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Preparation of a 1,2-isoxazolidine synthon for the synthesis of zetekitoxin AB

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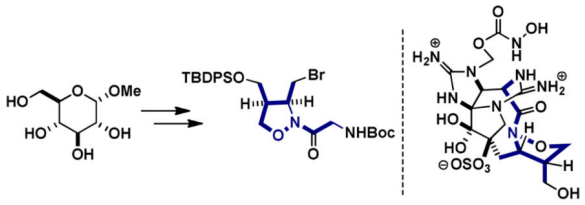
Abstract

A synthesis of the 1,2-isoxazolidine fragment of the potent voltage gated sodium channel blocker, zetekitoxin AB is described. The synthesis utilizes an intramolecular nitrone –olefin 1,3-dipolar cycloaddition to establish the stereochemistry of the *cis*-1,2-isoxazolidine. The oxidative cleavage of an all anti-triol with the excision of the central carbon is central to using α-D-glucopyranoside as a traceless stereochemical template. This route furnishes a suitably protected synthon for the synthesis of zetekitoxin AB.

Graphical Abstract



Keywords

Zetikitoxin AB; saxitoxin; dipolar cycloaddition; 1,2-isoxazolidine; nitrone

1. Introduction

In 1969, H. S. Mosher and colleagues isolated the potent sodium ion channel blocker zetekitoxin AB (ZTX) from the skin extracts of a Panamanian golden frog, *Atelopus zeteki*.[1] ZTX was isolated by bioassay guided fractionation for acute toxicity (LD_{50} = 11 μg/kg, i.p. mouse) and shown to be a very potent (pM) antagonist of the Na_V, with potencies ranging from ~60-500 fold more than those for STX (2) based on tissue type and isoform distribution. In 2004, 30+ years after isolation, Yamashita and coworkers disclosed the

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Supplementary Material

Supplementary data (experimental procedures, characterization data for new compounds) associated with this article can be found, in the online version.

magnificent structure of ZTX (1) using just 0.3 mg of the isolated sample.[2] Similar to the related natural product saxitoxin (2), ZTX also contains a tricyclic bis-guanidine core. However, ZTX also includes a 1,2-isoxazolidine group within a 10 membered macrocyclic ring, a pendant *N*-hydroxycarbamate and a sulfate ester (Figure 1).[2] Due to the classification of *A. zeteki* as an endangered species, this 0.3 mg sample remains the only purified of ZTX. Given the utility of ZTX as a probe for ion channel physiology, its unique structure and its exquisite rarity, total synthesis provides the only current avenue to secure more material for biological investigations and several groups have initiated programs in pursuit of the total synthesis of ZTX.[3,4] These synthetic approaches have also revealed unique spectroscopic anomalies in the structural features in ZTX. For example, Nishikawa first noted the discrepancy of the ^{13}C chemical shift of the amide carbonyl (C13) in ZTX (δ = 156.5 ppm) compared to most *N*-alkoxyamides (δ = 170-175 ppm) and showed that inductive effects of a tethered guanidinium ion in model 1,2-isoxazolidine systems could still not account for the unsusual chemical shift (δ = 167-173 ppm).[4a] This was further confirmed in a more elaborate bicyclic guanidine model.[4b]

Although neither report had the correct carbon framework of the 1,2-isoxazolidine fragment, it is clear that factors that affect the ^{13}C environment are unique and suggests that ZTX will provide a rich medium for structural and synthetic studies.

This and our immediate access to STX (**2**) and its analogues, piqued our interest in the structure of ZTX (**1**).[5] It is known that the pyrrolidine ring in STX is derived from arginine and thus likely the same in ZTX and further unlikely that nature elaborates this ring until late in its production.[6] Since C10 in STX is oxidized one could imagine that enol/keto tautomerization produces a high enol content at C11 and this reacts with an electrophilic carbon at C14 in the biosynthesis of ZTX (Figure 1). Herein we describe the synthesis of a suitably protected 1,2-isoxazolidine derivative, with the correct carbon framework, to explore this strategy for the synthesis of ZTX.

2. Results and discussion

Our synthetic approach toward the 1,2-isooxazolidine fragment began by regioselectively converting the primary hydroxyl group of methyl α-D-glucopyranoside (**3**) to the iodide and acetylation of the remaining alcohols to give α-D-2,3,4 triacetoxy-6-deoxy-6-iodoglucopyranoside **4** in 70% yield.[6] According to methods developed by Bernet and Vasella, reductive fragmentation of **4** with zinc in refluxing ethanol gave the aldehyde **5**.[7] Without further purification, the aldehyde was condensed with *p*-methoxybenzylhydroxylamine to afford the nitrone. Heating in ethanol triggered an intramolecular nitroneolefin [3+2] cycloaddition, proceeding through a predictable transition state in which all acetates adopt an equatorial arrangement, to afford the *cis*-fused bicyclic isoxazolidine **6** in 52% yield as a single stereoisomer.[7,8] Deacetylation of **6** was carried out with sodium methoxide to afford the product triol **7** in 80% yield (Scheme 1).[8]

