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Synthesis of the Paralytic Shellfish Poisons (+)-Gonyautoxin 2, (+)-Gonyautoxin 3, and (+)-11,11-Dihydroxysaxitoxin

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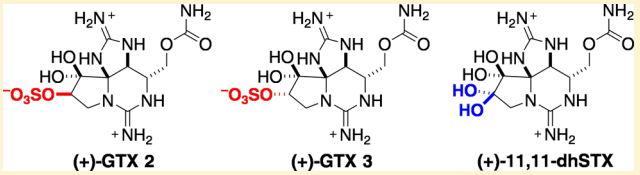
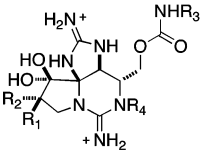
Supporting Information

ABSTRACT: The paralytic shellfish poisons are a collection of guanidine-containing natural products that are biosynthesized by prokaryote and eukaryote marine organisms. These compounds bind and inhibit isoforms of the mammalian voltage-gated Na^+ ion channel at concentrations ranging from 10^{-11} to 10^{-5} M. Here, we describe the de novo synthesis of three paralytic shellfish poisons, gonyautoxin 2, gonyautoxin 3, and 11,11-dihydroxysaxitoxin. Key steps include a diastereoselective Pictet−Spengler reaction and an intramolecular amination of an *N*-guanidyl pyrrole by a sulfonyl guanidine. The IC_{50}'s of GTX 2, GTX 3, and 11,11-dhSTX have been measured against rat $Na_V1.4$, and are found to be 22 nM, 15 nM, and 2.2 μM, respectively.



INTRODUCTION

Voltage-gated Na^+ ion channels (Na_Vs) are responsible for the rising phase of action potentials, and are essential molecular components for electrical transmission in neuronal cells.[1] The paralytic shellfish poisons (PSPs) are a family of small molecule neurotoxins that occlude the outer pore of these channels and inhibit Na^+ ion flux.[2] Detailed accounts of human intoxication by these agents can be found from the 18th century.[3] Researchers at the University of California correlated the occurrence of certain toxic mussels with blooms of *Alexandrium catenella*, a species of dinoflagellate algae, following an outbreak in San Francisco over the summer of 1927.[4] Since this pioneering report, a collection of >50 structurally related small molecule toxins has been isolated from a variety of dinoflagellate and cyanobacterial sources (Figure 1).[2b,5]



	R_1	R_2	R_3	R_4
STX	H	H	H	H
GTX 1	OSO_3^-	H	H	OH
GTX 2	OSO_3^-	H	H	H
GTX 3	H	OSO_3^-	H	H
GTX 4	H	OSO_3^-	H	OH
GTX 5	H	H	SO_3^-	H
GTX 6	H	H	SO_3^-	OH
11,11-dhSTX	OH	OH	H	H

Figure 1. Select naturally occurring paralytic shellfish poisons.

Structural elucidation, beginning with the elegant synthetic work disclosed by Rapoport in 1962 and culminating in the X-ray crystal structures published independently by Schantz and Clardy, and Rapoport in 1975, first revealed the molecular architecture of one poison, (+)-saxitoxin (STX).[6,7] A contemporaneous discovery by Shimizu identified two lethal compounds from the hepatopancreases of contaminated clams, which were initially assigned as epimeric C11-hydroxylated forms of STX.[8,9] These diastereomeric compounds were named gonyautoxin 2 (GTX 2) and gonyautoxin 3 (GTX 3) after the dinoflagellate from which they were isolated. The structures of GTX 2 and GTX 3 were later revised by Schantz to the corresponding 11α- and 11β-sulfate esters.[10]

Over the ensuing 30 years, additional PSPs have been identified, all having in common a tricyclic, bis-guanidinium core.[2b,5c] Electrophysiological and biochemical studies have revealed that STX and GTX function by binding in the extracellular pore of voltage-gated Na^+ ion channels, making contacts with the reentrant loops that form the Na^+ ion selectivity filter, and sterically blocking the ion permeation pathway.[11,12]

The availability of STX, along with the functionally related Na_V pore-blocker tetrodotoxin (TTX), enabled the earliest efforts to separate Na^+ and K^+ currents in intact nerve fibers.[2a,13] Our interest in these natural products follows from their utility as chemical probes of Na_Vs and the potential of de novo synthesis to deliver unique analogue structures to further explore channel structure and cellular function.[14] Despite the elegant work of Kishi, Jacobi, Nagasawa, Nishikawa, Looper, and others, access to the PSPs through asymmetric construction has been exclusive to STX with one exception.[15−20] Because of the difficulty of isolation and highly polar character of these molecules, chemical modification of the natural products has proven particularly challenging.[2b,21] By leveraging technologies developed in our lab for guanidine assembly, we have devised an efficient route to a versatile intermediate **1**, which encompasses the tricyclic perhydropyrrolopurine core of this family of neurotoxins (Figure 2).[22] Elaboration of this structure has enabled preparation of three naturally occurring PSPs, gonyautoxin 2 (GTX 2), gonyautoxin 3 (GTX 3), and 11,11-dihydroxysaxitoxin (11,11-dhSTX), the

