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

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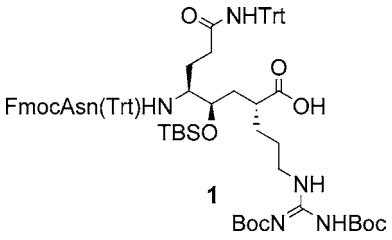
ABSTRACT



The protected hydroxyethylene dipeptide isostere of Gln-Arg and the tripeptide derivative 1 were synthesized as components of potential peptidase inhibitors.

The botulinum family of neurotoxins (BoNT-A through G) are among the most lethal toxins known with a mouse LD_{50} equal to 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] The native substrate for BoNT-A is SNAP-25, and the scissile bond lies between a Gln and Arg residue. During the course of an investigation aimed at developing BoNT-A inhibitors, we became interested in synthesizing the Gln-Arg hydroxyethylene isostere as a possible transition-state mimetic inhibitor.

The hydroxyethylene isostere is defined as a dipeptide unit in which the central amide bond has been replaced by a CH_2-CH(OH) group. First synthesized as a potential transition-state analogue inhibitor of renin,[4−6] the hydroxyethylene moiety (HE) has also been applied with success to develop HIV protease[7] and β-secretase[8] inhibitors. Although the synthesis of hydroxyethylene isosteres has received considerable attention in the literature, most HE analogues synthesized to date contain unfunctionalized side chains. Herein

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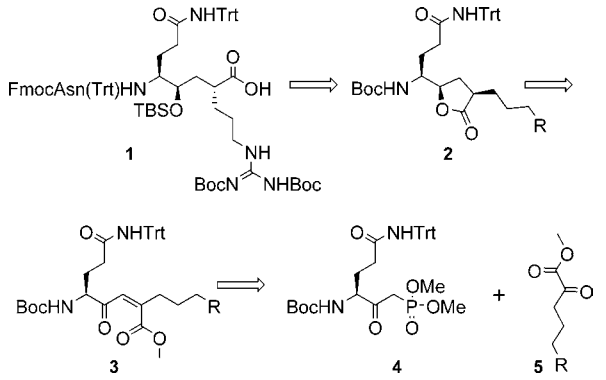
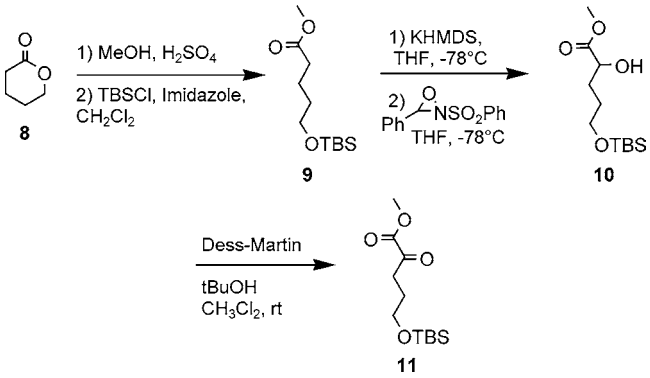
Scheme 1



Scheme 3

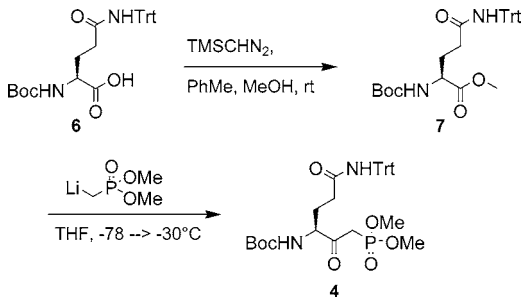


we report the synthesis of the fully functionalized Gln-Arg hydroxyethylene isostere as a tripeptide derivative.

