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Synthesis of saxitoxins*

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Abstract: Saxitoxin (STX), a neurotoxin isolated from Alaskan butter clam, *Saxidomus giganteus*, inhibits voltage-gated sodium channels by binding to the pore-forming p-loop region. Here we describe stereospecific syntheses of STX and its natural derivatives, (+)-decarbamoylsaxitoxin (dcSTX) and (+)-gonyautoxin3 (GTX3).

Keywords: alkaloid synthesis; guanidine alkaloid; intramolecular reactivity; natural product synthesis; saxitoxin.

INTRODUCTION

Saxitoxin (STX) **1**, originally isolated from toxic shellfish [1], is a neurotoxin that blocks ion influx via voltage-gated sodium channels by binding to the site-I region, to which tetrodotoxin also binds [2]. The structure of **1** was independently elucidated by means of X-ray analysis by Schantz's and Rapoport's groups (Fig. 1) [3]. STX **1** contains two cyclic guanidine structures located in five- and six-membered ring systems, which show different pK_a values of 8.22 and 11.28 (in H_2O), respectively [4]. The difference is due to the non-planarity of the five-membered guanidine ring. Other noteworthy structural features of **1** are the ketone hydrate stabilized by the neighboring guanidine, the anti-diaxial arrangement of the five-membered guanidine ring at C4 and C5 and the side chain at C6. More than 30 analogs of STX have been isolated, and most of them have modifications at C11, C13, and N1 [1,5]. Because of the complex structure of STX **1**, as well as its biological importance as a pharmaceutical tool for ion channel studies, the molecule has attracted a great deal of attention from synthetic chemists. The first total synthesis of (+/–)-STX **1** was reported by Kishi and his co-workers in 1977 [6]. Jacobi et al. also developed a strategy for the synthesis of (+/–)-**1** based upon intramolecular 1,3-dipolar cycloaddition (1,3-DC) of azomethine imine to an imidazolone [7]. Recently, Du Bois and his co-workers succeeded in the synthesis of natural (+)-STX **1** by using a catalytic C–H amination strategy [8]. Other natural STX analogs, (–)-decarbamoylsaxitoxin (dcSTX) **3** and (+)-gonyautoxin3 (GTX3) **7**, were also synthesized by Kishi and Du Bois, respectively [9]. Quite recently, Nishikawa and his co-workers reported a synthesis of (+)-dc-α-saxitoxinol (STXol) **4** [10].

In the course of our synthetic studies of STX and its analogs, we have reported syntheses of (–)- and (+)-decarbamoyloxysaxitoxin (doSTX) **2**, (+)-STX **1**, (+)-dcSTX **3**, and (+)-GTX3 **7** [11]. Herein, we present details of our synthesis of (+)-STX **1** and its analogs, focusing on (+)-dcSTX **3** and (+)-GTX3 **7** [11c].