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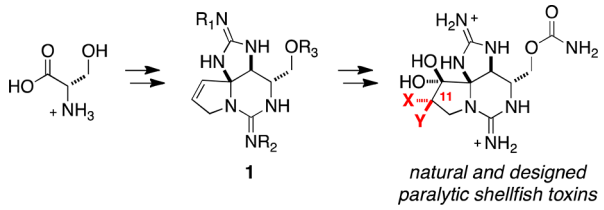


Figure 2. Divergent pathway to C11-derived guanidinium toxins from intermediate **1**.

syntheses of which are described below.[23,24] Information gained from these studies positions us to capitalize on **1** en route to more complex PSP congeners as well as a varied collection of modified toxin derivatives.

RETROSYNTHETIC ANALYSIS

Our desire to formulate a general synthetic approach to PSPs led to the identification of **1**, which could serve as a common precursor to both natural and unnatural targets. Having previously delineated a method to generate 2-iminoimidazolidines through oxidation of aliphatic C−H bonds (Figure 3),

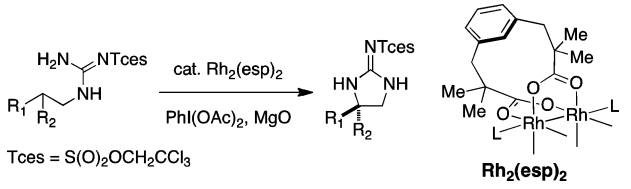


Figure 3. Intramolecular amination with 2,2,2-trichloroethoxy-sulfony-(tces)-protected guanidine substrates.

our analysis focused on the retrosynthetic disconnection of the five-membered cyclic guanidine.[22] In principle, compound **1** could be derived from 2,2,2-trichloroethoxysulfonyl (Tces)-guanidine **2** by stereospecific amination of the C4−H bond (Figure 4). Such a transformation, however, could also give an

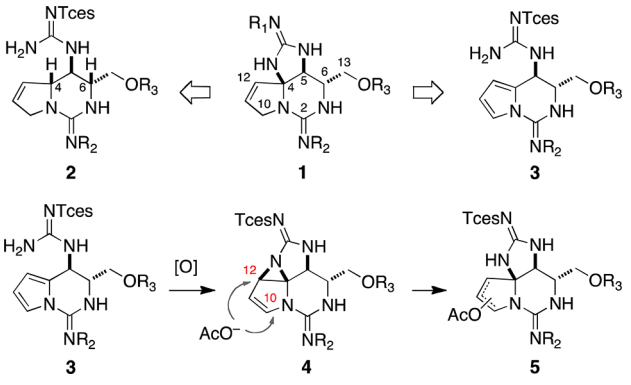


Figure 4. Strategies to prepare the tricyclic core of the PSPs.

isomeric product as a result of competing oxidation of the C6−H center. Our concerns regarding the control of chemoselectivity in this critical reaction prompted us to consider alternative ideas, from which a pyrrole oxidation strategy materialized.

Selective amination of **3** offers an attractive method for assembling the tricyclic core of the PSPs with concomitant incorporation of an oxygen group on the resulting pyrroline ring. In principle, Rh-catalyzed oxidation could deliver an aziridine intermediate **4**, which would open in one of two ways to afford either the C10- or the C12-substituted product. It is equally plausible that pyrrole oxidation occurs through a dipole intermediate in lieu of aziridine **4**.[25] Mechanistic considerations notwithstanding, for our purposes either product is suitably configured for subsequent elaboration of the π-bond to furnish C11-derived STX targets (e.g., GTX 2/3, 11,11-dhSTX).

The ability to access pyrrole **3** efficiently from commodity chemicals was an important factor in our strategic planning. By capitalizing on the nucleophilic reactivity of the pyrrole group, stereoselective formation of the bicyclic ring structure **6** could be achieved through a Pictet−Spengler-type reaction (Figure 5).[26] Analysis of transition state models for the cyclization

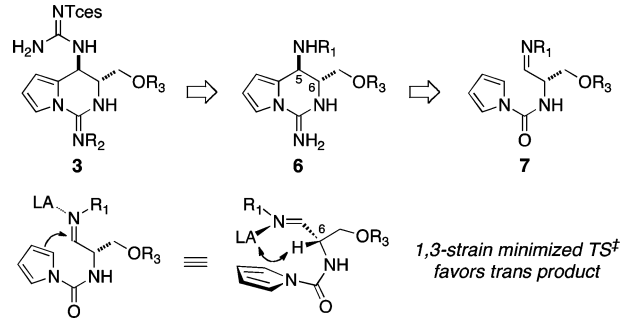


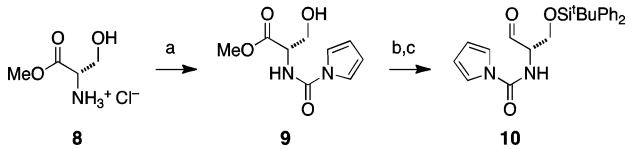
Figure 5. A proposed Pictet−Spengler reaction to access the desired precursor for Rh-catalyzed aziridination.

event in which allylic strain is minimized across the C6−C5−N7 bonds suggested that the stereochemistry at C5 would be established in the requisite configuration. Accordingly, the *N*-acyl pyrrole needed to test these ideas could be readily assembled from commercial serine methyl ester.