Our synthetic approach to Asn-Gln-HE-Arg **1** was based on a Horner−Wadsworth−Emmons strategy (Scheme 1).[9] In a retrosynthetic manner, the Gln-HE-Arg portion of derivative **1** would be derived from lactone **2** (Scheme 1). Clearing two stereocenters from lactone **2** reveals the unsaturated 1,4-dicarbonyl derivative **3**. This in turn could be derived from a Horner−Wadsworth−Emmons reaction that, in a single step, provides the entire carbon framework of the desired inhibitor. The compounds required for this reaction would be Gln-derived ketophosphonate **4** and an α-ketoester of type **5**.

The synthesis of ketophosphonate **4** is shown in Scheme 2. BocGln(Trt)OH (**6**)[10] was reacted with TMS−diazo-

Scheme 2



methane to form methyl ester **7** in quantitative yield. Claisen condensation of this ester with lithio dimethyl methylphosphonate afforded the desired ketophosphonate **4** in 87% yield.[11]

With the phosphonate in hand, several keto-esters were evaluated as partners for the Horner−Wadsworth−Emmons reaction. Of these, only the siloxy derivative **11** provided good yields of the desired product in a reproducible manner. The synthesis of siloxy **11** is shown in Scheme 3. Acid-catalyzed methanolysis of δ-valerolactone[12] afforded the corresponding hydroxyester, which was directly silyated to afford silyl ether **9** in 76% yield over two steps. Treating the potassium enolate of ester **9** with Davis oxaziridine[13] afforded α-hydroxyester **10** in a satisfactory yield of 56%. Oxidation of the hydroxyl group with Dess−Martin periodinane afforded α-ketoester **11** in high yield (96%) and excellent purity.[14,15]

Treatment of the sodium salt of phosphonate **4** with ketoester **11** provided the (*Z*)-isomer of desired olefin **12** in 80% yield with none of the (*E*)-isomer observed. This condensation was strongly temperature dependent; decomposition occurred when the temperature was raised above −30 °C. The next step in the synthesis was reduction of the ketone functionality. Treating ketone **12** with $NaBH_4$ at −30 °C and allowing the reaction to warm to room temperature produced an inseparable mixture of diastereomeric lactones **13a** and **13b** in a 2:3 ratio.[9b] Interestingly, the ratio of diastereomers obtained changes to 2:1 favoring diastereomer **13a** when the reaction was instead maintained at −30 °C and quenched at that temperature with a saturated aqueous solution of NH_4Cl. We hypothesize that on warming to room-temperature, adventitious methoxide causes epimerization of the allylic center, while at low-temperature, epimerization does not occur. If so, then lactone **13b** appears to be the thermodynamically favored product.

Attempts were made to control the product ratio using bulkier hydride sources.[16] Interestingly, DIBAL favored formation of **13b** over **13a** (6:1 ratio), while LS-Selectride and $LiAlH(OtBu)_3$ both favored **13a** (11.5:1 and 10:1, **13a** to **13b**, respectively). Unfortunately, reductions with DIBAL and LS-Selectride were both low-yielding reactions, providing only 37 and 30% yields of product, respectively.

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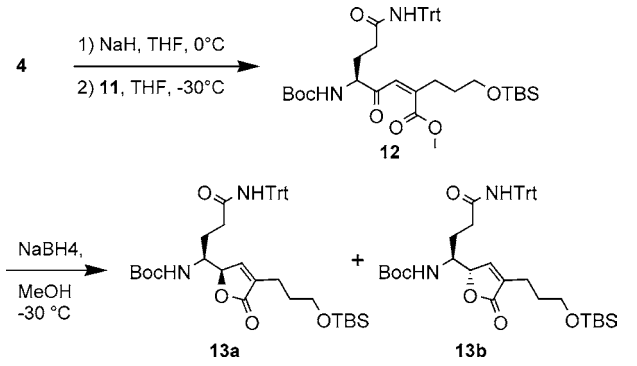
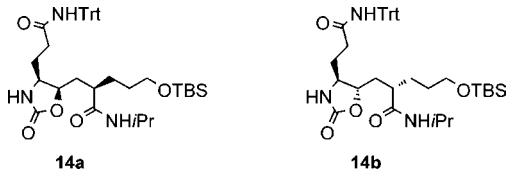
Scheme 4



Reduction with $LiAlH(OtBu)_3$ provided good product yield (84%), but the high diastereomeric ratio was not reproducible on scale-up.

