Natural Product Synthesis

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Total Synthesis of 11-Saxitoxinethanoic Acid and Evaluation of its Inhibitory Activity on Voltage-Gated Sodium Channels

*Chao Wang, Mana Oki, Toru Nishikawa, Daisuke Harada, Mari Yotsu-Yamashita, and Kazuo Nagasawa**

Abstract: *11-Saxitoxinethanoic acid (SEA) is a member of the saxitoxin (STX) family of paralytic shellfish poisons, and contains an unusual C–C bond at the C11 position. Reported herein is a total synthesis of SEA. The key to our synthesis lies in a Mukaiyama aldol condensation reaction of silyl enol ether with glyoxylate in the presence of an anhydrous fluoride reagent, $[Bu_4N][Ph_3SnF_2]$, which directly constructs the crucial C–C bond at the C11 position in SEA. The Na_VCh-inhibitory activities of SEA and its derivatives were evaluated by means of cell-based assay. SEA showed an IC_{50} value of (47 ± 12) nM, which is approximately twice as potent as decarbamoyl-STX (dcSTX).*

Saxitoxin (**1**; STX; Figure 1), which was first isolated as a paralytic shellfish poison,[1] is an inhibitor of voltage-gated sodium channels (Na_VCh).[2] So far, more than 50 analogues have been discovered[3] and they have attracted considerable interest from synthetic chemists.[1c,d,4] Among the STX analogues, only zetekitoxin AB (**6**; ZTX)[5] and 11-saxitoxinethanoic acid (**7**; SEA)[6] contain a C–C bond at the C11 position. It is extremely difficult to understand how this C–C bond arises in terms of proposed biosynthetic pathways for STXs.[7]

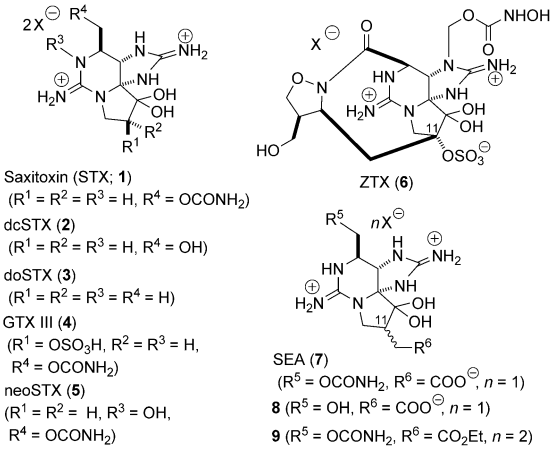
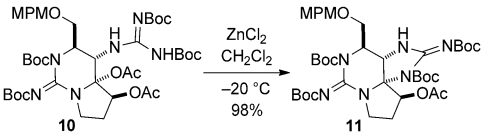


Figure 1. Structures of saxitoxin (**1**) and its derivatives **2**–**5**, zetekitoxin AB (**6**; ZTX), and 11-saxitoxinethanoic acid (**7**; SEA) and its derivatives **8** and **9**.

We are interested in developing subtype-selective Na_VCh inhibitors, and in this work we focused on the synthesis of SEA (**7**) and its analogues as candidate Na_VCh modulators.[8] SEA was originally isolated from xanthid crab *Atergatis floridus* in 1995,[6] and it contains a saxitoxin core with an acetic acid group at the C11 position. The natural product is a 9:1 mixture of stereoisomers. The toxicity of **7** was reported as 830 mouse units per μmol on i.p. injection into mice, and corresponds to approximately one-third of the toxicity of **1**.[3,6] Herein we describe the synthesis of **7**[9] and its derivatives **8** and **9**. The Na_VCh-inhibitory activity of these new STX derivatives in a cell-based assay is also reported.

We have recently developed a synthesis of the fully protected saxitoxinol **11**[4g] by utilizing neighboring acyl-group-assisted construction of the five-membered cyclic guanidine structure in STXs under mild reaction conditions (Scheme 1). The compound **11** is a key intermediate for the synthesis of STX and its derivatives, which have a highly polar nature because of the two guanidine groups. We have employed **11** in the syntheses of dcSTX (**2**), GTX III (**4**), and artificial STX derivatives.[4g,8b] Herein, we envisage application of **11** to the synthesis of **7** through C–C bond formation at the C11 position.



Scheme 1. Synthesis of the fully protected saxitoxinol **11**.[4g] Boc = *tert*-butoxycarbonyl, MPM = *p*-methoxyphenylmethyl.

Regarding synthetic approaches to **7**, there are two possibilities to construct the C–C bond at the C11 position (Scheme 2), that is, direct alkylation of the enolate **12** with halides in the presence of base, and Mukaiyama aldol reaction of the silyl enol ether **13** with glyoxylate. We initially investigated the alkylation strategy. However, the conversions were extremely low and only trace amounts of the corresponding alkylation product were obtained.

