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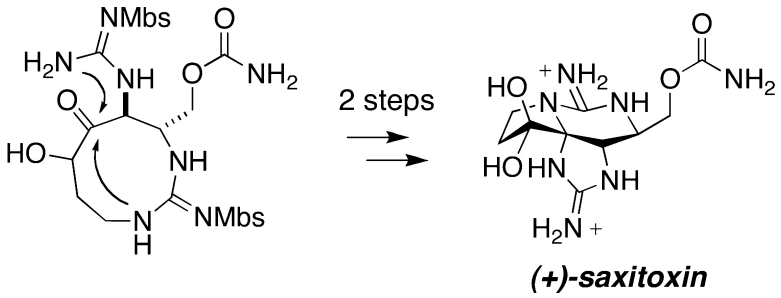

(+)-saxitoxin

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(+)-Saxitoxin: A First and Second Generation Stereoselective Synthesis

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Abstract: A stereoselective synthesis of the bis-guanidinium toxin (+)-saxitoxin (STX), the agent infamously associated with red tides and paralytic shellfish poisoning, is described. Our approach to this unique natural product advances through an unusual nine-membered ring guanidine intermediate **39** en route to the tricyclic skeleton that defines STX. The effectiveness of this strategy is notable, as only four steps are needed to transform **39** into the target molecule, including a four-electron alkene oxidation catalyzed by $OsCl_3$. Construction of the critical monocyclic guanidine has been achieved through two channels, the first of which makes use of Rh-catalyzed C−H amination and highlights a novel class of heterocyclic *N,O*-acetals as iminium ion equivalents for crafting functionalized amines. A second route to **39** relies on a stereoselective acetylide dianion addition to a serine-based nitrone, thereby facilitating the preparation of STX in just 14 linear steps from commercial material.

Introduction

Neurotoxic agents can serve as important pharmacological tools for understanding protein function associated with the highly complex ionic mechanisms of electrical transmission in cells. The guanidinium toxins, (+)-saxitoxin and (−)-tetrodotoxin (Figure 1), are exemplary in this regard and have been instrumental for the identification, characterization, and study of voltage-gated sodium channels (vg-Na^+ channels). As molecular targets, these natural products present incomparable challenges for chemical methods research given the degree of oxidation and the highly polar nature of the heteroatom substituents. Although our initial interest in these compounds drew principally from a desire to showcase new tactics for oxidative C−N bond construction, it was apparent from the outset of this work that efficient access to a molecule such as saxitoxin (STX) would create opportunities to explore Na^+ channel function through the systematic and rational design of non-natural STX-based structures. Herein, we present a detailed analysis of our synthetic strategies to this most unusual bis-guanidinium natural product.[3] The elegant and timeless works of the Kishi and Jacobi labs, which constitute the only other reported routes to the toxin, provided valuable guidance to our own synthetic planning.[4,5] Accordingly, our efforts have culminated in both first and second generation asymmetric syntheses of (+)-STX, the shortest of which requires 14 linear operations from commercial materials. The application of Rh-catalyzed C−H amination is one of several unique transformations that highlight this work and make available the desired target in a manner amenable to programmed modification.

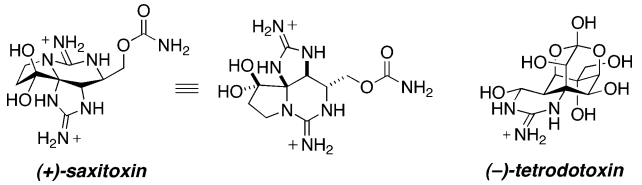

Figure 1. Structurally unique guanidinium toxins act as site I selective blockers of voltage-gated Na^+ ion channels.

Background

Ion channels are the molecular machinery that allow passive diffusion of ions across the cell membrane and are found in all animal, plant, and bacterial cells.[6] Such proteins intimately control the movement of Na^+, K^+, Ca^{2+}, and Cl^- ions to produce small, transient changes in ion concentrations. Ion flux in and out of a cell is, in turn, coupled to a diverse number of biochemical processes, which include nerve and muscle excitation, hormonal secretion, and sensory transduction. The ability to modulate ion passage through specific channels could greatly aid efforts to deconvolute the integrated circuitry of these remarkable transmembrane conduits.[6,7] One strategy to exact such control would capitalize on small molecules that interact

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(3) Fleming, J. J.; Du Bois, J. *J. Am. Chem. Soc.* **2006**, *128*, 3926−3927.
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(5) (a) Jacobi, P. A.; Martinelli, M. J.; Polanc, S. *J. Am. Chem. Soc.* **1984**, *106*, 5594−5598. (b) Martinelli, M. J.; Brownstein, A. D.; Jacobi, P. A.; Polanc, S. *Croat. Chem. Acta.* **1986**, *59*, 267−295.
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(7) (a) Cestele, S.; Catterall, W. A. *Biochimie* **2000**, *82*, 883−892. (b) Wang, S. Y.; Wang, G. K. *Cellular Signaling* **2003**, *15*, 151−159. (c) Yamaoka, K.; Vogel, S. M.; Seyama, I. *Curr. Pharm. Des.* **2006**, *12*, 429−442.

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with specific channel isoforms. Because of the general absence of detailed structural information on most ion channel proteins, however, the *de novo* design of such chemical agents is exceedingly difficult.[8] Fortunately, Nature has provided a number of topologically unique, low molecular weight compounds that act as modulators of ion channel proteins; such compounds can serve as scaffolds from which to craft new materials with unprecedented pharmacological properties.

The paralytic shellfish poison, (+)-saxitoxin, most notably associated with outbreaks of red tide, was first isolated in pure form by Schantz in 1957 and structurally characterized by X-ray analysis by the groups of Schantz/Clardy and Rapoport in 1975.[9,10] This small molecule is among the most lethal non-proteinaceous substances known, its acute toxicity resulting from its ability to disable ionic conductance through the voltage-gated sodium channel, a characteristic shared by the *fugu* poison, (−)-tetrodotoxin (TTX).[11,12] At a molecular level, STX and TTX act by occluding the channel pore, lodging with nanomolar affinity in the extracellular mouth of certain voltage-gated Na^+ channel isoforms.[11,13] Physiological studies of the Na^+ channel protein have been empowered with the availability of STX, TTX, and a small number of naturally occurring isolates having related guanidinium structures.[14] More information pertinent to understanding both Na^+ channel structure and function would be gained if specific chemical tailoring could be done to either toxin.[15] Semisynthetic protocols, however, have proven largely ineffective for modifying these heteroatom-rich, charged, hydrophilic natural products.[11a,16] The development of a concise, easily modified route to STX is therefore warranted, as it is only through total synthesis that the potential of STX to serve as a blueprint for new Na^+ channel blockers having designed function may be realized.

(8) Potassium and chloride ion channels have been characterized crystallographically. For recent reviews of this work, see: (a) Tombola, F.; Pathak, M. M.; Isacoff, E. Y. *Annu. Rev. Cell Dev. Biol.* **2006**, *22*, 23−52. (b) Dutzler, R. *Curr. Opin. Struct. Biol.* **2006**, *16*, 439−446. (c) Gouaux, E.; MacKinnon, R. *Science* **2005**, *310*, 1461−1465.

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(11) (a) *Tetrodotoxin, Saxitoxin, and the Molecular Biology of the Sodium Channel*; Kao, C. Y., Levinson, S. R., Eds.; Annals of the New York Academy Science: New York, 1986; Vol. 479. (b) Narahashi, T. *J. Toxicol., Toxin Rev.* **2001**, *20*, 67−84. (c) Novakovic, S. D.; Eglen, R. M.; Hunter, J. C. *Trends Neurosci.* **2001**, *24*, 473−478.

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(13) For a general discussion of Na^+ channel isoforms, see: (a) Catterall, W. A.; Goldin, A. L.; Waxman, S. G. *Pharmacol. Rev.* **2003**, *55*, 575−578. (b) Catterall, W. A.; Goldin, A. L.; Waxman, S. G. *Pharmacol. Rev.* **2005**, *57*, 397−409.

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(15) Kishi has prepared a small number of STX derivatives through de novo synthesis, see: Strichartz, G. R.; Hall, S.; Magnani, B.; Hong, C. Y.; Kishi, Y.; Debin, J. A. *Toxicon* **1995**, *33*, 723−737.

(16) (a) Koehn, F. E.; Ghazarossian, V. E.; Schantz, E. J.; Schnoes, H. K.; Strong, F. M. *Bioorg. Chem.* **1981**, *10*, 412−428. (b) Hu, S. L.; Kao, C. Y.; Koehn, F. E.; Schnoes, H. K. *Toxicon* **1987**, *25*, 159−165. (c) Sato, S.; Sakai, R.; Kodama, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1787−1789. (c) Watanabe, R.; Samusawa−Saito, R.; Oshima, Y. *Bioconjugate Chem.* **2006**, *17*, 459−465.

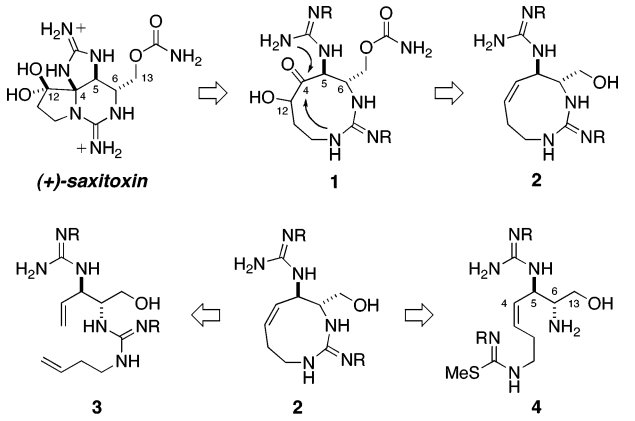


Figure 2. Retrosynthetic scheme for STX assembly: two strategies for accessing the nine-membered ring guanidine **2**.