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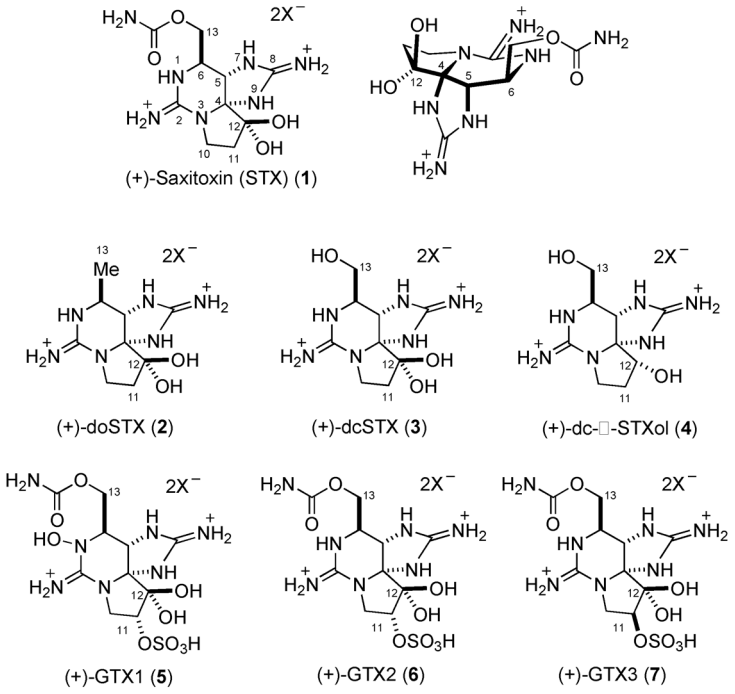
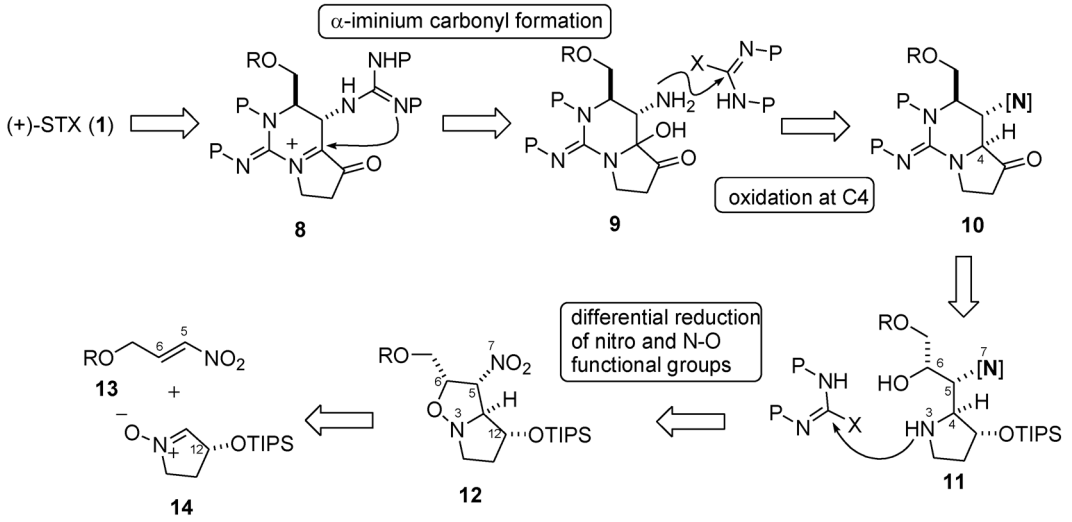


Fig. 1 Structures of STX **1** and representative naturally occurring analogs **2–7**.

SYNTHETIC PLAN FOR SAXITOXINS

Our synthetic plan for STX **1** is illustrated in Scheme 1. The stereochemistries at C5 and C6 in **1** are controlled by the chiral center at C12 in **14** during the intermolecular 1,3-DC reaction of nitroalkene **13** with nitrone **14**. By means of this reaction, the amino group can be directly installed at N3 and N7