Then, the Mukaiyama aldol reaction was investigated for construction of the C–C bond at the C11 position. The silyl

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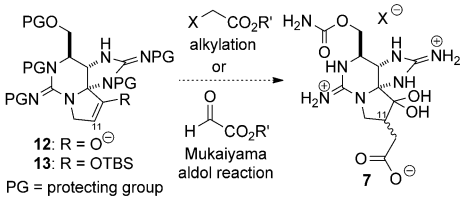
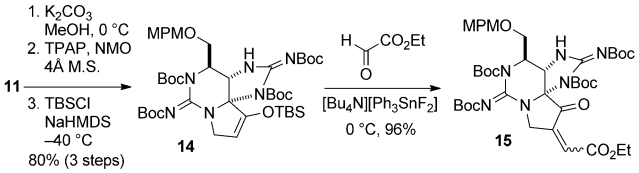


Scheme 2. Synthetic strategies to SEA (**7**). TBS = *tert*-butyldimethylsilyl.

enol ether **14** was synthesized from **11** in three steps: 1) hydrolysis of acetate with potassium carbonate, 2) oxidation of the resulting alcohol by means of Ley oxidation,[10] and 3) silyl enol ether formation from the resulting ketone with TBSCl in the presence of NaHMDS (Scheme 3). With **14** in



Scheme 3. Mukaiyama aldol reaction of **14** with ethyl glyoxylate. HMDS = hexamethyldisilazide, M.S. = molecular sieves, NMO = *N*-methylmorpholine-*N*-oxide, TPAP = tetrapropylammonium perruthenate.

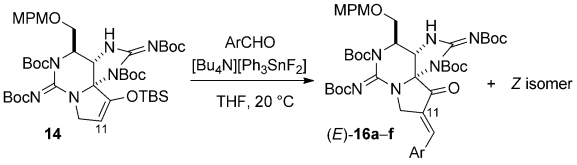
hand, the Mukaiyama aldol reaction was investigated with ethyl glyoxylate under various reaction conditions. At first, Lewis acid promoted conditions with either $TiCl_4$ or $BF_3{\cdot}Et_2O$ were tested,[11] but **14** decomposed to generate mostly Boc-deprotected compounds, and no aldol reaction products were obtained. Next, fluoride-anion-promoted reaction conditions were investigated. In the case of Bu_4NF,[12] no reaction occurred. In contrast, for the case of $[Bu_4N][Ph_3SnF_2]$,[13] which is an anhydrous fluoride anion reagent, the condensation the product **15** was obtained in 96% yield as a mixture of stereoisomers of the double bond (5:1).

Since the Mukaiyama aldol condensation reaction appears to be a powerful tool for constructing the C–C bond at C11 in STXs, the scope of the reaction was investigated. Thus, various aromatic aldehydes were subjected to reaction with **14**, and the corresponding aldol condensation adducts (**16a–f**) were obtained in 45–80% yields (Table 1).

After construction of the crucial C–C bond at the C11 position of **7**, the double bond in **15** had to be reduced with subsequent removal of the MPM group from the C13 position (Scheme 4). For the reduction, after extensive investigation,[15] L-selectride was found to be quite effective, thus giving **17** in 76% yield as a single diastereomer.[17] Unfortunately, subsequent removal of the MPM group at C13 failed under all the reaction conditions we investigated, for example, NBS-Et_3B,[4g] DDQ, or CAN.[18] Finally, we decided to change the protecting group from an MPM to TBS ether at an earlier stage.

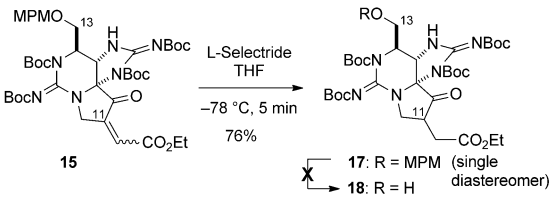
Thus, silyl enol ether **19** with a TBS ether at the C13 position was synthesized by following a similar procedure to

Table 1: Substrate scope of the Mukaiyama aldol condensation reaction of **14** with aromatic aldehydes.



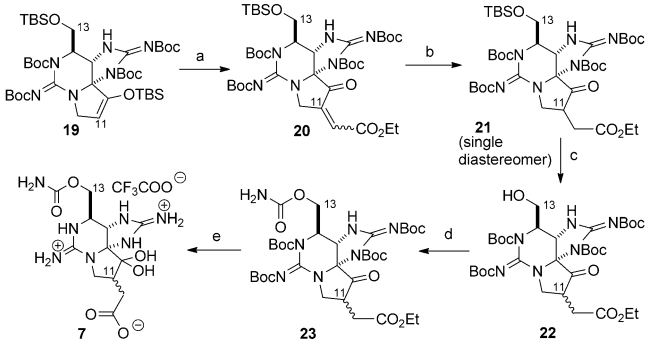
Entry	Ar	*t* [h]	**16**	*E*/*Z*[b]	Yield [%]
1	4-MeC_6H_4	48	**16a**	>10:1	45
2	C_6H_5	24	**16b**	>10:1	60
3	3-FC_6H_4	24	**16c**	>10:1	63
4	4-ClC_6H_4	24	**16d**	>10:1	65
5[a]	4-$NO_2C_6H_4$	2	**16e**	6:1	80
6[a]	2-furyl	2	**16f**	>10:1	80

[a] Reaction was carried out at 0 °C. [b] Ratios at C11 were determined by ^{1}H NMR spectroscopy.[14]



Scheme 4. Investigation of the reduction at C11 and removal of the MPM group at C13. THF = tetrahydrofuran.