DIASTEREOSELECTIVE PICTET−SPENGLER CYCLIZATION

Synthesis of the guanidinium toxins commences from serine ester **8**, which is converted selectively to urea **9** using the acid chloride derived from lithium pyrrolate (Scheme 1).[27] Attempts

Scheme 1[a]



[a]Reagents and conditions: (a) pyrrole-1-carboxylic acid, oxalyl chloride, cat. DMF, aq $NaHCO_3$, THF, 90%; (b) *t*-$BuPh_2SiCl$, imidazole, DMF, 93%; (c) *i*-Bu_2AlH, CH_2Cl_2, −90 °C.

to prepare an analogous guanidine derivative were unsuccessful, thus prompting a decision to advance the urea compound. Fortunately, the conversion of urea groups to corresponding guanidines or guanidinium salts finds ample precedent.[15,28,29] Alcohol protection and low temperature ester reduction with *i*-Bu_2AlH furnishes aldehyde **10**; this intermediate is used immediately in the subsequent Pictet−Spengler cyclization.

Initial experiments to effect imine formation and ring closure from aldehyde **10** employed allylamine in combination with Lewis acids. Monitoring of the reaction progress by ^1H NMR (CD_2Cl_2) revealed that allylamine addition to **10** occurs rapidly

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and forms a diastereomeric mixture of hemiaminals. Addition of 3.5 equiv of $BF_3{\cdot}OEt_2$ to this solution promotes iminium ion formation and cyclization to cyclic urea **11**. Other strong Lewis acids, such as $Sc(OTf)_3$, also proved effective in the transformation. A single product is obtained from this reaction mixture, which was confirmed by X-ray crystallography to be the *trans*-isomer (Figure 6). Assuming the *E*-imine is formed

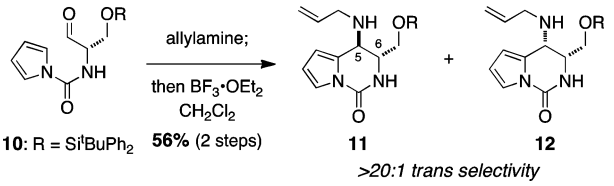
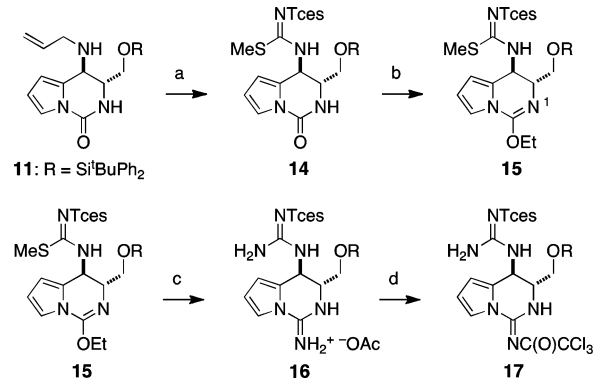


Figure 6. A diastereoselective Pictet–Spengler cyclization.

preferentially, minimization of allylic strain in a transition structure such as that depicted in Figure 5 rationalizes favored addition to the *re*-face of the activated imine. Related diastereoselective Pictet–Spengler reactions performed on amino acid-derived starting materials have found general use in the synthesis of isoquinoline and indole alkaloids.[26,30] This work served as the inspiration for our plan.

Cyclic urea **11** possesses two of the three rings and two of the three stereogenic centers that form the conserved core of the PSPs. To install the Tces-guanidine in preparation for our planned aziridination reaction, **11** is deallylated using $Pd(PPh_3)_4$ and *N*,*N*-dimethylbarbituric acid (Scheme 2).[31]

Scheme 2[a]



[a]Reagents and conditions: (a) 2 mol % $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, CH_2Cl_2, then TcesN=C(SMe)Cl **13**, aq Na_2CO_3, 96%; (b) EtOTf, 2,4,6-tri-*t*-butylpyrimidine, CH_2Cl_2, 79%; (c) NH_3, NH_4OAc, MeOH, 70 °C, 85%; (d) $Cl_3CC(O)Cl$, *i*-Pr_2NEt, CH_2Cl_2, 89%.

Following this operation, the unpurified primary amine is then treated with thiomethylchloroimidate **13** and aqueous Na_2CO_3 to give **14**. This protocol represents an extension of our previous work on Tces-guanidine synthesis, enabling convenient, one-pot amine deprotection and isothiourea formation.[23]

Conversion of the six-membered cyclic urea in **14** to the corresponding guanidine proved surprisingly difficult given the available precedent for analogous transformations.[28] In their synthesis of STX, Kishi et al. employed triethyloxonium tetrafluoroborate to convert a five-membered urea to an *O*-ethyl isourea.[15] This intermediate was subsequently treated with NH_4OAc at elevated temperature to yield the desired guanidinium adduct. Following a similar sequence with urea **14**, however, did not furnish an isolable *O*-alkyl product. Increasing reaction temperature, concentration, or time did nothing to improve the outcome. We surmise on the basis of these findings that the pyrrole group must strongly reduce the nucleophilicity of the urea oxygen toward alkylation.