Results and Discussion

Synthetic Plan. The challenges associated with STX as a target for chemical synthesis arise, in part, from the remarkably dense configuration of heteroatoms about its tricyclic core. As indicated by the molecular formula, $C_{10}H_{19}N_7O_4$, the total carbon count is in fact less than the combined number of oxygen and nitrogen atoms that comprise the natural product. Furthermore, the dicationic nature of STX adds complications to the handling and purification of this target. Nevertheless, shortly after the molecular structure of STX was revealed by X-ray analysis, two highly creative synthetic plans for its preparation were described.[4,5] In both instances the tricyclic core of the natural product is first assembled and the desired toxin is revealed following a common end-game strategy where pseudourea moieties are converted to their guanidinium equivalents. Accordingly, a more expeditious synthesis of STX might avoid these types of late-stage functional group exchange reactions and put directly in place the intact guanidinium groups. Following this line of analysis, unraveling the tetrasubstituted C4 aminal to its component parts, a C4 ketone and two guanidine units, thus became a defining disconnection in our approach (Figure 2). Such a plan posits that all three rings of the STX core can be formed from a nine-membered ring bisguanidine **1**. Importantly, the stereochemical configuration of the tetrasubstituted C4 aminal position would be controlled by the chirality of the attendant groups at C5 and C6. Although the decision to prepare a medium-sized ring intermediate such as **1** would not appear simplifying at first glance, such a move transforms a highly complex problem in stereocontrolled cyclic synthesis to the seemingly more manageable problem of preparing an acyclic polyamine (e.g., **3** or **4**) en route to **1**. The assembly of such an acyclic material could capitalize on the utility of our directed C−H amination methods.

The preparation of medium-sized rings, particularly heterocycles of eight and nine units in size, is challenging, and the construction of a nine-membered cyclic guanidine is without precedent.[17,18] In considering approaches to compounds such as **1**, two strategies were most appealing: ring closing-metathesis

(17) For general reviews on the synthesis of medium-sized rings, see: (a) Evans, P. A.; Holmes, A. B. *Tetrahedron* **1991**, *47*, 9131−9166. (b) Yet, L. *Chem. Rev.* **2000**, *100*, 2963−3007.

(18) The assembly of an eight-membered ring guanidine has been reported; however, the formation of a nine-membered ring was not possible under the same conditions, see: Fukada, N.; Takano, M.; Nakai, K.; Kuboike, S.; Takeda, Y. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 148−152.

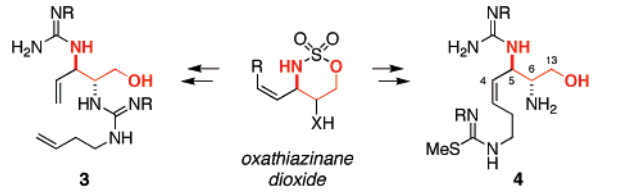


Figure 3. Highlighting the versatility of oxathiazinane dioxide heterocycles for amine synthesis.

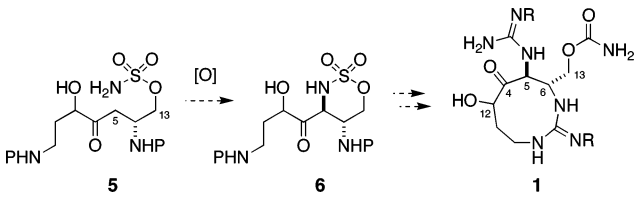


Figure 4. Possible strategy for employing C−H amination.

(RCM) and intramolecular isothiourea−amine condensation (Figure 2). Both of these plans require the design of functionalized acyclic substrates that contain a contiguous hydroxy diamine triad (i.e., C5−C6−C13, STX numbering).[19] The preparation of nitrogen-rich molecules by way of catalytic C−H amination is of special interest in our laboratory; thus, in considering paths to an *anti*-diamino alcohol intermediate, we recognized the unique potential of the [1,2,3]-oxathiazinane-2,2-dioxide heterocycle for such a purpose (Figure 3).[20] Structures of this type are valuable synthons for 1,3-substituted amine derivatives and, in the context of the STX work, appeared ideally suited for masking the C5,C13 amino alcohol and crafting an appropriate precursor to the nine-membered ring guanidine. As our lab has shown, oxidative C−H insertion of sulfamate esters enables construction of substituted oxathiazinanes;[20] however, the application of this method in the context of the STX assembly would provide a formidable test of reaction chemoselectivity and efficiency (*vide infra*).

C−H Insertion for Preparing Amine Derivatives. In principle, there exist a number of potentially viable routes using C−H amination to access cyclic guanidine **1** and related structures (Figure 4). One possibility is to establish the C5 amino group through oxidative cyclization of a C13 sulfamate ester **5**. Such a transformation would require that C−H insertion occur at a methylene center adjacent to a ketone moiety (or some protected form). In general, amination reactions at carbon centers proximal to electron-withdrawing groups are difficult and often result in depressed product yields.[21] As an alternative plan, masking the requisite C4,C12 α-ketol as an alkene would provide a more easily oxidized substrate, which could then be manipulated to an intermediate resembling **1**. While we were

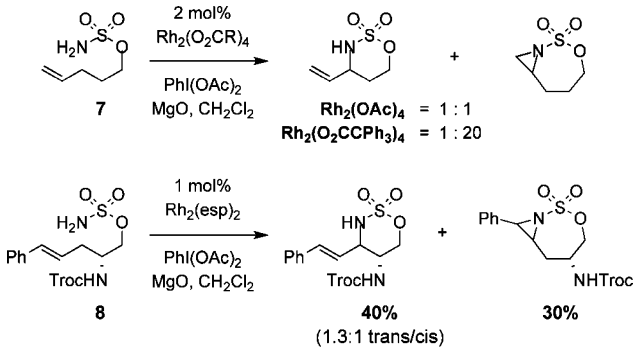


Figure 5. Exploring reaction chemoselectivity with sulfamate ester model systems.

attracted to such a strategy, at the time these studies were initiated, we had little experience with amination reactions of unsaturated sulfamates and were unsure as to whether olefin aziridination would compete with the desired allylic insertion pathway.[22,23] To examine questions pertaining to functional group chemoselectivity, model sulfamates **7** and **8** were subjected to standard reaction conditions that employed 2 mol % $Rh_2(O_2CR)_4$, $PhI(OAc)_2$, and MgO (Figure 5). In each case, the product of alkene aziridination formed in amounts approximately equal to or greater than the allylic amine, despite the larger-size ring formation. Changing catalysts from $Rh_2(OAc)_4$ to $Rh_2(O_2CCPh_3)_4$ or $Rh_2(esp)_2$ with the hope of favoring the oxathiazinane product did not have the intended effect.[24] Although the C−H insertion product from **8** would provide an ideal starting material for evolving a metathesis-based route to **1**, our inability to generate this compound in yields greater than 20% necessitated a new approach to such targets.

Confident in the "oxathiazinane-approach" for preparing STX, we considered a new tactic by which C−H amination could be employed to create highly functionalized, unsaturated heterocycles of this type. Knowledge accrued through mechanistic studies on the amination process had revealed that C−H bond reactivity follows the general order: 3° > α-ethereal ∼ benzylic > 2° ≫ 1°.[22] The high intrinsic activity of α-ethereal centers relative to most other C−H bonds suggested to us that such groups could be used to direct the insertion event in sulfamate esters bearing a large degree of attendant functionality.[25] Importantly, amination of an ethereal α-C−H bond would afford a novel *N*,*O*-acetal product that could serve as an imine or iminium ion surrogate for subsequent modification (Figure 6). Implementing this strategy would afford access to oxathiazinane materials not easily prepared through direct means; in addition, the utility of such a process might ultimately transcend the synthesis of STX itself.

The utilization of ethereal groups to bias the positional selectivity of the C−H amination reaction has been reduced to

(19) For the synthesis of *anti*-1,2-diamines, see: (a) Davis, F. A.; Deng, J. H. *Org. Lett.* **2004**, *16*, 2789−2792. (b) Luo, Y.; Blaskovich, M. A.; Lajoie, G. A. *J. Org. Chem.* **1999**, *16*, 6106−6111. (c) Rossi, F. M.; Powers, E. T.; Yoon, R.; Rosenberg, L.; Meinwald, J. *Tetrahedron* **1996**, *31*, 10279−10286.

(20) For the initial report on the oxidative cyclization of sulfamate esters, see: Espino, C. G.; Wehn, P. M.; Chow, J.; Du Bois, J. *J. Am. Chem. Soc.* **2001**, *28*, 6935−6936. Also, see: (a) Wehn, P. M.; Lee, J. H.; Du, Bois, J. *Org. Lett.* **2003**, *25*, 4823−4826. (b) Wehn, P. M.; Du, Bois, J. *Org. Lett.* **2005**, *7*, 4685−4688.

(21) For general reviews on C−H amination, see: (a) Espino, C. G.; Du Bois, J. In *Modern Rhodium-Catalyzed Organic Reactions*; Evans, P. A., Ed.; Wiley-VCH: Weinheim, 2005; pp 379−416. (b) Davies, H. M. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 6422−6425. (c) Lebel, H.; Leogane, O.; Huard, K.; Lectard, S. *Pure Appl. Chem.* **2006**, *78*, 363−375. (d) Halfen, J. A. *Curr. Org. Chem.* **2005**, *9*, 657−669. (e) Müller, P.; Fruit, C. *Chem. Rev.* **2003**, *103*, 2905−2920. (f) Dauban, P.; Dodd, R. H. *Synlett* **2003**, 1571−1586.

(22) Du Bois, J. *CHEMTRACTS−Org. Chem.* **2005**, *18*, 1−13.

(23) Alkene aziridination using sulfamate esters has been described, see: (a) Guthikonda, K.; Du Bois, J. *J. Am. Chem. Soc.* **2002**, *46*, 13672−13673. (b) Di Chenna, P. H.; Robert-Peillard, F.; Dauban, P.; Dodd, R. H. *Org. Lett.* **2004**, *6*, 4503−4505. (c) Guthikonda, K.; Wehn, P. M.; Caliando, B. J.; Du Bois, J. *Tetrahedron* **2006**, *62*, 11331−11342.

(24) Oxidation of **8** using $Rh_2(OAc)_4$ gave a ∼1.5:1 mixture favoring the six-membered ring product. Results using catalytic $Rh_2(O_2CCPh_3)_4$ were similar. The stereochemistry of the bicyclic aziridine was not determined.

(25) Nitrene insertion into an ethereal α-C−H bond parallels carbene reactivity, see: (a) Doyle, M. P.; McKervey, M. A.; Ye, T. *Modern Catalytic Methods for Organic Synthesis with Diazo Compounds: From Cyclopropanes to Ylides*; Wiley and Sons, Ltd.: New York, 1997. (b) Davies, H. M. L.; Beckwith, E. J. *Chem. Rev.* **2003**, *103*, 2861−2904. (c) Wang, P.; Adams, J. *J. Am. Chem. Soc.* **1994**, *116*, 3296−3305.