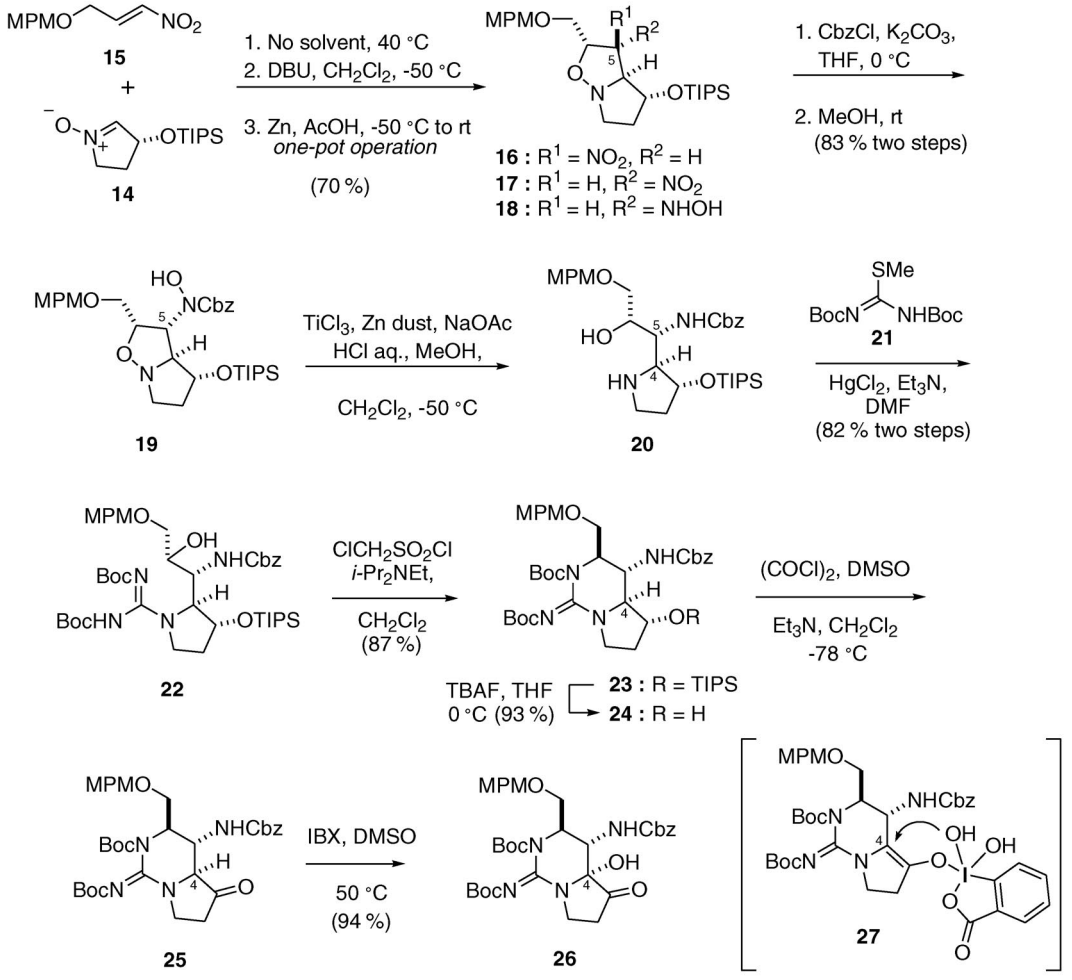


Scheme 1 Synthetic plan for STX **1**. [**N**] = nitrogen functional group, P = protecting group, TIPS = triisopropylsilyl.

with control of the stereochemistry, although further discriminatory transformation of the resulting functional groups, i.e., nitro and N–O groups, to other nitrogen functional groups is usually difficult. Thus, differential reduction of these functional groups is one of the challenging issues in this synthetic plan. Construction of the characteristic bicyclic guanidine structure (**9** to **1**) is also challenging. We planned to cyclize this guanidine ring system at C4 by generating the α-iminium cation **8**, which could be formed by oxidation at C4 (**10** to **9**).

SYNTHESIS OF BICYCLIC GUANIDINE 26 VIA 1,3-DIPOLAR CYCLOADDITION, DISCRIMINATIVE DIAMINE SYNTHESIS AND OXIDATION AT C4

The bicyclic guanidine **26**, which corresponds to **9** in Scheme 1, was synthesized as follows (Scheme 2). 1,3-DC of nitroalkene **15** with optically active nitrone **14** derived from D-malic acid gave *endo* adduct **16** in 95 % yield as a single diastereomer. In this reaction, the reaction was completed within 30 min under solvent-free conditions at 40 °C. Cycloadduct **16** underwent C5 epimerization with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at –50 °C to give thermodynamically stable **17** in a ratio of 95:5. Then, selective reduction of the nitro group in **17** was examined. After many attempts, we fortunately