An alternative procedure for *O*-functionalization of cyclic ureas appears in work by Overman.[28] In this example, $MeOSO_2CF_3$ is used as an alkylating agent in combination with a sterically hindered base, 2,6-di-*t*-butyl-4-methylpyridine. Only one equivalent of each reagent at a reaction concentration of 0.1 M is required to generate the *O*-alkyl product in excellent yield. Applying these conditions to urea **14**, we identified trace amounts of the desired *O*-methyl product when the reaction was performed at room temperature. Elevating the reaction temperature to 65 °C afforded a modest 33% yield of the *O*-methyl product **15**. The yield of **15** in this case was diminished by the competing formation of the *N1*-methylated compound (~1:1 isomeric mixture). Reasoning that a more hindered electrophile might improve reaction selectivity, $EtOSO_2CF_3$ was employed in place of the methyl derivative. This seemingly small change gave an improved ~3:1 ratio of the *O*- and *N1*-ethyl products. Additional reaction optimization, which included increasing the reaction concentration (1.0 M), lowering the temperature to 37 °C, and altering the base (2,4,6-tri-*t*-butylpyrimidine), further boosted product selectivity such that 79% of the *O*-ethylated isomer could be isolated as pure product (~9:1 ratio *O*-ethyl/*N1*-ethyl). Following this challenge, we were pleased to find that ethyl isourea **15** reacted smoothly with methanolic ammonia at 70 °C (sealed tube) to deliver the requisite bis-guanidine **16**.

INTRAMOLECULAR PYRROLE AMINATION

In our initial approach to GTX 2 and 3, the polar guanidinium salt **16** was *N*-protected with trichloroacetyl chloride (TCACl). This reaction proceeded efficiently and furnished **17** as a single product (Scheme 2). Attempts to functionalize **16** with other protecting groups (e.g., acetate, *tert*-butyloxycarbonyl) led to mixtures of products, presumably due to competing acylation of the N1 and N16 positions (vide infra). With the TCA-modified material **17**, cyclization to form the five-membered ring proceeded under the action of catalytic $Rh_2(esp)_2$, $PhI(OAc)_2$, and MgO (Figure 7). The putative aziridine **18** was not observed in this reaction; instead the resulting tricycle **20** was obtained as the acetoxylated C10-*N*,*O*-acetal, the result of

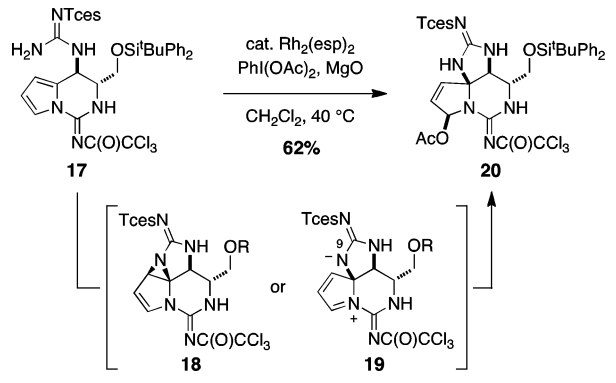


Figure 7. Intramolecular pyrrole–guanidine oxidative cyclization furnishes the tricyclic core of the PSPs.

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AcOH incorporation. This product is stable to chromatographic isolation and is generated as a single regio- and diastereoisomer (62%).

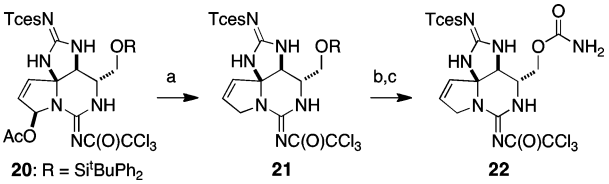
One plausible mechanism for amination of pyrrole **17** posits the intermediacy of aziridine **18**, which is displaced by AcOH in a S_N2' addition to give **20** (Figure 7). We have confirmed, however, through ROESY correlations that the stereochemistry at C10 is *S*-configured, a result that appears to discount a concerted ring-opening reaction. If the ring-strained aziridine does form, it is likely that rapid opening would ensue to give a zwitterionic species. It is also possible that this dipolar intermediate **19** is generated directly in the amination reaction.[25] In either case, assuming that the stereochemical configuration of the *N*,*O*-acetal is kinetically controlled, directed addition of AcOH by the N9 anion would account for the observed outcome. Alternatively, *si*-face addition of AcOH at C10 may be the result of a stereoelectronic preference for attack of the iminium ion antiperiplanar to the developing lone pair on N3 (Stevens'-type model).[32] While the mechanistic underpinnings of this reaction remain opaque, the formation of **20** through Rh-catalyzed oxidation represents a crowning success for the application of this technology in complex synthesis.