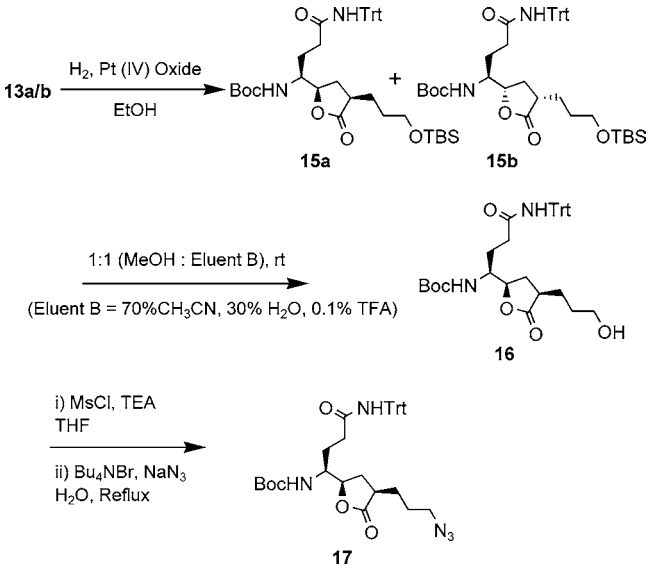
The stereochemical identification of these compounds was initially established by conversion to the corresponding oxazolidinones **14a** and **14b**, which have characteristic ^1H NMR spectra.[17] Comparison to analogous compounds in the literature allowed for identification of diastereomer **13a** as the (4*R*,5*S*)-lactone, and **13b** as the (4*S*,5*S*)-lactone.[18] This initial stereochemical assignment was confirmed by X-ray crystallography of the enantiomer of **15a**.[19] This also proved correct our initial assumption that hydrogenation of the unsaturated lactones occurs on the face opposite the bulky substituent at the C-4 position to provide the *cis*-lactone products.



Hydrogenation of the inseparable mixture of **13a** and **13b** occurs in a completely stereospecific manner, providing a separable mixture of only two diastereomeric lactones **15a** and **15b**. The preferred catalyst for this transformation was found to be Pt(IV) oxide, as Pd/C resulted in varying degrees of silyl ether cleavage and no conditions could be found that allowed separation of the diasteromeric free alcohols.

The lability of the silyl ether was also noted during HPLC studies of lactone **15a.** Incubation of **15a** in a 1:1 mixture of methanol and an HPLC eluent composed of acetonitrile (70%), water (30%), and TFA (0.1%) provided clean cleavage of the silyl ether. This proved to be an exceptionally mild and convenient method for alcohol deprotection and was thereafter used as the method of choice for the conversion of lactone **15a** to alcohol **16** (Scheme 5). The

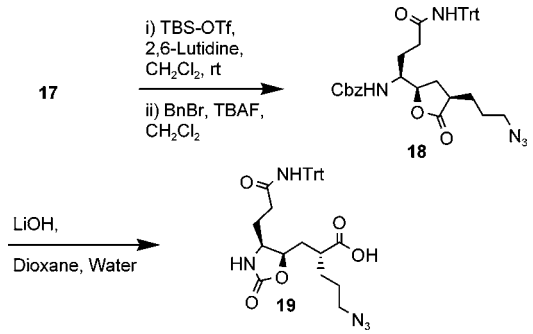
Scheme 5



successful use of an azide as a protected amine equivalent in our previous synthesis of the Phe-Arg HE[20] made this an attractive strategy for the current synthesis as well. Alcohol **16** was converted to azide **17** in 72% yield by a one-pot mesylation−displacement reaction (Scheme 5).[21]

The N-Boc protecting group of **17** was converted to the Cbz-protecting group in a one-pot procedure developed by Sakaitani and Ohfune[22] to provide **18** in 72% yield (Scheme 6). Unfortunately, lactone opening of this compound resulted in a significant amount of the undesired oxazolidinone **19**.