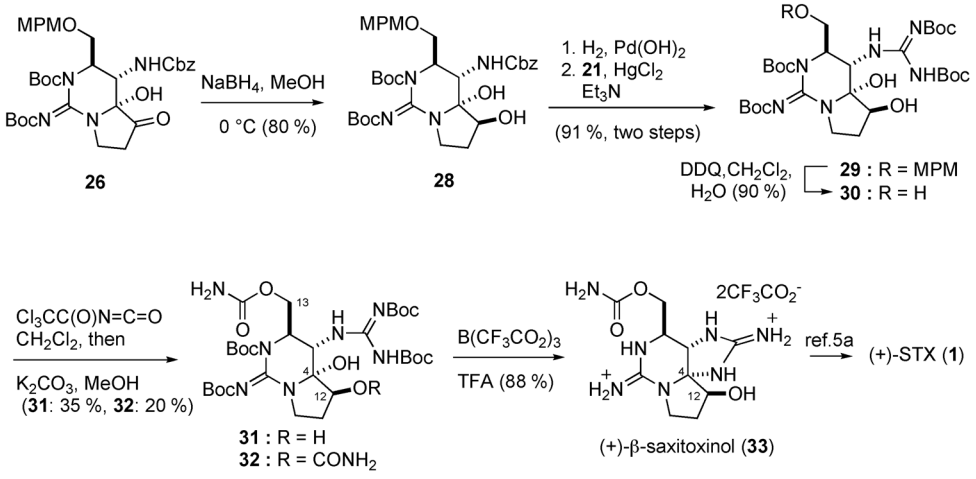


Scheme 2 Synthesis of bicyclic guanidine **26** via differential reduction and oxidation at C4.

found that the use of zinc powder in acetic acid gave the hydroxylamine **18** as the predominant product. These three steps could be performed in one pot by successive addition of reagents without any work-up, and gave **18** stereoselectively in 70 % yield. After protection of the amino group in hydroxylamine **18** with carbobenzoxy (Cbz) group, reduction of the two N–O bonds in the resulting *N*-protected hydroxylamine **19** was investigated. Among various conditions examined, we found that zinc powder in aqueous HCl reduced only the N–O bond of isoxazolidine, whereas Miller's conditions, i.e., $TiCl_3$-NaOAc in MeOH–HCl, reduced the N–O bond of hydroxylamine [12]. The combination of these two conditions was found to give diamine **20** exclusively. Since differential reduction of NO_2 and N–O groups could thus be carried out to generate diamine **20** efficiently, we next focused on oxidation at the C4 position (**10** to **9**). Guanidinylation of **20** with 1,3-bis(*t*-butoxycarbonyl)-2-methyl-2-thiopseudourea **21** gave guanidine **22**, which was subsequently cyclized to give bicyclic guanidine **23** by reaction with monochloromethanesulfonyl chloride in the presence of diisopropylethylamine in 87 % yield. Deprotection of the triisopropylsilyl (TIPS) group with tetra-*n*-butylammonium fluoride (TBAF) followed by oxidation of the resulting alcohol under the Swern conditions with a large excess of the reagents (10 equiv) gave the ketone **25**. With the ketone **25** in hand, oxidation at C4 was investigated, and we found that treatment with 2-iodoxybenzoic acid (IBX) (1.1 equiv) gave the desired aminal **26** regioselectively. This reaction is speculated to proceed from the enol intermediate **27** by intramolecular attack of the hydroxyl group on IBX at C4.

TOTAL SYNTHESIS OF (+)-STX 1

Total synthesis of (+)-STX **1** was achieved from **26** by constructing the five-membered cyclic guanidine (Scheme 3). Reduction of the ketone **26** with $NaBH_4$, followed by deprotection of the Cbz group with hydrogen in the presence of $Pd(OH)_2$ gave the amine, into which the *t*-butoxycarbonyl (Boc)-protected guanidine group was introduced by reaction with **21** and $HgCl_2$ to give **29** in 91 % yield. The *p*-methoxybenzyl (MPM) group was deprotected with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and the resulting alcohol was reacted with trichloroacetyl isocyanate, followed by hydrolysis with potassium carbonate in MeOH to give **31** in 35 % yield. In this reaction, bis-carbamoyl **32** was concomitantly formed in 20 % yield. Finally, deprotection of the four Boc groups and construction of the five-membered cyclic guanidine were achieved with boron tris(trifluoroacetate) in TFA, as developed by Du Bois [8], to give (+)-β-saxitoxinol **33** in 88 % yield. Since the synthesis of (+)-STX **1** from **33** was reported by Shantz with oxidation at C12 [5a], synthesis of **1** was formally accomplished.