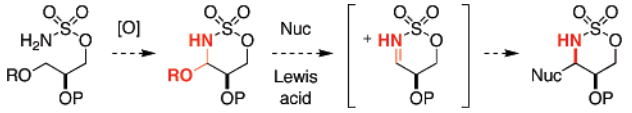


Figure 6. *N,O*-Acetal strategy for accessing substituted oxathiazinane heterocycles.

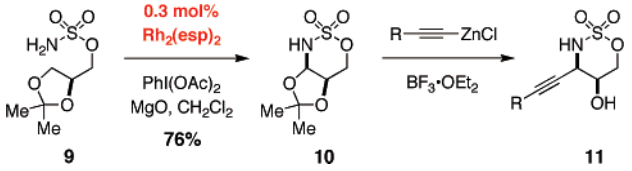


Figure 7. Organozinc addition to *N,O*-acetal **10** affords alkyne-derived oxathiazinane products.

practice, thus enabling facile preparation of unique oxathiazinane *N,O*-acetals.[26] These compounds, when treated with a Lewis acid (e.g., BF_3•OEt_2, $Sc(OTf)_3$), undergo smooth coupling with allylsilane, silyl enol ether, and silyl ketene acetal nucleophiles to give value-added oxathiazinane products. In the addition reaction, nucleophilic attack on the putative iminium ion intermediate is found to be highly diastereoselective, and the sense of induction is decidedly predictable. The development of this chemistry has been chronicled, and the reader is referred to previously published reports from this lab for a more extensive discussion on this topic.[26]

For the expressed purpose of the STX synthesis, a glycerol-derived sulfamate ester **9** was deemed an optimal starting material due to its ease of assembly in enantiomerically pure form on multigram scale and the facility by which it submitted to oxidative cyclization (Figure 7).[27,28] In fact, using our newest catalyst, $Rh_2(esp)_2$, sulfamate **9** oxidation is easily effected at 0.3 mol % catalyst loading.[29,30] The product *N,O*-acetal **10** is stable to isolation and storage and is formed in sufficiently high purity that it may be employed without purification. Finally, in the presence of alkynyl zinc reagents and BF_3•OEt_2, addition reactions proceed efficiently to give the coupled product (i.e., **11**). Such alkynyl oxathiazinane heterocycles represent the cornerstone of our first generation STX construction.

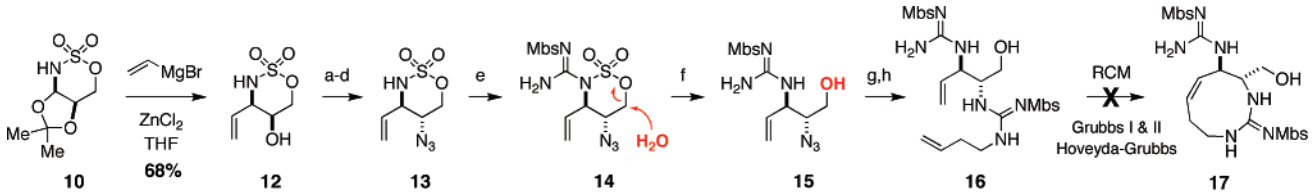
Ready access to differentially substituted alkynyl oxathiazinanes, as exemplified by **11**, has allowed us to examine numerous approaches for forming the nine-membered ring guanidine, and in this context the value of the *N,O*-acetal method cannot be overstated. With the absence of any prior art to guide our attempts to fashion a nine-membered ring guanidine, two disparate approaches were explored; each, however, would share a common origin in **10**.

Formation of the Nine-Membered Ring Guanidine. The power of ring-closing metathesis (RCM) for the construction of polar, nitrogen-containing heterocycles gave us ample cause to consider this process as a means for fabricating the desired nine-membered guanidine.[31] Myriad displays of medium-sized ring assembly by RCM notwithstanding, only a handful of examples of nine-membered ring formation dot the literature, the preparation of a nine-membered cyclic urea serving as perhaps the closest precedent to our desired goal.[32,33] Starting from *N,O*-acetal **10**, an appropriate RCM diene substrate was assembled through a sequence highlighted by the stereoselective addition of divinyl zinc to **10** (Scheme 1). This reaction represents our first example of a vinyl metal coupling to an *N,O*-acetal derivative and affords a particularly useful unsaturated oxathiazinane **12**. Bis-guanidine **16** was accessible from **12** through a series of straightforward functional group transformations. For reasons not entirely obvious to us, installation of the azido moiety proved quite difficult and was poor yielding. Still more vexing, diene **16** was an unwilling participant for RCM under conditions employing either first or second generation Grubbs or Hoveyda−Grubbs catalysts. Modification of the reaction conditions, including the use of $Ti(O^iPr)_4$ as a Lewis acid addend, also failed to provide any of the desired nine-membered ring product.[34] In all cases, starting material **16** and unidentifiable (assumed-to-be polymeric) products were obtained. The inability to execute RCM on **16** may be due to the polar nature of the two guanidine units and/or aggregation effects caused by the large number of hydrogen bond donor and acceptor groups. Different solvents, which included THF, did not influence the reaction outcome. As we would determine subsequently, the same protocols tried on an analogous urea substrate **18** were unable to induce nine-membered ring formation (Figure 8).[35]

Undeterred by the inability to prepare **17** or **19** through RCM, a second plan was adopted that would rely on a condensation reaction to drive ring-closure. Arguably, the most successful methods in the literature for the generation of medium-sized and macrocyclic rings involve the addition of an alcohol to an activated carbonyl (i.e., lactonization).[36] By analogy, a strategy for cyclic guanidine synthesis can be envisioned in which an amine is added to an isothiourea. This type of condensation is commonly employed for the preparation of substituted acyclic guanidines; in most cases, the reaction is presumed to operate through a reactive carbodiimide intermediate.[37] Accordingly, such a process could be staged in an intramolecular fashion to give **2** (Figure 9). The ability to access isothiourea-derived amine **4** from *N,O*-acetal **10** is an attractive feature of this design.

(26) (a) Fleming, J. J.; Fiori, K. W.; Du Bois, J. *J. Am. Chem. Soc.* **2003**, *125*, 2028−2029. (b) Fiori, K. W.; Fleming, J. J.; Du Bois, J. *Angew. Chem., Int. Ed.* **2004**, *43*, 4349−4352.
(27) (*R*)-(−)-2,2-Dimethyl-1,3-dioxolane-4-methanol (i.e., (*R*)-glycerol acetonide) was purchased from Oakwood Products Inc., West Columbia, SC 29172.
(28) (*R*)-Glycerol acetonide can also be obtained by resolution of (±)-glycerol acetonide or from (*S*)-glyceraldehyde acetonide, see: (a) Pallavicini, M.; Valoti, E.; Villa, L.; Piccolo, O. *Tetrahedron: Asymmetry* **1994**, *5*, 5−8. (b) Pallavicini, M.; Valoti, E.; Villa, L.; Piccolo, O. *Tetrahedron: Asymmetry* **1996**, *7*, 1117−1122. (c) Hubschwerlen, C.; Specklin, J. L.; Higelin, J. *Org. Synth.* **1995**, *72*, 1−5.
(29) An earlier, optimized preparation of **9** required 3 mol% $Rh_2(O_2CCPh_3)_4$, see ref 26a.
(30) $Rh_2(esp)_2$ = $Rh_2(\alpha,\alpha,\alpha',\alpha'$-tetramethyl-1,3-benzenedipropionate$)_2$, see: Espino, C. G.; Fiori, K. W.; Kim, M.; Du Bois, J. *J. Am. Chem. Soc.* **2004**, *126*, 15378−15379.
(31) (a) *Handbook of Metathesis*; Grubbs, R. H., Ed.; Wiley-VCH: Weinheim, 2003; Vol. 2. (b) Deiters, A.; Martin, S. F. *Chem. Rev.* **2004**, *104*, 2199−2238. (c) Conrad, J. C.; Fogg, D. E. *Curr. Org. Chem.* **2006**, *10*, 185−202.
(32) For some recent examples, see: (a) Hirama, M.; Oishi, T.; Uehara, H.; Inoue, M.; Maruyama, M.; Guri, H.; Satake, M. *Science* **2001**, *294*, 1904−1907. (b) Clark, J. S.; Marlin, F.; Nay, B.; Wilson, C. *Org. Lett.* **2003**, *5*, 89−92. (c) Crimmins, M. T.; Powell, M. T. *J. Am. Chem. Soc.* **2003**, *125*, 7592−7595. (d) Gaich, T.; Mulzer, J. *Org. Lett.* **2005**, *7*, 1311−1313. (e) Sato, K.; Sasaki, M. *Org. Lett.* **2005**, *7*, 2441−2444. (f) Pansare, S. V.; Adsool, V. A. *Org. Lett.* **2006**, *8*, 5897−5899. (g) Li, Y.; Hale, K. J. *Org. Lett.* **2007**, *9*, 1267−1270.
(33) McReynolds, M. D., Ph.D. Thesis, University of Kansas, Lawrence, 2004.
(34) Fürstner, A.; Langemann, K. *J. Am. Chem. Soc.* **1997**, *119*, 9130−9136.
(35) Urea **18** was synthesized from **12** following a sequence of steps analogous to those shown in Scheme 1.
(36) (a) Meng, Q. C.; Hesse, M. *Top. Curr. Chem.* **1992**, *161*, 107−176. (b) Parenty, A.; Moreau, X.; Campagne, J.-M. *Chem. Rev.* **2006**, *106*, 911−939.
(37) For a recent comprehensive review on guanidine synthesis, see: Katritzky, A.; Rogovoy, B. V. *Arkivoc* **2005**, 49−87. Also, see: Ma, D. W.; Xia, C. F.; Jiang, J. Q.; Zhang, J. H.; Tang, W. J. *J. Org. Chem.* **2003**, *68*, 442−451.

Scheme 1[a]



[a] Reagents and conditions: (a) PMBCl, K_2CO_3, $^nBu_4NI$, 71%; (b) Tf_2O, C_5H_5N; (c) NaN_3, DMF, −15 °C, 8% (two steps); (d) $(NH_4)_2Ce(NO_3)_6$, ^tBuOH, 55 °C, 92%; (e) NaO^{t}Bu, MbsN=CCl_2, CH_2Cl_2; then $(Me_3Si)_2NH$, 63%; (f) H_2O, CH_3CN, 65 °C, 70%; (g) Me_3P, H_2O/THF; (h) MbsN=C(SMe)NHCH$_2$CH$_2$CH=CH$_2$, $AgNO_3$, 80% (two steps).