Attempts to transpose the acetate group in **20** from C10 to C12 proved unsuccessful, a result that is perhaps unsurprising in light of the thermodynamic stability differences between isomeric dihydropyrroles. As such, a decision was made to simply reduce *N*,*O*-acetal **20**. This transformation is best accomplished using $BF_3{\cdot}OEt_2$ and triethylsilane. The tricyclic core structure **21** obtained in this way is suitably disposed with the alkene group at C11−C12 for elaboration to different PSP targets.

■ ELABORATION TO GTX 2 AND GTX 3

To complete the syntheses of GTX 2 and 3 from alkene **21**, we elected to first replace the silyl ether group at C13 with the requisite carbamate (Scheme 3). These two steps were

Scheme 3[a]



[a]Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, Et_3SiH, 83%; (b) *n*-Bu_4NF, THF; (c) $Cl_3CC(O)N{=}C{=}O$, CH_2Cl_2, then MeOH, 76% (two steps).

smoothly effected without intermediate purification of the C13 alcohol. Conversion of carbamate **22** to any of the C11-sulfated PSPs requires formal ketohydroxylation of the C11−C12 olefin. Plietker and co-workers have described a Ru-catalyzed method for alkene ketohydroxylation that employs $RuCl_3$ as the catalyst in combination with a stoichiometric persulfate oxidant.[33] In prior work from our lab to synthesize STX, olefin ketohydroxylaton was achieved by employing catalytic $OsCl_3$ as a substitute for the ruthenium salt.[20] Application of either ruthenium or osmium ketohydroxylation conditions for oxidation of **22**, however, gave only diol **24** (Figure 8), the same product obtained using more traditional alkene dihydroxylation methods (e.g., cat. OsO_4, NMO).

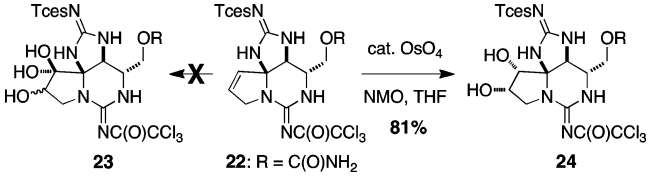


Figure 8. Alkene dihydroxylation prevails over attempts to effect single-step hydroxyketone formation.

The inability to promote single-step ketohydroxylation of **22** compelled us to identify an alternative, stepwise approach for preparing **23**. Dihydroxylation with 2 mol % OsO_4 and *N*-methylmorpholine-*N*-oxide (NMO) proceeded efficiently to afford **24** as a single diastereomer, the result of addition from the alkene face opposite the 5-membered guanidine (Figure 8). The product stereochemistry was assigned on the basis of analysis of ROESY correlations between N9−H and C12−H in a related compound, and by comparison of the ^1H NMR shifts and coupling constants with corresponding spectral data for 11α- and 11β-hydroxysaxitoxin.[34,35] Attempts were made to perform a two-electron oxidation of this diol, reasoning that even if the incorrect hydroxy ketone isomer was formed initially, tautomerization would transform this intermediate to the desired product **23**. All conditions examined for this oxidation (e.g., Dess−Martin periodinane, TEMPO/PhI-$(OAc)_2$, DMSO/$(COCl)_2$), however, gave either unreacted starting material or confounding product mixtures (Figure 9). Ultimately, we concluded that masking the C11 hydroxyl group would be necessary to achieve the desired oxidation reaction.

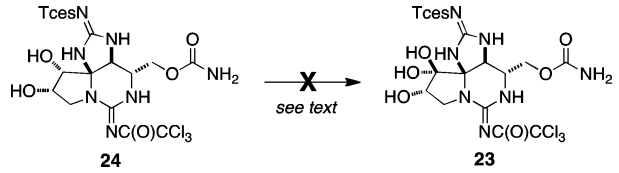


Figure 9. Initial efforts to oxidize diol **24** prove unsuccessful.

Our initial plan for blocking the C11-alcohol in diol **24** aimed to utilize a sulfate-derived protecting group. In a later transformation, selective cleavage of a sulfate diester would leave the C11-sulfate anion found in GTX 2 and 3.[36] Attempts to generate **25** were made using 2,2,2-trichloroethoxysulfonyl chloride and the corresponding imidazolium salt (Table 1).[37] Neither electrophile was sufficiently reactive to engage diol **24**, and only starting material was recovered from these experiments. Searching for an alternative solution for selectively blocking the C11−OH group, a collection of silylating and acylating conditions was screened against **24** (and a related C13 silyl ether). From these experiments, the combination of benzoyl cyanide and 4-dimethylaminopyridine was identified. By employing this protocol, C11-benzoate **26** was obtained as a single product in 69% yield with the remainder of the material identified as unreacted diol and bis-acylated side products (entry 8).

