone **18** with lithium hydroxide was protected as the TBS silyl ether using TBSCl and imidazole after extensive drying to give **1** in 82% yield over the two steps.

Most reported HE syntheses contain aliphatic or aromatic side chains corresponding to the $P_1$ and $P_1'$ positions of the native dipeptide, whereas our route allows for incorporation of functionalized side chains in the $P_1$ position. The present synthesis is particularly nice as it provides late-stage synthetic intermediates (in which all of the chiral centers have been set), with functionality that can be easily manipulated to provide an array of HE analogues differing at the $P_1$ residue. This is highlighted by the late-stage conversion of the side-chain alcohol in lactone **15** into the Gln-amide side chain.

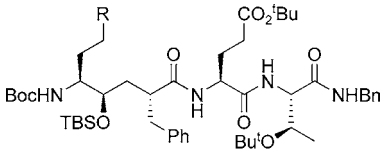
One derivative was obtained by direct hydrolysis of lactone **3** (Scheme 7) followed by TBS protection of the secondary alcohol to give protected HE isostere **2** in 84% yield over the two steps. The cold workup under basic conditions[23] also was required in order to prevent relactonization of this isostere.

**Scheme 7**




**2**

Both HE derivatives **1** and **2** were coupled with $H_2N$-Glu-(OtBu)-Thr(tBu)-NHBn to give the fully protected pseudotetrapeptides **19** and **20** (Figure 1) as short protected




**19** R = CONHTmob
**20** R = $CH_2$-OTBS

**Figure 1.** Protected pseudotetrapeptides.

analogues of the BoNT/B VAMP [60−94] substrate in 75 and 72% yields, respectively. The biological activity of pseudopeptides containing the HE isosteres will be reported elsewhere.

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**Supporting Information Available:** Full experimental details and characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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