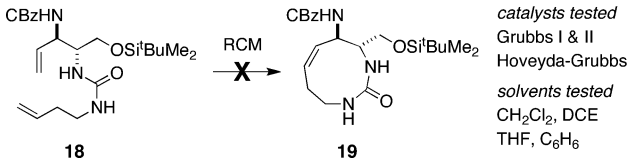


Figure 8. Urea analog also fails to provide nine-membered ring through metathesis reaction.

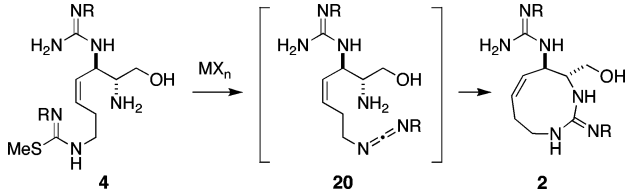


Figure 9. Cyclic guanidine formation via carbodiimide addition.

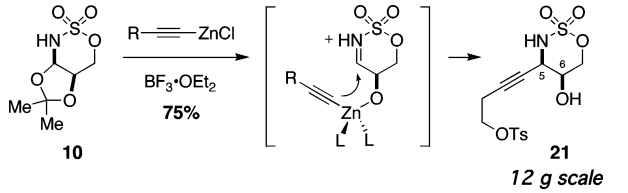
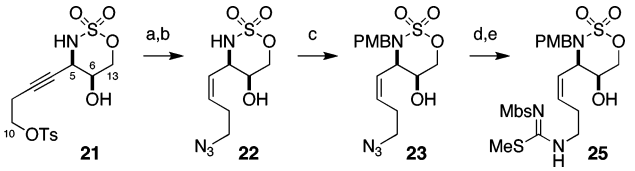


Figure 10. Stereoselective alkynylzinc addition furnishes a highly versatile intermediate.

Capitalizing on earlier work from our lab, oxathiazinane *N*,*O*-acetal **10** was transformed to the corresponding alkyne derivative in a highly stereoselective addition process (>20:1 ds, Figure 10).[26a] Following an optimized protocol, more than 10 g of **21** was synthesized in a single pass without recourse to chromatography. X-ray analysis of this crystalline material confirmed the desired cis-stereochemistry between C5 and C6 (STX numbering). The sense of induction in this reaction is consistent with directed attack of the alkynyl nucleophile by the C6 heteroatom. Optically pure **21**, obtained in just three steps from commercial material, affords an extremely versatile building block from which to elaborate **4**.

To forward our synthetic plan, transforming the C10 tosylate to an isothiourea was considered the most direct and practicable means to enact nine-membered ring closure. Azide displacement of the C6 alcohol could provide the necessary amine nucleophile for this intramolecular condensation. Accordingly, partial hydrogenation of oxathiazinane **21** was accomplished using Lindlar's catalyst (Scheme 2). The efficiency of this process was sufficiently high that subsequent azide displacement of the 1° tosylate was performed on the unpurified material (80%, two steps). Our exploratory studies aimed at trying to manipulate **22**, and in particular the C6 hydroxyl center, made evident the need to first block the NH moiety of the oxathiazinane nucleus (*vide infra*). For this purpose, the *p*-methoxybenzyl (PMB) protecting group was chosen and could be easily installed by

Scheme 2[a]



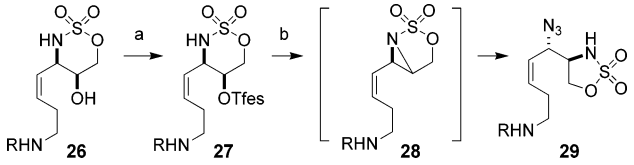
[a] Reagents and conditions: (a) Pd/$CaCO_3$/Pb, quinoline, THF, H_2; (b) NaN_3, $^nBu_4NI$, DMF, 80% (two steps); (c) PMBCl, K_2CO_3, $^nBu_4NI$, 80%; (d) Me_3P, H_2O/THF; (e) MbsN=C(Cl)SMe **24**, $^iPr_2NEt$, CH_3CN, 70% (two steps).

alkylation of **22** under mild base treatment (K_2CO_3, 80%). Reduction of azide **23** using Me_3P followed by treatment of the intermediate amine with an imidoyl chloride reagent, MbsN=C(Cl)SMe **24**, cleanly furnished isothiourea **25** (70%, two steps). Rapoport has explored the general use of imidoyl chloride electrophiles such as **24** for guanidine synthesis, although this particular derivative had not been previously prepared.[38] Despite the sparing use of such compounds in the literature, **24** and a related dichloro-form (*vide infra*) have been of exceptional service in our STX effort. In addition, and as will be highlighted in a later section, the *p*-methoxybenzenesulfonyl (Mbs) group proved ideal for protecting the polar guanidine residues.

Access to isothiourea **25** now possible, replacement of the C6 alcohol with the requisite amine unit remained the final task prior to attempting nine-membered ring closure. The added need to invert the stereochemistry at this C6 position suggested a sequence involving alcohol activation (Tf_2O or $CF_3CH_2SO_2Cl$, pyridine) and displacement with NaN_3. Attempts to employ other activating groups (i.e., mesylate, tosylate) failed, as such substrates were found to be unacceptable for the S_N2 reaction. Although a solution involving triflate formation ultimately proved successful, our initial attempts to install the C6 azide revealed an unexpected roadblock. Through earlier routes that lacked PMB-protection of the oxathiazinane nitrogen, we learned that activation of the C6 alcohol and treatment with azide (or other mild bases) resulted in intramolecular displacement to give the unusual bicyclic aziridine **28** (Scheme 3). This material has been isolated and characterized; however, under most reaction conditions, nucleophilic opening at the allylic center ensued to afford the five-membered sulfamidate product **29**. Our desire to avoid the introduction of an oxathiazinane NH protecting group caused us to examine many different reaction conditions for introducing the C6 amine; unfortunately, all attempts were complicated by the rather remarkable facility by which the aziridine forms. In the final analysis, blocking the NH position allowed this problem to be circumvented (Scheme 4). Notably,

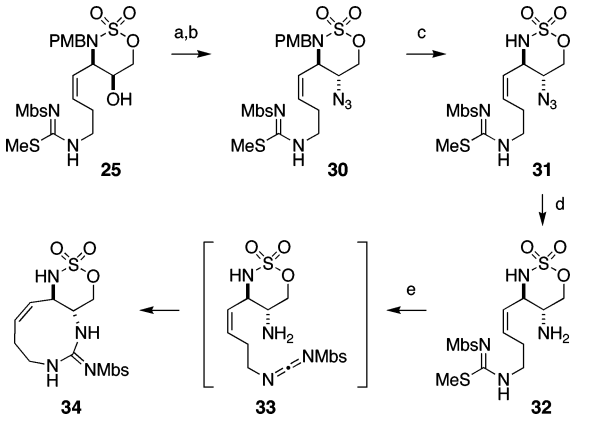
(38) Bosin, T. R.; Hanson, R. N.; Rodricks, J. V.; Simpson, R. A.; Rapoport, H. *J. Org. Chem.* **1973**, *38*, 1591−1600, and references therein.

Scheme 3. An Unexpected Ring Contraction [a]



[a] Reagents and conditions: (a) $CF_3CH_2SO_2Cl$, C_5H_5N, DMAP, CH_2Cl_2, 0 °C, 89%; (b) NaN_3, DMF, 0 °C, 70%; R = MbsN=C(SMe).

Scheme 4 [a]



[a] Reagents and conditions: (a) Tf_2O, C_5H_5N, DMAP, CH_2Cl_2, 0 °C; (b) NaN_3, DMF, −15 °C, (70%, two steps); (c) $(NH_4)_2Ce(NO_3)_6$, ^{t}BuOH, CH_2Cl_2, 74%; (d) $SnCl_2$, THF, MeOH; (e) $AgNO_3$, ^{i}Pr$_2$NEt, CH_3CN, 40% (two steps).

our completed synthesis of STX relies exclusively on this lone *p*-methoxybenzyl and just two *p*-methoxybenzenesulfonyl protecting groups.

Successful introduction of the C6 azide rendered the PMB group unnecessary, and therefore its removal was effected under oxidative conditions (ceric ammonium nitrate, 74%, Scheme 4).[39] Closure of the nine-membered guanidine ring could now be attempted from the oxathiazinane intermediate **31**. Initial reduction of the azide using $SnCl_2$ was effective for preparing the C6 amine without recourse to purification. Under highly optimized conditions, treatment of this compound with $AgNO_3$ and ^{i}Pr$_2$NEt provided the sought-after bicycle **34** in 40% yield. This material was unstable to chromatography and storage, presumably due to strain-promoted ring opening of the fused oxathiazinane. The strain induced by the trans-substituted oxathiazinane ring may also have a deleterious influence on the cyclization reaction itself. Given our inability to optimize this process, we decided to investigate nine-membered ring formation from an acyclic starting material (e.g., **4**, see Figure 9). Such an intermediate could be prepared by first converting oxathiazinane **31** to the Mbs-guanidine **36** (Figure 11). This unusual guanidinylation reaction was made possible with the use of a second imidoyl chloride, MbsN=CCl_2 **35**.[40,41] Initial condensation of **31** with MbsN=CCl_2 followed by quenching

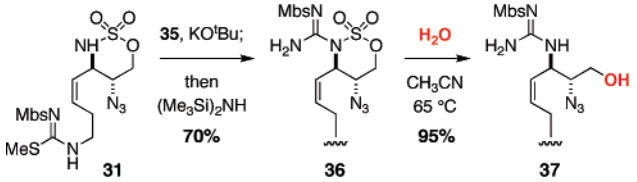


Figure 11. Oxathiazinane N-guanidinylation facilitates subsequent ring opening with H_2O.

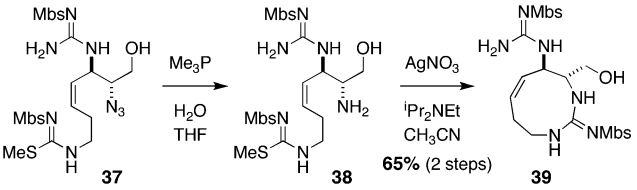


Figure 12. Efficient nine-membered ring formation from an acyclic starting material.

with $(Me_3Si)_2NH$ afforded the desired product in 70% yield (+20% of recovered **31**). By activating the oxathiazinane heterocycle in this way, subsequent hydrolysis is greatly facilitated and acyclic alcohol **37** can be obtained. It is interesting to note that this rather uncomplicated structure, **37**, contains all of the carbons found in the tricyclic core of STX.

