Published in final edited form as:

Org Lett. 2019 October 04; 21(19): 7999–8002. doi:10.1021/acs.orglett.9b02986.

A Direct C11 Alkylation Strategy on the Saxitoxin Core: A Synthesis of (+)-11-Saxitoxinethanoic Acid

Srinivas R. Paladugu, Chintelle K. James, Ryan E. Looper*

Department of Chemistry, University of Utah, 315 S 1400 E, Salt Lake City, Utah 84112, United States

Abstract

The bis-guanidinium ion family of natural products are revered for their utility in the study of ion channel physiology. While many congeners have been isolated with various oxidation and sulfation patterns, only two members of this family have been isolated bearing a carbon–carbon bond at C11, namely 11-saxitoxinethanoic acid and zetekitoxin AB. Herein we described a synthetic platform capable of efficiently targeting (+)-saxitoxin and 11-saxitoxinethanoic acid with an embedded C11 carbon–carbon bond. We demonstrate that this strategy enables direct enolate coupling in both an inter- and intramolecular fashion to create the C11–C15 carbon–carbon bond.

Graphical Abstract

Direct inter- and intra-molecular enolate couplings

(+)-Saxitoxin (STX) was the first representative of the bis-guanidinium ion natural product family to be isolated as a causative agent of paralytic shellfish poisoning (Figure 1A).¹ The potent inhibitory effects of STX on the voltage gated sodium channels (Na_vs) has rendered it an important tool to understanding the complex physiology of these channels.² Since the isolation of STX, almost 60 related natural products have been characterized, varying largely in oxidation and sulfation patterns or substitution at the C13 hydroxyl group.^{2c} Remarkably, among all of these analogs, STX remains the most potent. To date only two analogs have been identified that bear substitution at C11 in the form of a carbon–carbon bond; (+)-11-saxitoxinethanoic acid (11-SEA)³ and zetekitoxin AB (ZTX).⁴ Nagasawa⁵ and

*Corresponding Author: r.looper@utah.edu.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02986.

General experimental procedures and spectroscopic data for all new compounds (PDF)

The authors declare no competing financial interest.

Du Bois⁶ independently demonstrated that (+)-11-SEA is approximately 10–20-fold less potent than STX, while initial reports suggest that ZTX is between 50- and 600-fold more potent than STX depending on the ion channel subtype.⁴ Clearly, these represent important structural variants to probe the potency and specificity of these toxins for eukaryotic Na_vs.

Nagasawa and Du Bois have completed impressive syntheses of (+)-11-SEA, both aiming to install the requisite C11–C15 bond on the intact STX core to enable access to multiple congeners.^{5,6} The most obvious disconnection harnesses the inherent reactivity of the ketone/hydrate to construct the C–C bond. However, the laboratory derived enolates of this system are inherently difficult to react, often flanked by multiple nucleophilic nitrogens, and prone to decomposition under basic conditions. To obviate these reactivity problems Nagasawa demonstrated the ability of the silyl enol ether (preformed at low temperatures) to undergo a Mukaiyama aldol with a variety of aromatic aldehydes and glyoxals (Figure 1B).⁵ Du Bois' solution utilized the C11 iodo-enaminone to participate in a Stille reaction with a novel tributyltin-ketene acetal reagent.⁶ While these represent practical solutions to C–C bond formation at C11, our proposed entry to 11-SEA and ZTX necessitates the C15 carbon electrophile to be introduced as an sp³ hybridized carbon.⁷ Herein, we report the execution of this strategy in both an inter- and intramolecular fashion to forge the C11–C15 bond and ultimately 11-SEA (Figure 1C).

We previously reported an efficient synthesis of (+)-STX featuring a Ag(I)-catalyzed cyclization cascade from bis-guanidine **1** to prepare a valuable tricyclic intermediate **2** (Scheme 1).⁸ This intermediate could be prepared on gram scale and processed to STX in just five synthetic operations.