Subsequent exposure of **26** to Dess−Martin periodinane (DMP) efficiently transformed the C12 alcohol to the desired ketone (Scheme 4). Product analysis by ^{13}C NMR revealed that ketone **27** exists in a fully hydrated form. Similarly, when **27** is

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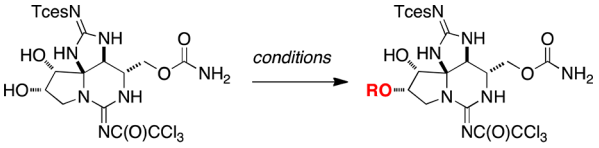
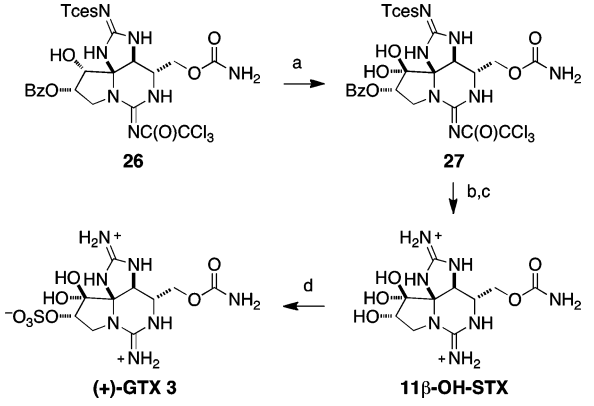
Table 1. Reaction Conditions Tested for Selective Protection of the C11−OH



entry	electrophile	base	temp (°C)	% yield
1	TcesCl	NEt_3, DMAP	23	
2[a]	$TcesNR_2^+OTf^-$	NMI[b]	23	
3	Et_3SiCl	2,6-lutidine	23	30−50
4[c]	Ac_2O	pyridine	23	
5[c]	Ac_2O	TTBP[d]	40	trace
6[c]	$(n\text{-PrCO})_2O$	TTBP[d]	66	40
7[c]	$(PhCO)_2O$	TTBP,[d] DMAP	0	40
8	**PhC(O)CN**	**DMAP**	**−78**	**69**

[a]NR_2^+ = *N*-methylimidazolium. [b]NMI = *N*-methylimidazole. [c]Experiments performed on an analogous C13-OSi^{t}BuPh$_2$ derivative in place of **24**. [d]TTBP = 2,4,6-tri-*t*-butylpyrimidine.

Scheme 4[a]



[a]Reagents and conditions: (a) Dess−Martin periodinane, CH_2Cl_2, 78%; (b) H_2, cat. Pd/C, MeOH, CF_3CO_2H; (c) NH_3, MeOH, 83% (two steps); (d) DMF·SO_3, 2,6-di-*t*-butyl-4-methylpyridine, 71%.

dissolved in d_4-methanol, two diastereomeric hemiketal adducts are generated. In d_6-acetone, the ^{13}C NMR shift of the C12 carbon appears at 98.4 ppm, indicating that even in organic solvents, ketone hydration occurs due to the presence of adventitious water.

Having addressed the problem of C12 oxidation, protecting group cleavage and alcohol sulfation remained to complete the preparation of GTX 3 from **27**. Given the nature of the blocking groups in **27**, we wished to identify a one-pot method for transforming **27** to C11β-hydroxysaxitoxin (11β-OH-STX). Initial examination of Zn/AcOH for removing the Tces group, however, was unsuccessful and gave complex product mixtures. Clean deprotection of the Tces guanidine can be induced under hydrogenolytic conditions (H_2, cat. Pd/C) in acidic methanol, conditions that also excise the trichloroacetyl group.[36] Filtration of the reaction mixture followed by treatment with methanolic ammonia promotes cleavage of the C11-benzoate ester. This latter reaction occurs quickly and must be carefully monitored; acidification of the reaction mixture is necessary to minimize base-promoted decomposition of the product.[38] Isolation of the bis-guanidinium salt by reversed-phase HPLC affords pure 11β-OH-STX.[39] Electrophysiological recordings have been performed with this compound on Chinese Hamster Ovary Cells expressing recombinant rat $Na_V1.4$ and confirm that 11β-OH-STX blocks sodium ion flux (IC_{50} = 9.3 ± 0.7 nM against $rNa_V1.4$) with 3-fold reduced potency as compared to STX (IC_{50} = 2.9 ± 0.1 nM).[11b]

The synthesis of GTX 3 is completed upon treatment of purified 11β-OH-STX with SO_3·DMF and 2,6-di-*t*-butyl-4-methylpyridine in *N*-methylpyrrolidinone. Other sulfating reagents such as SO_3·pyridine are not effective in this transformation, affording only unreacted starting material. 2,6-Di-*t*-butyl-4-methylpyridine was included as a sterically hindered base to buffer the reaction mixture. Following our discovery of these conditions, we became aware of a report by Laycock in which GTX was desulfated and subsequently resulfated using H_2SO_4 and *N*,*N*′-dicyclohexylcarbodiimide.[40] These conditions are also effective, but in our hands give lower conversion and isolated product yield (~30%) than the SO_3·DMF procedure. Synthetic GTX 3 was purified by reversed-phase HPLC and could be epimerized to a ~3:1 mixture of GTX 2/GTX 3 upon prolonged treatment with aqueous NaOAc (0.3 M).[9] Separation of the diastereomeric guanidinium toxins was accomplished by RP-HPLC (Figure 10), and