The decision to prepare alcohol **37** proved advantageous, as cyclization of this intermediate to the desired nine-membered guanidine was considerably more effective than prior attempts to close **32** (Figure 12). In this instance, azide reduction with Me_3P was optimal over other methods tested (e.g., $SnCl_2$). Treatment of the unpurified amine with $AgNO_3$ triggered carbodiimide formation and ring closure in 65% yield for the two-step process. Unlike its predecessor **34**, this monocyclic compound **39** was stable to purification. With the improved performance of this cyclization reaction, and the ability to prepare gram quantities of the nine-membered guanidine **39**, the final elements of the synthetic plan could now be tried.

Transforming the Monocyclic Guanidine to STX. Conversion of a nine-membered ring guanidine such as **39** to the tricyclic core of STX is a problem laden with potential complications (Figure 13). In the natural product, both C4 and C12 centers reside at the ketone oxidation level. The direct six-electron oxidation of an alkene to a diketone is, however, a reaction with very little precedent.[42,43] Moreover, it is likely that any method for transforming an alkene to an α-dione would proceed through intermediate species of varying oxidation states (i.e., 1,2-diol, α-hydroxy ketone). Selectivity in the formation of a C4,C12-hydroxy ketone **40** is essential, as the regioisomeric structure **42** has the potential for transannular collapse to give an undesired bicyclic product **43**. In principle, the isomeric bicycle could be made to equilibrate to the proper form, but such a reaction would require ring opening back to the nine-

(39) To our knowledge, *p*-methoxybenzyl cleavage of an N-substituted oxathiazinane heterocycle or sulfamate ester has not been reported. For deprotection of an *N*-PMB amide, carbamate, and thiazolidinone, see respectively: (a) Williams, R. M.; Armstrong, R. W.; Dung, J. S. *J. Am. Chem. Soc.* **1984**, *106*, 5748−5750. (b) Alcon, M.; Moyano, A.; Pericas, M. A.; Riera, A. *Tetrahedron: Asymmetry* **1999**, *10*, 4639−4651. (c) Smith, A. B.; Leahy, J. W.; Noda, I.; Remiszewski, S. W.; Liverton, N. J.; Zibuck, R. *J. Am. Chem. Soc.* **1992**, *114*, 2995−3007.

(40) For the general preparation of reagents such as **35**, see: (a) Neidlein, R.; Haussman, W. *Tetrahedron Lett.* **1965**, 1753−1755. (b) Gompper, R.; Kunz, R. *Chem. Ber. Recl.* **1966**, *99*, 2900−2904. (c) Merchan, F. L.; Garin, J.; Tejero, T. *Synthesis* **1982**, 984−986.

(41) Compound **35** was prepared following a published protocol, see: Tanga, M. J.; Bradford, W. W.; Bupp, J. E.; Kozocas, J. A. *J. Heterocycl. Chem.* **2003**, *40*, 569−573.

(42) For examples, see: (a) Sharpless, K. B.; Lauer, R. F.; Repic, O.; Teranishi, A. Y.; Williams, D. R. *J. Am. Chem. Soc.* **1971**, *93*, 3303−3304. (b) Yusubov, M. S.; Filimonov. V. D.; Vasilyeva, V. P.; Chi, K.-W. *Synthesis* **1995**, 1234−1236. (c) Yusubov, M. S.; Krasnokutskaya, E. A.; Vasilyeva, V. P.; Filimonov, V. D.; Chi, K.-W. *Bull. Korean Chem. Soc.* **1995**, *16*, 86−88. (d) Clayton, M. D.; Marcinow, Z.; Rabideau, P. W. *Tetrahedron Lett.* **1998**, *39*, 9127−9130.

(43) Methods for alkene ketohydroxylation have been recently reviewed, see: Plietker, B. *Tetrahedron: Asymmetry* **2005**, *16*, 3453−3459. Also, see: Plietker, B. *Synthesis* **2005**, 2453−2472.

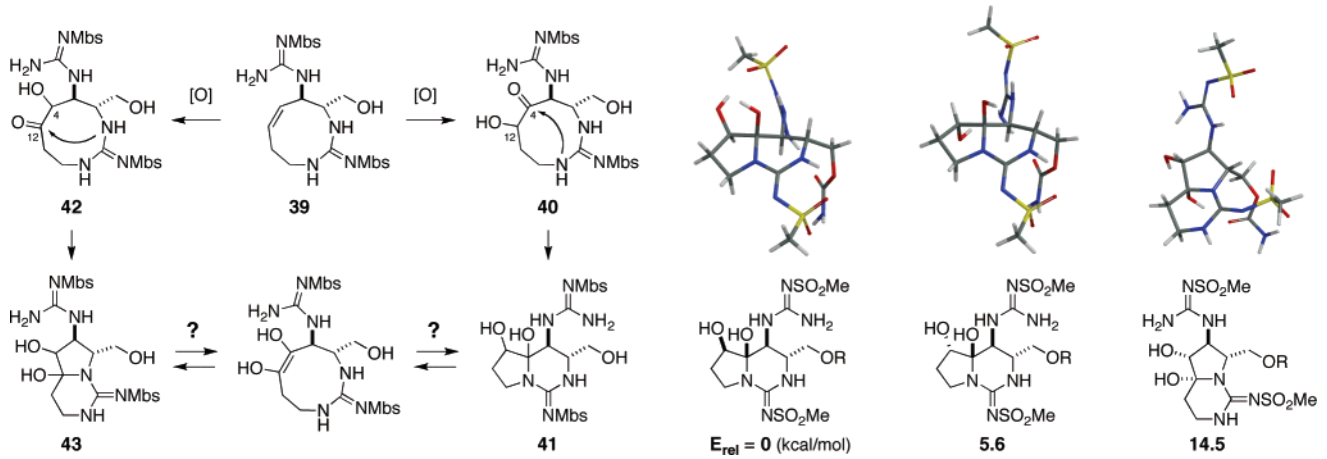


Figure 13. Potential complications associated with C4,C12-alkene oxidation. DFT analysis (B3LYP/6-31G*) suggests that the desired isomer **41** is thermodynamically preferred. Calculations were performed on all four diastereomers of each configurational isomer (i.e., **41** and **43**). The three lowest energy structures are shown (R = C(O)NH_2), see Supporting Information for details.

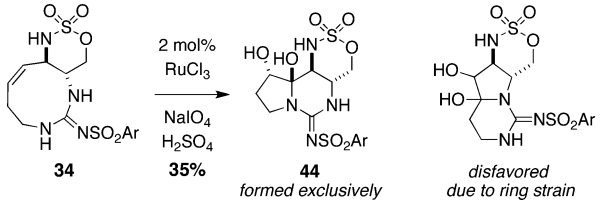


Figure 14. Regioselective alkene ketohydroxylation gives the desired hemiaminal **44**.

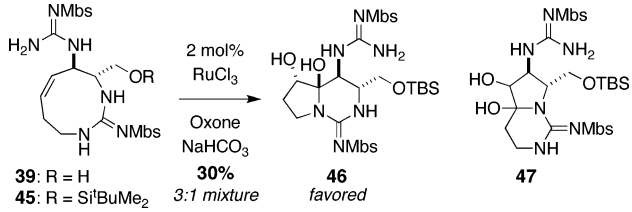


Figure 15. Ru-catalyzed oxidation of **45** affords a mixture of hemiaminal products.

membered guanidine. Also, relying on thermodynamic equilibration between structures **41** and **43** presupposes that an energetic difference between these two compounds exists and is biased toward the requisite product **41**. In this regard, density functional theory (DFT) calculations (B3LYP/6-31G*) do, in fact, confirm that the desired bicyclic isomer **41** is strongly preferred, irrespective of the stereochemical consequences of the ketohydroxylation reaction (Figure 13).[44]

It bears mentioning that the potential problems surrounding the oxidation of the monocyclic guanidine **39** guided our earliest approach to STX. With bicyclic oxathiazinane **34**, we surmised that generation of the undesired hemiaminal product following alkene oxidation would be disfavored due to the strain energy associated with forming a fused trans-5,6 ring system (Figure 14). This expectation was indeed realized, as oxidation of **34** under conditions described by Plietker (2 mol % $RuCl_3$, $NaIO_4$, H_2SO_4) furnished only the tricyclic compound **44**, albeit in a rather modest 35% yield.[45] Compound **44** is produced as a single diastereomer, the structure of which was confirmed by X-ray analysis.[46] Our excitement with having prepared **44** was, unfortunately, tempered by the suboptimal yields for both the nine-membered guanidine assembly (*vide supra*) and this Ru-catalyzed four-electron oxidation. Accordingly, focus turned toward ketohydroxylation of the more readily accessible monocyclic compound, **39**.

As our initial experiments would indicate, the absence of the oxathiazinane ring in guanidine **39** jeopardizes the ability to control isomer formation in the transannular ring-closing event. Treatment of **45** with catalytic $RuCl_3$ and Oxone furnished both the desired hemiaminal **46** and a second product tentatively assigned as the undesired isomer **47** (~3:1 selectivity, Figure 15).[47,48] More troubling than the low product selectivity, however, was the poor overall yield of this reaction (30%). In spite of these results, a sufficient quantity of the bicyclic compound **46** was obtained to enable forward progress. To complete the synthesis of the STX core, **46** was exposed to *p*-TsOH, conditions that we anticipated would induce iminium ion formation and ring-closure of the five-membered guanidine. This reaction did indeed furnish a new product (Figure 16), but a definitive structural assignment of this material was not possible in the absence of unambiguous coupling constant data. Thus, we advanced under the assumption that formation of the desired *N*,*N*-aminal at C4 had occurred in the expected fashion, conducting the subsequent oxidation of the C12 alcohol with $C_5H_5N{\cdot}SO_3$ in DMSO.[49] This transformation proved quite revealing, as it became clear that the bond connectivity in the isolated material **48** was different from that of the natural product. Perhaps the most telling discrepancy between the two structures was the C5,C6 coupling constant, measured at 3.4 Hz for **48** and ~1 Hz for decarbamoylsaxitoxin (dcSTX).[16a]

(44) (a) Calculations performed using Gaussian 03, Revision C.02, Frisch, M. J. et al. Gaussian, Inc., Wallingford, CT, 2004. (b) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652.