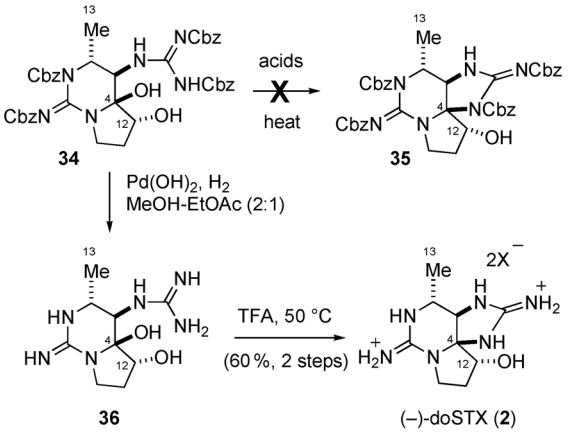


Scheme 3 Synthesis of (+)-STX **1**.

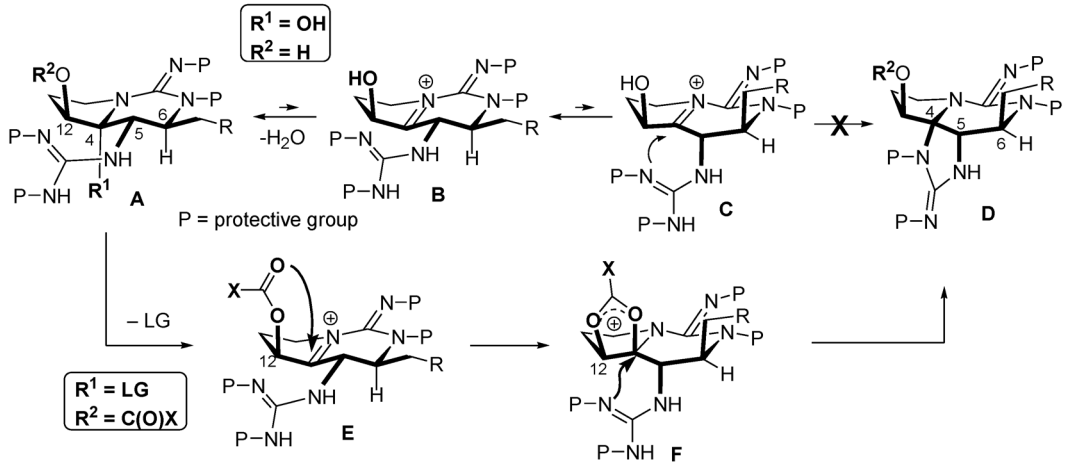
SYNTHESIS OF STX SKELETON IN PROTECTED FORM

As described above, the five-membered cyclic guanidine was constructed at the final stage of the synthesis of STX **1**. During this transformation, all protecting groups were removed, and the resulting saxitoxinol was highly water-soluble. For application of the present strategy to the synthesis of other, more functionalized STX derivatives, however, cyclization should be carried out without removal of the protecting groups in order to avoid solubility and purification problems [13]. Thus, the development of a mild method to promote the key cyclization is essential for synthesis of a range of STX derivatives.

In our early studies on the synthesis of (–)-doSTXol **2**, construction of the STX skeleton in the protected form, i.e., conversion of **34** into **35**, was investigated under a variety of acidic conditions (Scheme 4). Unfortunately, no cyclization reaction took place. On the other hand, cyclization proceeded under strongly acidic conditions after removal of the Cbz guanidine protecting groups (**34** to **35** to **36**). Based on these results, we considered that two key processes should take place for the cyclization reaction to occur efficiently (Scheme 5).



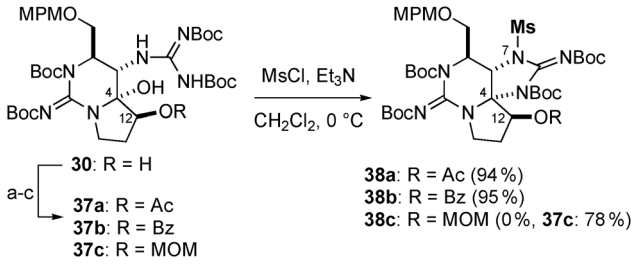
Scheme 4 Early attempts to construct the STX skeleton in protected form.



Scheme 5 Hypothetical scheme of five-membered guanidine cyclization reaction.