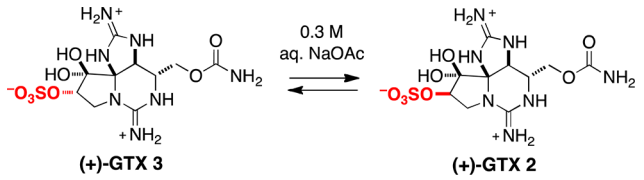


Figure 10. Epimerization of GTX 3 affords GTX 2 as a ~1:3 mixture at equilibrium. The two diastereomers are separable by RP-HPLC.

IC_{50} values were measured independently for each compound against heterologously expressed $rNa_V1.4$ (CHO cell). GTX 2 and GTX 3 have measured IC_{50} values of 22 ± 1.7 and 15 ± 2.1 nM, respectively, in agreement with literature reports of their relative potency.[41] To our knowledge, these studies complete the first de novo synthesis of any of the sulfated gonyautoxins. A preparation of GTX 3 by Nagasawa is the only other report of this kind.[17]

MODIFICATION OF THE SYNTHETIC PLAN AIDS ANALOGUE SYNTHESIS

One goal in developing a preparative synthesis of PSPs was to make possible access to certain rare and difficult to isolate natural toxins, such as 11,11-dihydroxySTX.[24] A second objective was to facilitate the preparation of PSP analogues to enable structure−activity studies against sodium ion channel subtypes.[11,42] Both of these aims motivated our decision to consider specific alterations to our first-generation synthesis of GTX that would facilitate our scale-up efforts.

Alkene **22** (see Scheme 3) represents a versatile intermediate for preparing both natural and unnatural PSPs. The ability to manipulate this compound, however, using a number of standard functional group transformations is impeded by lability of the *N16*-trichloroacetyl moiety. Accordingly, an alternative, more robust protecting group was sought.

Successful pyrrole oxidation under the action of $Rh_2(esp)_2$ necessitates a strongly electron-withdrawing protecting group on the six-membered guanidine unit. This type of substitution mitigates a deleterious, uncatalyzed reaction between the pyrrole unit and $PhI(OAc)_2$. For this reason, a 2,2,2-

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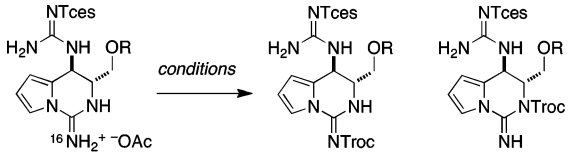
trichloroethoxycarbonyl (Troc) group was selected to replace the trichloroacetyl in **17**. Unlike trichloroacetyl installation, however, treatment of **16** with TrocCl at 0 °C generated a mixture of isomeric acyloxy-guanidines **28** and **29**, which favored the latter product (Table 2). Lowering the reaction

Table 2. Conditions for Selective Protection of N16



entry	electrophile	base	temp (°C)	28/29	% yield
1[a]	TrocCl	*i*-Pr$_2$NEt	0	1:5	84
2	TrocCl	*i*-Pr$_2$NEt	−78	1:10	80
3[b]	TrocNR$_2^+$ $^-$OTf		23	1:1	80
4[c]	TrocNR′$_2^+$ $^-$OTf		23	6:1	88
5[c]	**TrocNR′$_2^+$ $^-$OTf**		**55**	**>20:1**	**75**

[a]TrocCl = $Cl_3CCH_2OC(O)Cl$. [b]NR_2^+ = *N*-methylimidazolium. [c]NR'_2^+ = *N*-methyl-2-phenylbenzimidazolium.

temperature to −78 °C marginally increased the ratio in favor of the *N1*-Troc product **29**. By switching from TrocCl to a Troc-imidazolium or Troc-benzimidazolium salt, the *N16*-Troc compound could be formed preferentially.[43] The product ratio was improved through further reaction optimization, which included raising the reaction temperature to 55 °C. Identification of conditions to favor **28** was necessary, as the *N1*-Troc isomer appears to be quite labile toward hydrolysis and, more importantly, does not engage effectively in the Rh-catalyzed pyrrole oxidation. As such, the ability to invert selectivity in the Troc-protection of **16** was a critical advance.

Dirhodium-catalyzed amination of **28**, while lower yielding than the equivalent transformation of the trichloroacetyl substrate **17** (41% vs 62%, respectively), is easily performed on multigram scale to afford large quantities of the desired tricyclic product (Scheme 5). The same reactions conducted with **20** and all subsequent trichloroacetyl intermediates can be performed with the equivalent Troc-derivatives and are generally more efficient. The availability of **30** has enabled us to assemble a number of unique PSP analogues, the structures and activities of which will be disclosed in a future publication.