(45) The conditions employed were analogous to those described for olefin dihydroxylation, see: (a) Plietker, B.; Niggemann, M. *Org. Lett.* **2003**, *5*, 3353−3356. (b) Plietker, B.; Niggemann, M.; Pollrich, A. *Org. Biomol. Chem.* **2004**, *2*, 1116−1124.

(46) X-ray analysis was performed on the *p*-toluenesulfonyl-protected form of **44**. The tosyl-protecting group was employed in our earliest studies to prepare STX and later replaced with *p*-methoxybenzenesulfonyl (Mbs).

(47) Oxone has been shown to be a more effective oxidant than $NaIO_4$ for alkene ketohydroxylation, see: (a) Plietker, B. *J. Org. Chem.* **2003**, *68*, 7123−7125. (b) Plietker, B. *J. Org. Chem.* **2004**, *69*, 8287−8296. (c) Plietker, B. *Eur. J. Org. Chem.* **2005**, 1919−1929.

(48) The broadened signals in the ^{1}H NMR spectrum of this product made difficult an accurate determination of the relative amount of the minor isomer.

(49) The ketone was isolated in the hydrated form as determined by IR and ^{13}C NMR. For examples of guanidine-derived structures possessing a hydrated ketone, see: Houghten, R. A.; Simpson, R. A.; Hanson, R. N.; Rapoport, H. *J. Org. Chem.* **1979**, *44*, 4536−4543.

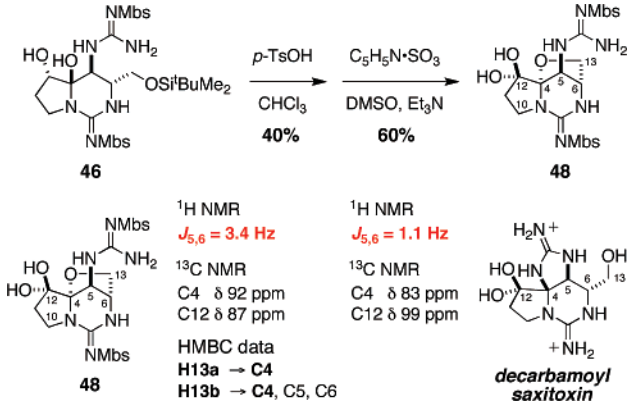


Figure 16. An attempt to form the tricyclic core of STX gives an undesired isomeric structure.

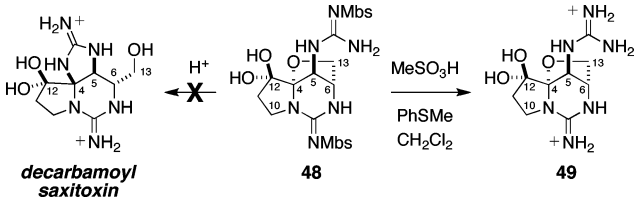
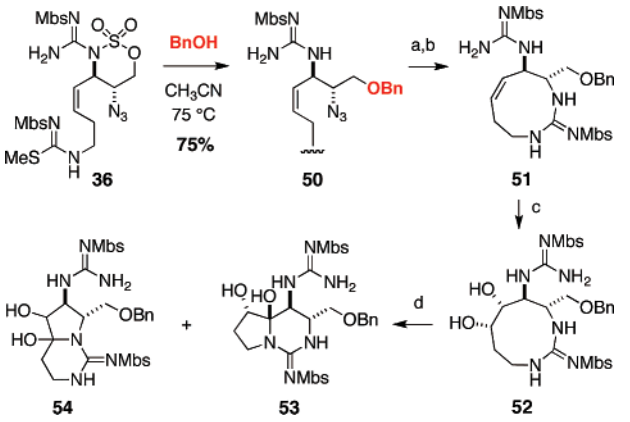


Figure 17. Acid treatment of **48** does not afford decarbamoyl STX.

Although mass spectral data could not distinguish between the two potential isomeric forms, additional NMR analysis, including $^1H-^{13}C$ heteronuclear multiple bond correlation (HMBC), revealed that the C13-hydrogens were within three contiguous bonds of the C4 center, suggesting that *N*,*O*-acetal **48** had formed instead of the desired *N*,*N*-aminal.[50] We speculated that the Mbs-protecting groups on both guanidine units might be responsible for the unexpected preference of the *N*,*O*-acetal linkage; accordingly, their removal was smoothly effected using CH_3SO_3H and thioanisole.[51] This transformation, however, failed to promote the anticipated isomerization reaction of the recalcitrant *N*,*O*-acetal **48** (Figure 17); thus, a new tactic was needed to facilitate the desired cyclization pathway.

Blocking the C13-hydroxyl moiety with an acid-stable protecting group presented the most straightforward means for circumventing the formation of *N*,*O*-acetal **48**. Fortunately, our synthetic plan to the nine-membered ring guanidine was sufficiently flexible to allow for facile introduction of an ether or ester group at C13. Benzyl ether **50** could be prepared by direct displacement of oxathiazinane **36** with BnOH (Scheme 5), and by following our earlier protocol (Me_3P, then $AgNO_3$), the nine-membered ring olefin **51** was readily assembled. Ketohydroxylation of **51**, however, using catalytic $RuCl_3$ and Oxone lead only to intractable material, possibly owing to competitive oxidation of the benzylic unit. Recourse to a two-step process of dihydroxylation and monooxidation with Dess–Martin periodinane (DMP) furnished hemiaminal **53** in 50% yield along with 35% of its structural isomer **54**.[52] Having isolated hemiaminal **54** in pure form, the prospect of equilibrating this compound to the requisite bicycle **53** could now be discerned. No such reaction was observed under neutral or acidic

Scheme 5[a]



[a] Reagents and conditions: (a) Me_3P, H_2O/THF; (b) $AgNO_3$, $^iPr_2NEt$, CH_3CN, 65% (two steps); (c) 20 mol % OsO_4, NMO, DABCO, H_2O, ^{t}BuOH/acetone, 84%; (d) DMP, CH_2Cl_2, 85% (50% **53**, 35% **54**).

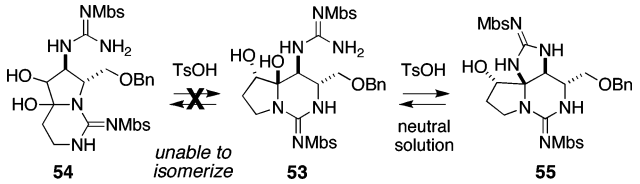


Figure 18. Acid-promoted ring closure gives an unstable tricyclic product **55**, which reverts to starting material **53** upon standing.

conditions (TsOH), and thus we speculate that the selectivity observed for **53** (albeit small) is kinetic in origin (Figure 18).[53] While a method for selective diol oxidation would be needed to improve material throughput in this step, we were quite pleased to find that treatment of the correct hemiaminal **53** with TsOH resulted in ring closure to the tricyclic frame of STX **55**. Thus, our strategy for preparing STX through late-stage dehydrative aminal formation appeared viable. Rather surprisingly, however, it was not possible to further manipulate tricycle **55**, as it reverted back to starting material **53** upon attempted purification. Given the known stability of β-STXol (a dication), the Mbs guanidine protecting groups in **55** were presumed to be responsible for disfavoring the equilibrium between **53** and **55**. Our next move, it therefore seemed, was to identify reaction conditions that would induce ring closure and simultaneous guanidine deprotection without cleaving the C13 benzyl ether.

Prior success at deblocking the two Mbs-guanidines in **48** with CH_3SO_3H (see Figure 17) prompted our decision to test this acid for the single-step conversion of **53** to decarbamoyl β-saxitoxinol (β-dcSTXol). The strength of CH_3SO_3H in CH_2Cl_2 is rather remarkable and, while some of the desired product was indeed obtained, an equivalent amount of *N*,*O*-acetal **56** was generated (Figure 19). Assuming that **56** forms irreversibly, the observed product ratio suggests that the relative rate of benzyl ether acidolysis and *N*,*O*-acetal formation is comparable to that of ring closure and guanidine deprotection. Thus, in an attempt to alter favorably the course of this reaction, Lewis acidic conditions were explored. Prior work by Pless, Bauer, and Nishimura suggested to us that boron tris(trifluoroacetate) ($B(O_2CCF_3)_3$) might be a uniquely effective reagent for

(50) Complete HMBC data for compound **48** can be found in the Supporting Information.
(51) Structural assignment of intermediate **49**, as determined by ^{1}H NMR and mass spectral analysis, is tentative.
(52) Although the undesired hemiaminal **54** formed as a single diastereomer, its stereochemistry was never established conclusively.
(53) It is not possible to discount a mechanistic scenario in which rapid tautomerization of the α-ketol isomers precedes transannular ring closure. We believe, however, that the collective results of our studies to oxidize substrates such as **51** are more consistent with an oxidation reaction in which α-ketol selectivity is kinetically controlled.

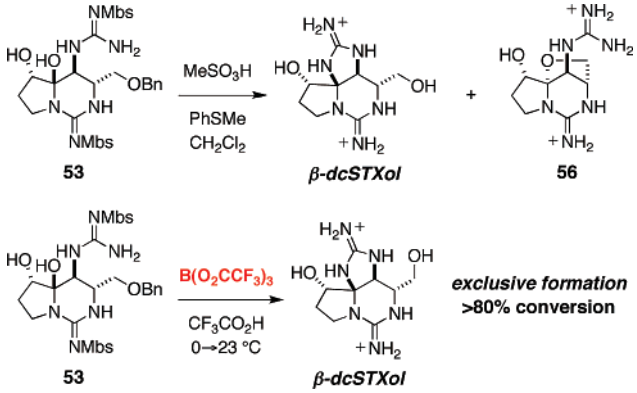
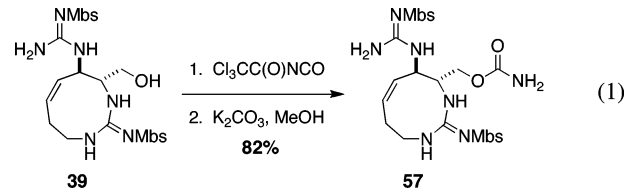


Figure 19. Guanidine deprotection affords decarbamoyl β-saxitoxinol.

transforming **53** to β-dcSTXol.[54] Although milder than BBr_3, $B(O_2CCF_3)_3$ has been used to deprotect Mbs-guanidines.[54b] Such a Lewis acid would undoubtedly cleave the C13 benzyl ether as well, but here it was assumed that the intermediate alkoxy boron species would be incapable of closing to form the unwanted *N*,*O*-acetal **56**. When put to practice, $B(O_2CCF_3)_3$ exceeded all expectations, affording β-dcSTXol in >80% yield and giving no trace of the isomeric structure **56** in the ^{1}H NMR spectrum of the unpurified reaction mixture. Now only two steps remained to complete the natural product. Selective carbamoylation, however, of the C13 alcohol with the 2° alcohol free at C12 would be necessary to accomplish this feat. The highly polar nature and poor solubility of the bis-guanidinium salt in organic solvents further complicated this problem.