A unique feature of our first approach to (+)-STX was the early installation of the carbamoyl group which obviated the need for protecting groups. However, this proved disadvantageous to access the free C13 hydroxyl group for the preparation of targeted congeners (e.g., ZTX). We also noted that the concerted hydrogenolysis of the N7-benzyl group and C10-benzyl ether in **2** was capricious and often led to the concomitant removal of the N9-Boc group. This complicated the formation of the correct pyrrolidine regioisomer upon activation and displacement of the C10-alcohol.

In order to address these synthetic challenges, we installed a more versatile silicon-derived protecting group to mask the C13-alcohol (Scheme 2). To decouple the activation of the C10 alcohol for pyrrolidine formation and to facilitate N7-deprotection we chose to enter the synthesis directly with the C10 alcohol activated as the tosylate and N7-masked with an acid labile 2,4-dimethoxybenzyl (DMB) group. Coupling of the DMB-nitrone **3** with the metalated alkyne of **4** gave the antidiamine **5** in good yield and 9:1 *d.r.*⁹ Reduction of the N–O bond and removal of the Boc group gave **7**. Installation of the bis-guanidine with *N,N'*-di-Boc-*S*-Me-isothioureia proceeded smoothly to give **8**.¹⁰ Although the primary tosylate had survived a variety of conditions already, we studied the cyclization cascade stepwise to ensure reaction conditions did not deliver unwanted side reactions. Treatment of **8** with AgOAc cleanly delivered the five-membered *ene*-guanidine **9** as a single regioisomer by ¹H NMR spectroscopy.¹¹ Iodine and AgOAc promoted the iodoguanylation to give the iodide **10** in 70% yield as a single diastereomer. Removal of the iodide by heating with AgOAc was

accompanied by selective cyclization of the N3-Boc group to give the oxazolidinone **11** in 60% yield. We were delighted to see that the tosylate survived all of these transformations and were indeed able to demonstrate the one-pot variant of this sequence whereby **8** was transformed directly into **11** in 40% yield which is directly comparable to the efficiency of individual transformations. With the C10-alcohol activated from the beginning of the synthesis, we were able to execute the pyrrolidine formation directly by treatment with Cs₂CO₃ in ethanol, giving **12** in 82% yield. Oxidation with Dess-Martin periodinane oxidation delivered the C12 ketone **13**.¹² Having arrived at the key tricyclic intermediate **13** we targeted decarbamoyl saxitoxin (+)-dc-STX to confirm that the N7-DMB group could be removed under acidic conditions (as compared to the capricious hydrogenolysis of the corresponding benzyl derivative). While deprotection of the Boc protecting groups can be accomplished at room temperature with trifluoroacetic acid, the DMB requires elevated temperature to affect complete cleavage. Nevertheless, refluxing this intermediate in TFA delivered analytically pure (+)-dc-STX in 60% yield.

We also aimed to complete the synthesis of (+)-STX to confirm this synthetic sequence can effectively tolerate the required functional group transformations (Scheme 3). Acetylation of the tricyclic alcohol **12** afforded **14** in good yield. Deprotection of the C13 alcohol was accomplished in TBAF buffered with acetic acid which prevented partial removal of the N9-Boc group. The primary carbamate was then installed with trichloroacetyl isocyanate followed by K₂CO₃ in methanol to cleave both the trichloroacetyl and acetyl groups, providing **16**.¹³ Oxidation of C12 alcohol gave the ketone **17** in good yield. Again, refluxing **17** in TFA was required to complete the global deprotection, successfully delivering (+)-STX.

We were now confident that all functional groups could be successfully manipulated to access these targets. To begin our study of the C11 alkylation we utilized the tricyclic alcohol **12** (Scheme 4). Anticipating the free guanidine N-H may interfere with attempts to alkylate C11, it was masked by formation of the corresponding oxazolidinone **18**.¹⁴ After significant screening efforts we found that the phosphazene base, *tert*-butylimino-tri(pyrrolidino)phosphorane (BTPP), was an excellent base for generating the C11 enolate.¹⁵ Addition of *tert*-butyl iodoacetate generated the C-alkylated product **19** in 54% yield. However, all attempts to open the oxazolidinone to the primary carbamate were unsuccessful. Attempts to open the oxazolidinone with more basic nucleophiles caused significant decomposition.