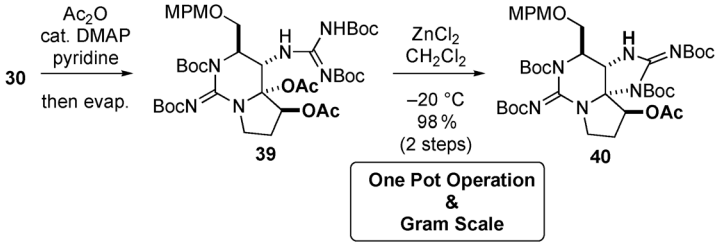
For the cyclization reaction to proceed, firstly, the iminium cation **B** should be generated from compound **A**. Secondly, conformational change must take place from **B** to **C**, where the guanidine side chain is oriented axially, to construct the five-membered guanidine moiety. In the case of **34**, there is an electron-withdrawing group in the six-membered cyclic guanidine, which could suppress iminium ion formation even under acidic conditions. Moreover, conformational change from **B** to **C** might be difficult because of the presence of bulky side chains at C5 and C6 in **B**. With these considerations in mind, we assumed that activation of the leaving group at C4 in **A** (R^1) and stabilization of conformer **C** would facilitate construction of the five-membered guanidine under mild conditions. Thus, we planned to install a leaving group at C4 to accelerate iminium ion generation and an acyloxy group at C12 that would facilitate the conformational change by forming a bridged acyloxonium ion intermediate **F**.

The above working hypothesis was examined by using the bis-guanidine **37a–c** with various R groups at C12, and by employing reaction with methanesulfonyl chloride to install methanesulfonate as a leaving group (Scheme 6). In the cases of **37a** and **37b**, cyclization reaction took place smoothly to generate **38a** (94 %) and **38b** (95 %) as N7 methanesulfonamide derivatives without loss of the Boc protecting group. In contrast, reaction of **37c**, having the methoxymethyl (MOM) protecting group at C12, with methanesulfonyl chloride did not result in cyclization, and the starting **37c** was recovered in 78 % yield.

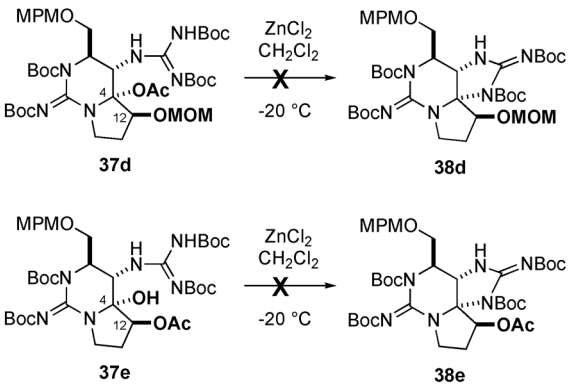


Scheme 6 Construction of five-membered cyclic guanidine by activation at C4 with methanesulfonate. *Reagents and conditions*: (a) Ac_2O, pyridine (95 %); (b) Bz_2O, DMAP, pyridine (92 %); (c) MOMCl, NaI, iPr$_2$NEt, THF (64 %).

Encouraged by these results, we sought more practical conditions to avoid the methanesulfonylation at N7. We eventually found that cyclization reaction of bis-acetate **39**, derived from **30** and acetic anhydride in the presence of *N*,*N*-dimethyl-4-aminopyridine (DMAP), with zinc(II) chloride in dichloromethane at –20 °C proceeded to form **40**, a protected saxitoxinol, in 98 % yield from **3** (Scheme 7). It is noteworthy that this two-step cyclization reaction could be performed in one pot on a gram scale. Since removal of either of the acetates (**37d** and **37e**) blocked the cyclization, the two acetate groups in **39** were indispensable for the cyclization reaction under mild conditions (Scheme 8).