As a consequence of our inability to manipulate β-dcSTXol, an alternative end-game strategy was devised that would attempt the critical Mbs-deprotection/guanidine cyclization step with the C13-carbamate in place. The reported functional group compatibility of $B(O_2CCF_3)_3$ toward carboxylic esters encouraged us to examine such a strategy.[54] Moreover, such a plan, if successful, would afford a most expeditious path from **39** to STX.

Alkene Ketohydroxylation: An Optimal Solution. Conversion of the C13-alcohol in **39** to the requisite 1° carbamate was readily accomplished using trichloroacetyl isocyanate (eq 1).[55] It should be noted that in both the Kishi and Jacobi routes to STX, carbamate installation was conducted as a modest-yielding, final step on dcSTX, a bis-guanidiniun salt that is insoluble in most solvents.[4,5] With the guanidines suitably masked, the ability to synthesize, isolate, and purify carbamate **57** is facile and, as an added benefit, straightforward access to carbamate derivatives of STX also becomes possible.



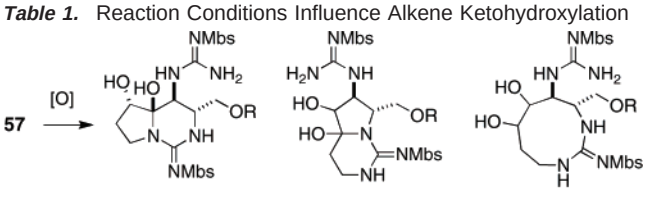
(1)

Initial attempts to execute the critical ketohydroxylation of alkene **57** would capitalize on a two-step sequence developed in our earlier studies (see Scheme 5). Accordingly, dihydroxylation of **57** with OsO_4/*N*-methylmorpholine *N*-oxide (NMO) proceeded smoothly and furnished a single diol product. The subsequent reaction with DMP, however, was marred by an unfavorable isomer product ratio (~1:2 desired/undesired). The discernible difference between this reaction and oxidation of **52** (see Scheme 5), while small, is quite surprising in light of the number of carbon centers separating the C13−O protecting group and the C4/C12 alkene. Fortunately, this selectivity was reversed in the one-step ketohydroxylation of **57** with catalytic $RuCl_3$ and Oxone, conditions that afforded a 2.3:1 product mixture favoring **58** (entry 1, Table 1). The combined yield for this reaction, however, could never be made to exceed 40% (entry 2). Determined to solve this problem in a direct fashion, efforts focused on improving the one-step, four-electron conversion of **57** to the desired hemiaminal **58**. The low yield and mass recovery for the Ru-catalyzed oxidation was likely due to competitive C−C bond cleavage of the intermediate Ru-glycolate. Changing the oxidant and pH of the solution did little to improve the reaction performance. The switch to an Os catalyst in place of Ru, thus, followed as a logical progression.[56]

Direct conversion of an olefin to an α-ketol using OsO_4 can be traced as far back as 1942 in seminal work from Prins and Richstein.[57] Later studies at Upjohn would demonstrate more fully the power of this transformation in the context of modifying unsaturated steroidal systems.[58] This same group and others have noted that Os-catalyzed dihydroxylation using H_2O_2 or ^{t}BuOOH gives varying amounts of the vicinal keto-alcohol as a side product.[59] To our knowledge, however, no systematic investigation of OsO_4 for ketohydroxylation of nonsteroidal olefins has been reported. By employing a combination of 8 mol % OsO_4 and ^{t}BuOOH, efficient four-electron oxidation of alkene **57** was achieved (70%); regrettably, it soon became clear that the product had formed exclusively as the undesired hemiaminal **59** (entry 3). Buoyed by earlier data from our studies with Ru-catalysts, which had shown that oxidant and base additives could alter selectivity in the ketohydroxylation event, we continued to examine this unique reaction. Further guided by a report by Murahashi describing alkene ketohydroxylation, we turned to $OsCl_3 \cdot H_2O$ as a catalyst in combination with different terminal oxidants including Oxone.[60] Rather astonishingly, treatment of **57** with a mixture of 10 mol % $OsCl_3 \cdot H_2O$, Oxone, and $NaHCO_3$ reversed the reaction outcome and favored the wanted bicycle **58** as a 5:1 isomeric mixture along with a substantial fraction of diol **60** (entry 4). The amount of this latter product was sensitive to the base employed, and the addition of excess Na_2CO_3 in lieu of $NaHCO_3$ almost completely suppressed its formation. Subsequent optimization studies revealed conditions in which hemiaminal **58** could be obtained in good yield (57%) and with high regio- (12:1) and stereocontrol (>20:1) using 10 mol % $OsCl_3 \cdot H_2O$, 10 equiv of Na_2-

(54) (a) Pless, J.; Bauer, W. *Angew. Chem., Int. Ed.* **1973**, *12*, 147−148. (b) Nishimura, O.; Fujino, M. *Chem. Pharm. Bull.* **1976**, *24*, 1568−1575.
(55) Kocovsky, P. *Tetrahedron Lett.* **1986**, *27*, 5521−5524.
(56) For a discussion of C−C bond cleavage by Ru and Os oxidants, see: Frunzke, J.; Loschen, C.; Frenking, G. *J. Am. Chem. Soc.* **2004**, *126*, 3642−3652.
(57) (a) Prins, D. A; Reichstein, T. *Helv. Chim. Acta* **1942**, *25*, 300−322. (b) Miescher, K. *Helv. Chim. Acta* **1950**, *6*, 1840−1847. (c) Miescher, K.; Schmidlin, R.; Schmidlin, J. U.S. Patent 2,668,816, 1954.
(58) Harkema, J. U.S. Patent 3,582,270, June 1, 1971, and references cited therein.
(59) (a) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *23*, 1973−1976. (b) Lohray, B. B.; Bhushan, V.; Kumar, R. K. *J. Org. Chem.* **1994**, *6*, 1375−1380.
(60) Murahashi, S. I.; Naota, T.; Hanaoka, H. *Chem. Lett.* **1993**, *10*, 1767−1770. Murahashi reports the use of $OsCl_3$ with peracetic acid in EtOAc for α-ketol formation. We did not investigate these specific conditions due to the inability to obtain a commercial supply of peracetic acid in EtOAc.

Table 1. Reaction Conditions Influence Alkene Ketohydroxylation



entry	catalyst[a]	oxidant	base	58	59	60[b]	% yield[c]
1	$RuCl_3$	Oxone	$NaHCO_3$	2.3	1	1	35
2	$RuCl_3$	Oxone	$NaHCO_3$	2.3	1	1	40[d]
3	**OsO_4**	**^tBuOOH**	**none**	**1**	**10**	**–**	**70**
4	$OsCl_3$	Oxone	$NaHCO_3$	5	1	2.3	35
5	$OsCl_3$	Oxone	Na_2CO_3	12	1	1	62
6	$RuCl_3$	Oxone	Na_2CO_3	5	1.5	1	33

[a] Reactions performed with 2−10 mol % catalyst at 25 °C. [b] Product ratio determined by HPLC analysis. [c] %Yield is combined for **58** and **59**. [d] Reaction performed at 10 °C.

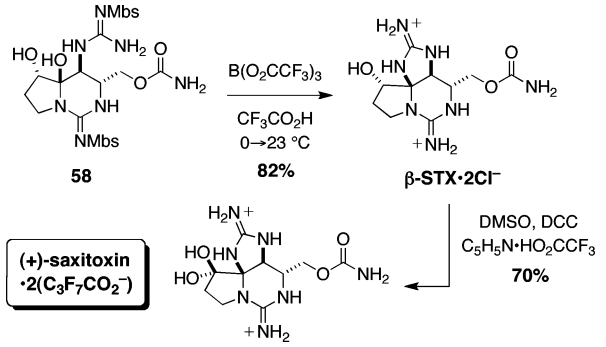


Figure 20. Completed first-generation synthesis of the desired target.

CO_3, and 7 equiv of Oxone (entry 5). The fact that this method delivered the oxidized product **58** out of a mixture of eight possible isomers with selectivity completely opposite that of the OsO_4/^{t}BuOOH combination deserves special comment. As a final note, $OsCl_3$ can be substituted with $RuCl_3$, but not without a cost to both product yield and selectivity (entry 6).

The four-electron oxidation of alkene **57** would ultimately distinguish itself as one of the most intriguing and critical transformations in our completed synthesis of STX. The side-chain element at C13, catalyst, oxidant, and base all transpire to influence the reaction outcome.[61] Optimization of this unique oxidation process at such a late stage underscores the efficiency of the synthetic route to this point.

The Completed Synthesis. With access to hundreds of milligrams of hemiaminal **58** as a single structural and stereoisomer, completion of the STX synthesis now seemed at hand. Conditions previously developed for the deprotection and cyclization of benzyl ether **53** (Figure 19) were anticipated to function with **58**, although the stability of the carbamate side chain to $B(O_2CCF_3)_3$ was uncertain. These concerns were quickly assuaged when exposure of the bicyclic starting material to 30 equiv of $B(O_2CCF_3)_3$ in trifluoroacetic acid provided the known compound, β-STXol, in 82% yield (Figure 20). For this step, the unpurified reaction mixture was exceptionally clean, as judged by ^{1}H NMR analysis, and revealed none of the undesired *N*,*O*-acetal product **56** that had plagued our earlier routes. Facile isolation of the pure bis-hydrochloride salt of β-STXol was easily accomplished using ion-exchange chromatography.