Having set the requisite *cis*-stereochemistry of the isoxazolidine we focused our attention on the cleavage of anti-triol **7** with the excision of C5 to remove the initial stereochemical information provided by the pyranoside. After exploring various conditions, sodium

periodate and $NaHCO_3$ gave an unstable bis-aldehyde hydrate **8** as a mixture of diastereomers. *In-situ* reduction with $LiAlH_4$ reduction at room temperature afforded the 3,4-dihydroxymethyl isoxazolidine **9** in 70% overall yield.[9,10] Attempts to differentially protect the C4 and C6 alcohols at this point proved unsuccessful. Oxidative strategies to cyclize the C6 alcohol on the benzylic carbon of the PMB group were also unsuccessful, leading to decomposition of the substrate. An alternative strategy would engage the C6 alcohol and the amine as an oxazolidinone. Protection of two primary hydroxyl groups with tert-butyldiphenylsilyl chloride gave the di-TBDPS protected species **10** in 97% yield. Von Braun type cleavage of the PMB group via the reaction with 2,2,2-trichloroethoxycarbonyl chloride (TrocCl) gave the Troc protected 1,2-isoxazolidine derivative **11** in 82% yield (Scheme 3). The Troc group allowed us to effectively distinguish the two primary hydroxyl groups by selectively trapping one of the primary hydroxyl groups as a cyclic carbamate. This was accomplished by treatment of 11 with tetra-*n*-butylammonium fluoride to give **12** in 92% yield.

Re-silylation of the C4 hydroxyl group as the TBDPS ether gave the protected cyclic carbamate **13** in 85% yield (Scheme 3). Base mediated hydrolysis (Cs_2CO_3 in EtOH) of cyclic carbamate **13** furnished the amino alcohol **14** in 72% yield. Using *N*-Bocglycine as a surrogate for the saxitoxin core, we attempted a coupling with the amino alcohol **14** using EDCI, HOBt or HATU. This resulted in a mixture of undesired isoxazolidineglycine ester and amide. Whether this mixture arises from direct acylation of the alcohol or a facile $N \rightarrow O$ acyl migration from the isoxazolidine is unclear but argued for activation of the C6 hydroxyl group prior to peptide coupling. Treatment of **14** with carbon tetrabromide and triphenylphosphine provided the aminobromide **15** which was unstable when isolated. Coupling of this intermediate, *in situ*, with *N*-Boc-Gly-OH using standard EDCI, HOBt coupling conditions afforded the protected isoxazolidineglycine amide **16** in 40% overall yield.

We had some concern with the stability of the sensitive *bis*-aldehyde hydrate **8** and the potential for epimerization. Comparison of the coupling constants for H_3 in our final product **16** (H_3: J = 14, 8, 5 Hz) with Nishikawa's *N*-acetylisoxazolidine **17** ((H_3: J = 12, 8, 6 Hz) indicates that the *cis*-stereochemistry has been maintained. The ^{13}C chemical shift of the isoxazolidine amide carbonyl in **16** is 171.0 ppm, consistent with the previously published models[4a,b] and suggest that if the structure of ZTX is correct, a significant decoupling of the isoxazolidine nitrogen non-bonding electrons from the carbonyl, or complex shielding phenomenon imposed by the rigid three dimensional structure of ZTX, significantly effects this carbonyl.