An alternative strategy to prevent potential interference or alkylation of the guanidine would be to present a single equivalent of the electrophile via an intramolecular delivery (Figure 2). To that end, we prepared the iodoester **20** after desilylation of **13** and coupling of the alcohol to iodoacetic acid with EDC. Again, BTPP served as an efficient base to generate the enolate and to our delight alkylation proceeded to produce the macrolactone **21** in 48% yield. The success of this challenging C11–C15 bond formation was supported by HMBC correlation between H11 → C16 and H15a/b → C12. It was subsequently confirmed by hydrolysis and deprotection of **22** with TFA at 70 °C, which gave decarbamoyl-11-saxitoxinethanoic acid (dc-11-SEA).¹⁶ To advance the lactone, it was saponified with LiOH, which also resulted in the removal of the N9-Boc group. Treatment of **22** with trichloroacetylisocyanate led to a

complex mixture of products, and we conceded that we could not install the carbamate on this substrate.

An important observation from this sequence was that the guanidine, even when presented with a potential six-membered ring, cyclization under basic conditions does not interfere with the alkylation. This prompted us to evaluate the intramolecular variant on the TBDPS protected ketone **13** (Scheme 5). Attempted alkylation of this substrate with *tert*-butyl iodoacetate and BTPP provided <20% yield of the alkylated product and led to extensive decomposition of the starting material. Attempts to optimize this alkylation with a multitude of strong bases (LDA, LiHMDS, NaHMDS, KHMDS), solvents, and activated electrophiles (haloacetates, allylic and propargylic halides) failed to yield any of the desired alkylation products consistent with previous observations on the reactivity of related ketones.⁵

We noted Smith's successful utilization of zinc enolates in challenging alkylations of ketones with haloacetates implemented in the synthesis of (–)-oleocanthal.¹⁷ Careful preparation of the zinc enolate with LiHMDS and Et₂Zn followed by addition of the enolate to *tert*-butyl bromoacetate consistently gave a 35–45% yield (60% yield brsm) of an inseparable mixture of **23** and **24**, with partial loss of the N9-Boc group. Unlike the BTPP reaction, this approach did not lead to decomposition permitting recovery of ~30–35% of the starting material. With the *tert*-butyl ester in place, desilylation and carbamoylation proceeded cleanly on the mixture of **25** and its free N9 complement **26**. Final deprotection with refluxing trifluoroacetic acid gave (+)-11-SEA in 24% yield over the three-step sequence.

In conclusion, we have developed an improved entry to the saxitoxin core based on our silver mediated cyclization cascade. Entering this sequence with the C10 alcohol activated as its tosylate and processing through the cyclization cascade greatly facilitate formation of the final pyrrolidine. Replacement of the N7-benzylgroup with a DMB enables its removal in the final acid mediated global deprotection avoiding capricious high pressure hydrogenolysis conditions. These reactions were optimized in the successful synthesis of (+)-dc-STX and (+)-STX. We have demonstrated that important STX analogs bearing a carbon–carbon bond at C11 are also accessible from this sequence. This strategy delivers (+)-11-SEA in just 14 steps from L-serine-OMe and 2% overall yield. This compares favorably to previous syntheses by Nagasawa⁵ (25 steps, 4% yield) and DuBois⁶ (19 steps and 0.2% yield). Herein, the first direct coupling of the STX core with an sp³-hybridized electrophile has been demonstrated. This reactivity has been harnessed in an intramolecular sense to produce the macro-cyclic carbon–carbon bond which holds significant promise to enable the synthesis of ZTX.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the National Institutes of Health (2R01GM090082-A1). S.R.P. is grateful for fellowship support from the Eastman Chemical Company. C.K.J. is grateful for financial support from the Office of the Navajo Nation and the American Indian Graduate Center.