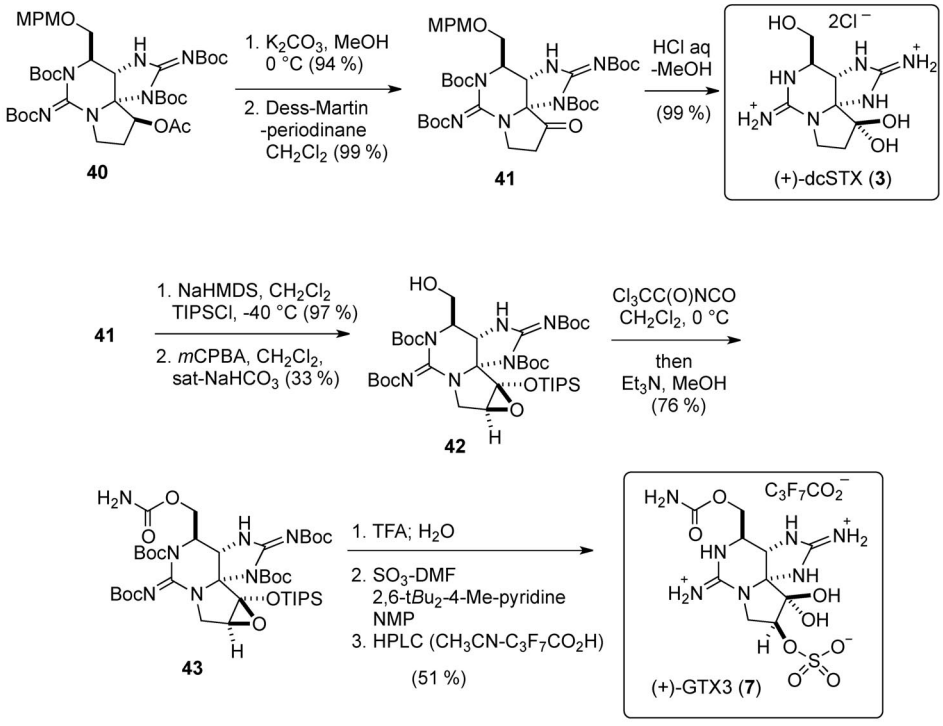
Scheme 7 Construction of five-membered guanidine by activation at C4 with acetate in the presence of zinc(II) chloride.



Scheme 8 Cyclization reaction of mono-acetates **37d** and **37e** in the presence of zinc(II) chloride.

SYNTHESIS OF (+)-dcSTX 3 AND (+)-GTX3 7

The protected saxitoxinol **40**, obtained via our newly devised conformationally controlled guanidine cyclization process, was applied to the synthesis of naturally occurring STX derivatives (+)-dcSTX **3** and (+)-GTX3 **7** (Scheme 9). (+)-dcSTX **3** was synthesized as follows. Removal of the acetate in **40** by methanolysis afforded the alcohol, which was oxidized with Dess–Martin reagent to give ketone **41** in 92 % yield from **40**. The Boc and MPM ether protecting groups in **41** were removed by reaction with HCl to give (+)-dcSTX **3** in quantitative yield. (+)-GTX3 **7** was also synthesized from **41**. Reaction of **41** with TIPS chloride in the presence of sodium hexamethyldisilazide (NaHMDS), followed by oxidation with *m*-chloroperoxybenzoic acid (*m*CPBA), afforded the siloxy epoxide **42**. In this reaction, MPM



Scheme 9 Synthesis of (+)-dcSTX **3** and (+)-GTX3 **7**.

ether was oxidatively cleaved to generate the primary alcohol, and the hydroxyl group was converted to carbamate **43** in 24 % yield from **41**. Then, the Boc and TIPS groups were removed with TFA, and sulfation of the resulting alcohol at C11 was carried out with SO_3-DMF in the presence of 2,6-di-*t*-butyl-4-methylpyridine, as developed by Du Bois [9b], affording (+)-GTX3 **7** in 51 % yield from **43**.

CONCLUSION

In conclusion, we present a total synthesis of (+)-STX **1** based upon 1,3-DC of nitroalkene with cyclic nitrone, and oxidation at C4 with IBX. In this synthesis, discriminative reduction of the nitro group and N–O bond in nitro-isoxazolidine was developed to provide efficient access to the key diamine intermediate for STX. Furthermore, mild construction conditions for the five-membered cyclic guanidine, using our newly developed conformationally controlled cyclization process, enabled us to access STX derivatives, i.e., (+)-dcSTX **3** and (+)-GTX3 **7**, through protected saxitoxinol **40**.

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