In conclusion we have developed a synthesis of the 1,2-isoxazolidine fragment of ZTX. Our synthesis features an intramolecular nitrone-olefin cycloaddition to establish the *cis*-fused isoxazolidine. This strategy uses α-D-glucopyranoside as a sterochemical template to relay the absolute stereochemistry required for this fragment. The oxidative cleavage of an all *anti*-triol with excision of the central carbon serves to remove all of the stereochemical information presented in the original template. This approach allows us to prepare gram quantities of **14**, a suitably protected synthon to explore a C11 - C14 coupling strategy for the synthesis of ZTX.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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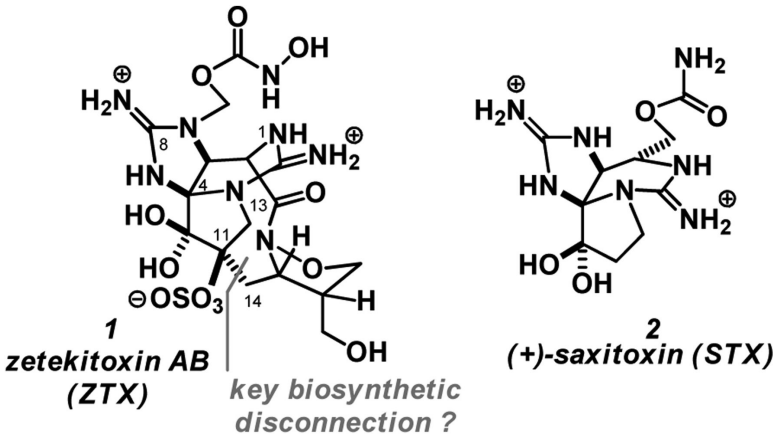
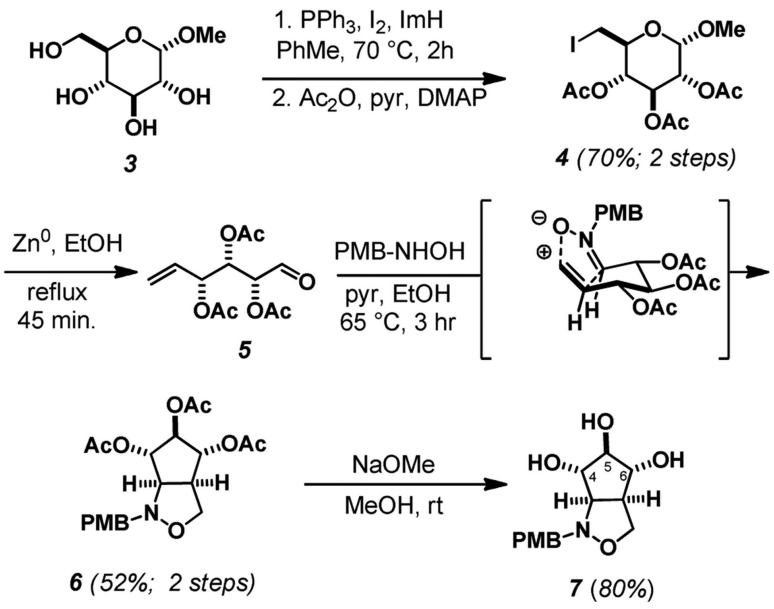
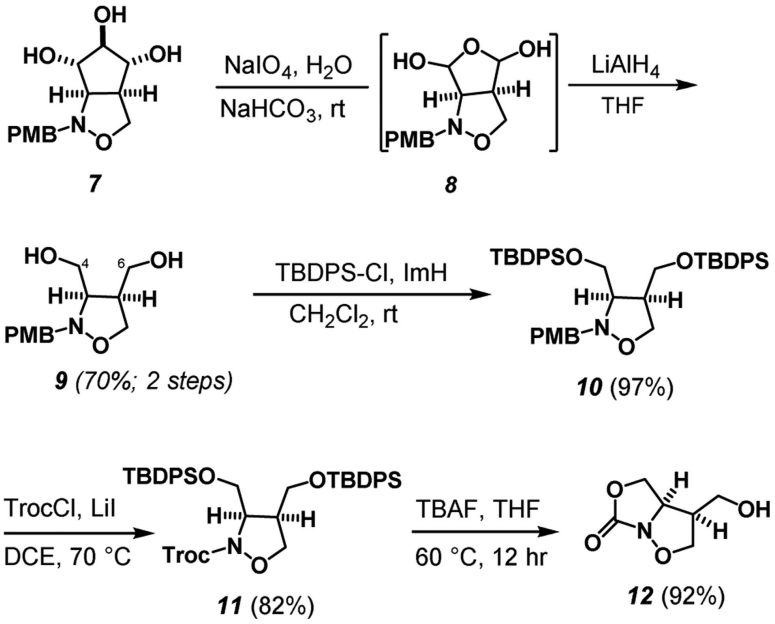


Figure 1.
Structures of zetekitoxin AB **1** and saxitoxin **2**.

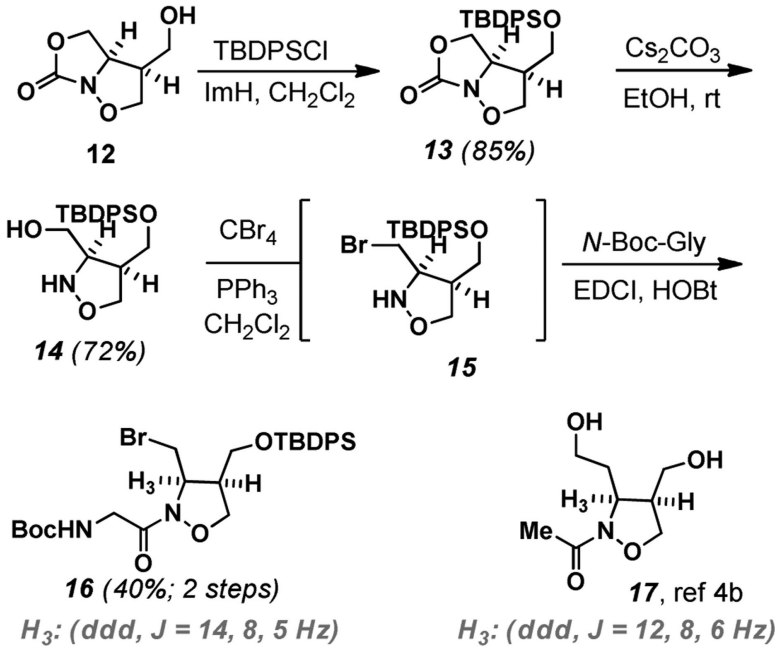


Scheme 1.
The key 1,3-dipolar cycloaddition.



Scheme 2.

Synthesis of a differentially protected diol.



Scheme 3.
Synthesis of a peptide coupling substrate.