Scheme 6



As the final goal of this synthesis was to form a 17-mer peptide containing the Gln-Arg dipeptide analogue, the next amino acid in the 17-mer sequence was added at this point. TBSOTf-mediated Boc deprotection of **17** followed by EDCI/HOBT-mediated peptide coupling provided Asn-derived tripeptide **20** in 94% yield over two steps (Scheme

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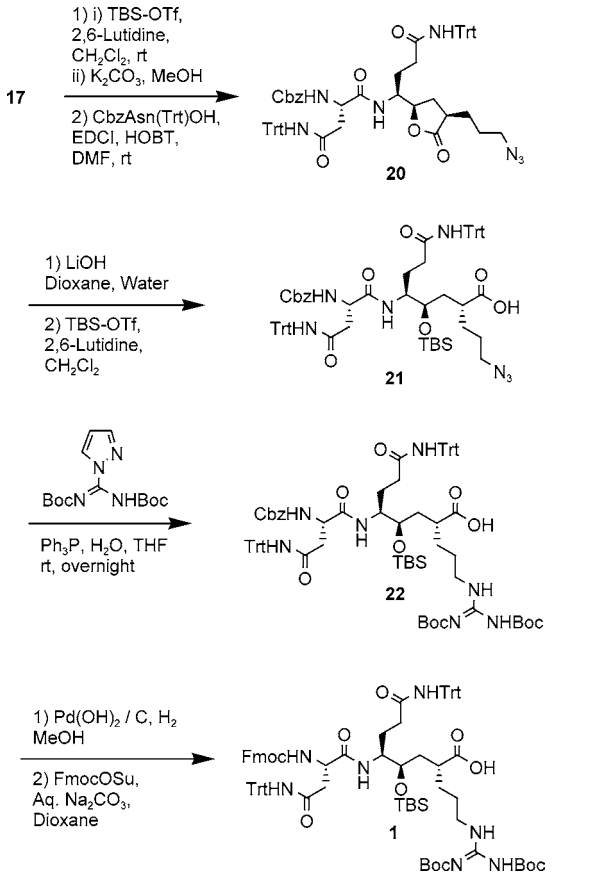
Scheme 7



7). Lactone opening to provide the free carboxylic acid initially proved to be problematic, as a significant amount of the reverse reaction was found to occur during workup. Carefully monitoring the pH of the reaction during the quench step was unsuccessful and formation of lactone **20** was still observed. Ultimately, an optimized workup procedure was developed that allowed isolation of the free acid under basic conditions to provide the desired product in near quantitative yield. The secondary alcohol was subsequently protected as the TBS-silyl ether to provide acid **21** in 80% yield over two steps.

Converting azide **21** to guanidine **22** was successfully achieved in 56% isolated yield via the Staudinger reduction-based protocol previously described by us.[20] The synthesis was finished by removing the Cbz-protecting group via hydrogenolysis over $Pd(OH)_2$ on carbon and reprotecting the resulting primary amine as the Fmoc derivative. In this manner, the desired Asn-Gln-Arg HE derivative **1** was obtained in 31% yield over two steps in a form suited for peptide synthesis.

The protected Gln-Arg HE analogues **1** and **22** reported here contain two highly functionalized natural amino acid side chains and are the most complex HE derivatives synthesized to date. Several of the intermediates in this synthesis are versatile building blocks that may prove to be useful for the synthesis of other side chain-modified HE analogues. Incorporation of protected HE analogue **1** into the 17-mer peptide substrate of BoNT-A and the bioactivity of the resulting substrate based inhibitor will be reported elsewhere.

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Supporting Information Available: Detailed experimental procedures for the synthesis and characterization data of compounds **1**, **4**, **7**, **9**−**13**, **15**−**18**, and **20**−**22** and crystallographic data for compound *ent*-**15a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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