The final transformation in the path to (+)-STX relied on a method reported by Schantz et al. for oxidation of α-STXol·2Cl$^-$ under Pfitzner−Moffat conditions (DCC, DMSO, C_5H_5N·HO_2CCF_3).[16a,62] We were optimistic that such a reaction would perform successfully on β-STXol, as the three-dimensional arrangement of the STX skeleton leaves the β-OH group more accessible than its α-counterpart. By employing this protocol, high conversion (>90% as determined by ^{1}H NMR analysis) of the starting material to STX was successfully accomplished, and the natural product was isolated in 70% yield. Initially, purification of STX·2Cl$^-$ by reverse phase HPLC was quite difficult to reproduce as a result of its extremely short retention time (<1 min) on a C18 column. We later discovered that an eluent system composed of CH_3CN, H_2O, and 10 mM heptafluorobutyric acid dramatically extended HPLC t_R (13.0 min @ 6 mL/min, C18, 10 × 250 mm), thereby providing highly pure samples of the target as the bis-perfluorobutyrate salt ($C_3F_7CO_2^-$). The synthetic material matched the physical data reported for natural (+)-STX·2Cl$^-$ in all respects (^{1}H and ^{13}C NMR, HRMS). In addition, an IC_{50} of 2.3 nM was measured against heterologously expressed $Na_V1.4$ channels (CHO cells), a value consistent with literature reports.[63]

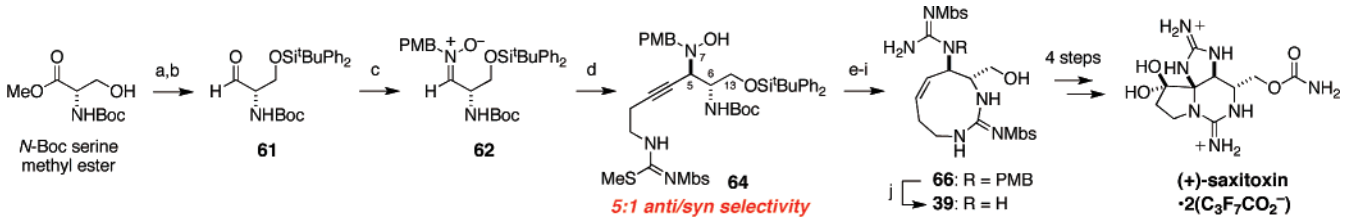
Enantioselective preparation of the paralytic shellfish poison, (+)-STX, has been accomplished through a 19-step sequence and in 1.3% overall yield from commercially available (*R*)-glycerol acetonide. Critical to the success of this program was the discovery of a new class of heterocyclic iminium ion surrogates for the rapid assembly of highly functionalized propargylic amine derivatives. The intermediate alkynyl oxathiazinane **21** (Figure 10) proved an exceptionally versatile substructure, allowing us to explore and evaluate disparate strategies for assembling an unprecedented nine-membered ring guanidine. These findings add to the general methods that we have described for the synthesis and selective manipulation of oxathiazinane *N*,*O*-acetals.[26] When combined with the demonstrated utility of oxathiazinanes for crafting substituted amine products, it is expected that the value of such chemistries will extend beyond the work presented herein. Nevertheless, with hindsight, we recognized that a preparative route to an acyclic target such as **38** (Figure 12) could be conducted with greater step-efficiency by employing alternative protocols for vicinal diamine synthesis. Propelled by an overarching interest to develop STX-like molecules for investigating the structure and integrated function of Na^+ channels in neuronal circuits, a second-generation approach to the nine-membered ring guanidine **39** was conceived. Successful implementation of this new strategy thus reduces the assembly of (+)-STX to only 14 linear steps. The details of this work are outlined below.

A Second Generation Route to (+)-Saxitoxin. A retrospective analysis of our initial route to STX underscores the power of the cyclodehydrative strategy for quickly (four steps) accessing the natural product from a monocyclic guanidine precursor **39** (Figure 21). The preparation of **39** itself, however, requires 15 unique transformations from commercial material,

(61) We speculate that the combination of $OsCl_3$ and Oxone leads to the formation of an Os(V)=O species, which serves as the operative oxidant. The pendant guanidine at C5 may have a critical role as a base to promote selective elimination from the C4 center. Studies are on-going to explore the mechanistic details of this process and will be reported in due course.

(62) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661−5670.
(63) Moran, O.; Picollo, A.; Conti, F. *Biophys. J.* **2003**, *5*, 2999−3006.

Scheme 6. (+)-Saxitoxin in 14 Steps from *N*-Boc-L-Serine Methyl Ester[a]



[a] Reagents and conditions: (a) $^{t}BuPh_2SiCl$, imidazole, DMF, 95%; (b) $^{i}Bu_2AlH$, CH_2Cl_2, 71%; (c) PMBNHOH, $MgSO_4$, CH_2Cl_2, 76%; (d) MbsN=C(SMe)NHCH$_2$CH$_2$C≡CH **63**, ^{i}PrMgCl, THF, −78 °C, 78%; (e) p-TsNHNH$_2$, NaOAc, THF, H_2O, 100 °C, 78%; (f) Zn, $Cu(OAc)_2$, HOAc, H_2O, 70 °C, 81%; (g) MbsN=C(SMe)NHBoc **65**, $HgCl_2$, Et_3N, CH_2Cl_2, 74%; (h) HCl, MeOH, 52%; (i) $AgNO_3$, Et_3N, CH_3CN, 73%; (j) CF_3CO_2H, 60 °C, 91%.

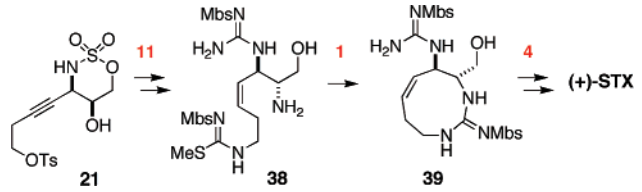


Figure 21. Step-count analysis of the finished route to saxitoxin.

a somewhat lengthy operation for a structure containing only two stereogenic centers. It is worth recalling that the initial role of the oxathiazinane, which ultimately allowed for the assembly of **39**, was not only as a synthetic intermediate but as a structural restraint to bias formation of the correct hemiaminal isomer in the ketohydroxylation/transannular cyclization event. Having established the viability of oxidizing regioselectively the monocylic guanidine **39** without imposed constraints, the plan for synthesizing this material bears reconsidering. To this end, our attention focused on isothiourea **38** (or a closely related structure) given the success of the subsequent carbodiimide ring-closure to fashion the nine-membered ring **39**. Borrowing from earlier work of Merino, addition of an organometallic nucleophile to the nitrone derived from L-serine **62** appeared aptly suited for achieving a more direct means to the desired intermediate (Scheme 6).[64]

Serine-derived aldonitrones analogous to **62** undergo smooth attack by a variety of simple organomagnesium and organolithium compounds to furnish hydroxylamine products with good to excellent levels of anti selectivity. In our minds, an ideal path to an intermediate such as **38** could make use of **62** to assemble **64** in a single step that would import the entire isothiourea substructure via addition of the corresponding metal acetylide. Importantly, preparation of nitrone **62** could be accomplished from commercially available *N*-Boc-L-serine methyl ester in just three transformations.[65] Although additions of dianionic nucleophiles to this starting material are unprecedented, the reaction of the Mg salt of **63** with **62** proceeded nearly quantitatively to afford the product as a 4−5:1 mixture of diastereomers favoring the anti-stereoisomer. Following purification, the desired hydroxylamine **64** was isolated in 78% yield. This dianion coupling step highlights our second generation route of STX and eliminates all functional group manipulations associated with assembling the isothiourea moiety from an alkyl tosylate (*vide supra*).[66]

Partial reduction of alkyne **64** using diimide and subsequent Zn-induced N−O bond cleavage proceeded without event and gave an intermediate 2° PMB-amine onto which the first guanidine unit could be installed. In this instance, a differentially protected isothiourea reagent **65** was prepared to facilitate the coupling reaction. In situ generation of the carbodiimide derived from **65** was effected using $HgCl_2$ and Et_3N, which reacts efficiently with the PMB-amine to afford the N7-guanidine (74%, STX numbering). Removal of both *N*-Boc and $^{t}BuPh_2$-Si-protecting groups using methanolic HCl gave an acyclic structure that was suitably configured for closure to the nine-membered ring. Although attempts were made to cleave the *p*-methoxybenzyl moiety in addition to the other three protecting groups by employing reagents such as cerium ammonium nitrate or CF_3CO_2H/Δ, yields for this process were generally poor. Accordingly, cyclization of the intermediate C6-amine to guanidine **66** was first executed ($AgNO_3$, Et_3N, 73%) followed by removal of the PMB group with CF_3CO_2H. The isolated compound **39** intercepts our initial STX preparation in just 10 linear operations from *N*-Boc-L-serine methyl ester and is easily processed to the optically pure natural product in four steps. This new route has enabled multigram production of the nine-membered guanidine and has greatly facilitated access to modified forms of the toxin.

Conclusion

The completed asymmetric synthesis of the guanidinium poison, (+)-STX, marks a significant first step in our effort to understand the complex structure and function of vg-Na^+ channels using novel pharmacological tools made available through de novo synthesis. A short, modular route to STX was deemed essential to accomplish these longer-term goals and, through the work described herein, such a recipe for assembling the toxin is now in place. Through these studies, new chemical tools and strategies for crafting highly polar, nitrogen-rich small molecules were delineated, some of which should enjoy application beyond the STX assembly. In particular, the development of unique heterocyclic *N,O*-acetals as iminium ion equivalents expands greatly the ability to employ C−H amination for accessing highly functionalized amine derivatives. Other highlights of this work include the first reported preparation of a nine-membered ring guanidine, catalytic ketohydroxylation methods for regioselective alkene oxidation, the application of $B(O_2CCF_3)_3$ to deblock Mbs-guanidine groups, stereoselective aminal formation through cyclodehydration, and acetylide dianion−nitrone addition to generate polyfunctionalized vicinal diamines. The combination of these tactics has reduced a complicated problem in cyclic stereocontrolled synthesis to one of assembling a heteroatom-

(64) (a) Merino, P.; Franco, S.; Merchan, F. L.; Tejero, T. *Synlett* **2000**, 442−454, and references cited therein. (b) Merino, P.; Franco, S.; Merchan, F. L.; Tejero, T. *J. Org. Chem.* **1998**, *16*, 5627−5630.
(65) Merino, P.; Lanaspa, A.; Merchan, F. L.; Tejero, T. *Tetrahedron: Asymmetry* **1998**, *4*, 629−646.
(66) All attempts to add the zinc acetylide of **63** to oxathiazinane **10** (see Figure 10) were unsuccessful.

substituted *cis*-heptene derivative. As such, the finished route to STX requires only 14 linear transformations, affording the natural product with unprecedented efficiency and empowering efforts to construct unique, non-natural toxin designs.

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Supporting Information Available: Complete ref 44a (page S24, ref 12); experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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