PREPARATION OF (+)-11,11-DIHYDROXYSAXITOXIN

A recent report by Quilliam and co-workers describes the isolation and structure elucidation of three new PSPs from contaminated mussels gathered during an intense bloom of *Alexandium tamarense* in Eastern Canada.[24] These investigations relied on a new analytical technique, hydrophilic interaction liquid chromatography and tandem mass spectrometry (HILIC-MS/MS), which made possible the identification of minuscule quantities of material. One of these isolates, 11,11-dhSTX, is a most unusual structure bearing three contiguous carbons at the ketone oxidation level (C4, C11, C12). The lack of available material has precluded toxicity studies, and no electrophysiological data measuring the potency of this compound against Na_V subtypes have been reported.

Synthesis of 11,11-dhSTX is possible in just two steps from diol **32** (Figure 11). Initial removal of the guanidinium

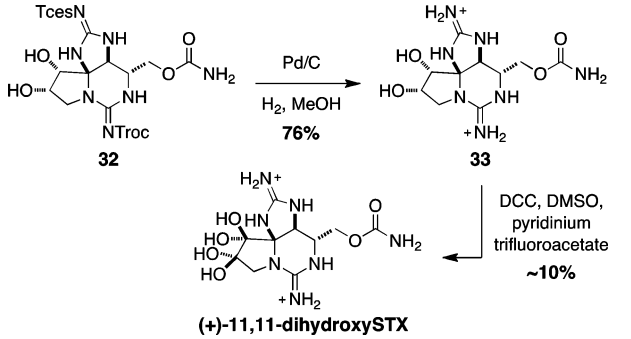
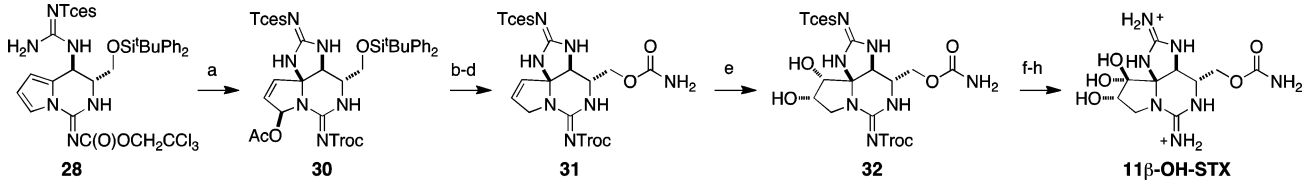


Figure 11. Elaboration of diol **32** to 11,11-dihydroxysaxitoxin.

protecting groups under hydrogenolytic conditions affords bis-guanidnium salt **33**. In previous work from our lab, we have employed DMSO and *N,N′*-dicyclohexylcarbodiimide (DCC), conditions originally described by Schantz, for C12-alcohol oxidation on bis-guanidium salts analogous to **33**. Using this combination of reagents, the diol group in **33** is doubly oxidized to give the C11,12-bis-hemiketal.[20,44] Although the isolated yield of 11,11-dhSTX is low, the reaction itself is reasonably efficient based on NMR analysis of the unpurified sample (~30% conversion). The product is weakly chromophoric (at best) and thus presents a significant challenge for chromatographic purification on a conventional HPLC instrument. Nevertheless, the analytic data we have obtained on a pure sample are entirely consistent with those reported in the literature.[24] In addition, we have determined the IC_{50} of this compound against rNa_V1.4 (CHO cells) to be 2.2 ± 0.2 μM, a value that is seemingly consistent for a PSP derivative that is speculated to be a product of detoxification by the host shellfish. Additional experiments are in progress to measure the affinity of 11,11-dhSTX against other Na_V isoforms and mutant channels.

Scheme 5[a]



[a]Reagents and conditions: (a) 10 mol % $Rh_2(esp)_2$, $PhI(OAc)_2$, MgO, CH_2Cl_2, 40 °C, 41%; (b) $BF_3{\cdot}OEt_2$, Et_3SiH, CH_2Cl_2, 35 °C, 72%; (c) ^{n}Bu$_4$NF, THF; (d) $Cl_3CC(O)NCO$, CH_2Cl_2, then MeOH, 91% (two steps); (e) OsO_4, NMO, THF, 83%; (f) $C_6H_5C(O)CN$, DMAP, 61%; (g) Dess−Martin periodinane, 91%.

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CONCLUSION

De novo synthesis of natural toxins, including gonyautoxin 2 and 3, and 11,11-dihydroxysaxitoxin has been successfully achieved through an intermediate that is available in gram quantities and in just nine steps from L-serine. Highlights of this route include diastereoselective Pictet−Spengler cyclization and pyrrole amination reactions to form the tricyclic core structure common to all PSPs. These chemistries are enabling access to unique PSP derivatives designed to probe molecular ligand−receptor interactions with the voltage-gated sodium channel.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b02343.

Complete experimental procedures and characterization (PDF)

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Notes

The authors declare the following competing financial interest(s): John Mulcahy and Justin Du Bois are cofounders and own equity shares in SiteOne Therapeutics, a pharmaceutical startup company aimed at developing sodium channel selective inhibitors as anti-nociceptive agents.

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