REFERENCES

- (1). (a)Schantz EJ; Ghazarossian VE; Schnoes HK; Strong FM; Springer JP; Pezzanite JO; Clardy J J. Am. Chem. Soc 1975, 97, 1238–1239. [PubMed: 1133383] (b)Bordner J; Thiessen WE; Bates H; Rapoport AH J. Am. Chem. Soc 1975, 97, 6008–6012. [PubMed: 1176726]
- (2). For recent reviews, see:(a)Llewellyn LE Nat. Prod. Rep 2006, 23, 200–222. [PubMed: 16572228] (b)Wiese M; D'Agostino PM; Mihali TK; Moffitt MC; Neilan BA Mar. Drugs 2010, 8, 2185–2211. [PubMed: 20714432] (c)Cusick KD; Saylor GS Mar. Drugs 2013, 11, 991–1018. [PubMed: 23535394] (d)Thottumkara AP; Parsons WH; Du Bois J Angew. Chem., Int. Ed 2014, 53, 5760–5784.
- (3). Arakawa O; Nishio S; Noguchi T; Shida Y; Onoue Y Toxicon 1995, 33, 1577–1584. [PubMed: 8866615]
- (4). Yotsu-Yamashita M; Kim YH; Dudley SC; Choudhary G; Pfahnl A; Oshima Y; Daly JW Proc. Natl. Acad. Sci. U. S. A 2004, 101, 4346–4351. [PubMed: 15070720]
- (5). Wang C; Oki M; Nishikawa T; Harada D; Yotsu-Yamashita M; Nagasawa K Angew. Chem., Int. Ed 2016, 55, 11600–11603.
- (6). Walker JR; Merit JE; Thomas-Tran R; Tang DTY; Du Bois J Angew. Chem., Int. Ed 2019, 58, 1689–1693.
- (7). Paladugu SR; Looper RE Tetrahedron Lett. 2015, 56, 6332–6334. [PubMed: 26594065]
- (8). Bhonde VR; Looper RE J. Am. Chem. Soc 2011, 133, 20172–20174. [PubMed: 22098556]
- (9). (a)Merino P; Lanaspa A; Merchan FL; Tejero T Tetrahedron: Asymmetry 1998, 9, 629–646. (b)Merino P; Franco S; Merchan FL; Tejero T J. Org. Chem 1998, 63, 5627–5630. (c)Fleming JJ; McReynolds MD; Du Bois J J. Am. Chem. Soc 2007, 129, 9964–9975. [PubMed: 17658800]
- (10). Bhonde VR; Looper RE eROS 2013, DOI: 10.1002/047084289X.rm01529.
- (11). Gainer MJ; Bennett NR; Takahashi Y; Looper RE Angew. Chem., Int. Ed 2011, 50, 684–687.
- (12). Dess DB; Martin JC J. Org. Chem 1983, 48, 4155–4156.
- (13). Kocovsky P Tetrahedron Lett. 1986, 27, 5521–5524.
- (14). Andresen B; Du Bois J J. Am. Chem. Soc 2009, 131, 12524–12525. [PubMed: 19678702]
- (15). (a)Schwesinger R; Willaredt J; Schlemper H; Keller M; Schmitt D; Fritz H Chem. Ber 1994, 127, 2435–2454. (b)Schwesinger R eROS 2007, DOI: 10.1002/9780470842898.rm00671.
- (16). ^1H and ^{13}C NMR spectra were in agreement with that reported by Nagasawa (ref 5) after H/D exchange for (+)-dc-11-SEA. However, the ratio of α - and β -isomers of the hydrate and the ketone was different, 9:1:2.5 respectively. In our hands, the equilibration between these three isomers was extremely slow requiring 1 week to reach equilibrium, likely a reflection of pH.
- (17). (a)Smith AB; Han Q; Breslin PAS; Beauchamp GK Org. Lett 2005, 7, 5075–5078. [PubMed: 16235961] (b)Smith AB; Sperry JB; Han Q J. Org. Chem 2007, 72, 6891–6900. [PubMed: 17685574]

Figure 1.
Saxitoxin and naturally occurring C11-alkylated variants.

Figure 2.

(A) Synthesis of key macrolactone **21**. (B) Partial HMBC data for **21**; key correlations between H11 $\rightarrow$ C16 and H15a/b $\rightarrow$ C12.

Scheme 1.
Silver-Catalyzed Cyclization Cascade to the Saxitoxin Core

Scheme 3.
Completion of the Synthesis of (+)-STX

Scheme 4.
First Attempted Intermolecular Alkylation

Scheme 5.
 Completion of the Synthesis of (+)-11-SEA