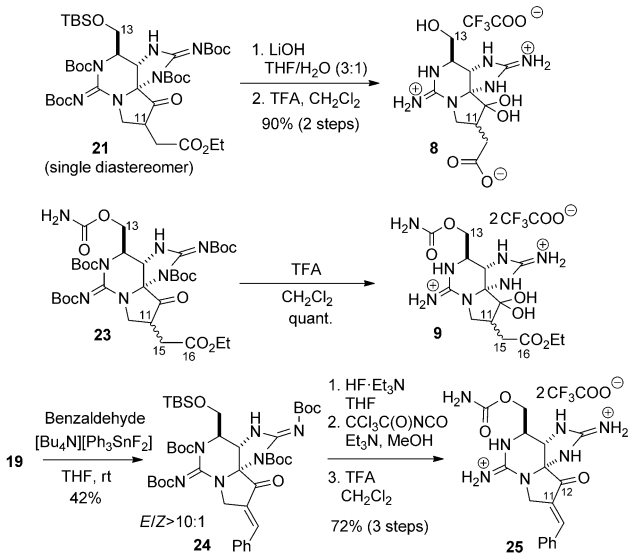
that used for **14**,[19] and the Mukaiyama aldol condensation reaction with ethyl glyoxylate was examined in the presence of $[Bu_4N][Ph_3SnF_2]$ (Scheme 5). Under these reaction conditions, the aldol condensation adduct **20** was obtained in 85% yield. Interestingly, the TBS ether at C13 remained intact under these reaction conditions. After reduction of the double bond with L-selectride, removal of the silyl ether in **21** at C13 took place smoothly upon treatment with the $HF{\cdot}Et_3N$ complex to give **22** in 87% yield.[20] The resulting hydroxy group was further converted into a carbamoyl group by



Scheme 5. Synthesis of **7**. Reagents and conditions: a) ethyl glyoxylate, $[Bu_4N][Ph_3SnF_2]$, THF, 0 °C, 85%; b) L-Selectride, THF, −78 °C, 76%; c) $HF{\cdot}Et_3N$, THF/Et_3N (5:1), RT, 87%; d) $CCl_3C(O)NCO$, CH_2Cl_2, 0 °C, then Et_3N, MeOH, RT, 68%; e) LiOH, THF/H_2O (3:1), 0 °C; then, TFA, CH_2Cl_2, RT, 90%. L-Selectride = lithium tri-*sec*-butyl(hydrido)borate-(1−), TFA = trifluoroacetic acid.

reaction with trichloroisocyanate followed by hydrolysis with triethylamine in methanol to give **23** in 68% yield. Finally, **7** was obtained, by further hydrolysis of the ethyl ester with lithium hydroxide and removal of all four Boc groups with TFA, in 90% yield.[21] The stereochemistry at C11 of synthetic **7** was found to be a mixture of about a 9:1 ratio, which is identical with that of the natural product reported by Onoue and co-workers.[6]

The inhibitory activities towards Na_VCh of **7** and its synthetic derivatives, decarbamoyl-11-saxitoxinethanoic acid (**8**; dcSEA) and 11-saxitoxinethanoic ethyl ester (**9**; SEE), which were obtained from **21** and **23**, respectively (Scheme 6),



Scheme 6. Synthesis of dcSEA (**8**), SEE (**9**), and (**25**).

were evaluated in a cell-based assay. In addition, 11-benzyliden-saxitoxin (**25**; 11-benzylidenSTX), synthesized from **19** via **24** (Scheme 6), was also tested.[22] Specifically, the Na_VCh-inhibitory activity of ligands was evaluated in terms of cytotoxicity to mouse neuroblastoma Neuro-2a cells, which express Na_VCh.[23] In this cell-based assay, Neuro-2a is treated with a sodium channel activator, veratridine, in the presence of ouabain, an inhibitor of Na^+/K^+ ATPase. This treatment blocks sodium ion efflux, and decreases the cell viability. Na_VCh inhibition by tetrodotoxin (TTX), STX, and related compounds antagonizes this effect and rescues the cells in a dose-dependent manner.[23e,8] The inhibitory activities of **7**, **8**, **9**, and **25** were calculated from the cell viability in the above assay, and the results are summarized in Table 2. SEA (**7**), dcSEA (**8**), SEE (**9**), and 11-benzylidenSTX (**25**) showed a concentration-dependent Na_VCh-inhibitory effect, and their IC_{50} values were determined to be (47 ± 12) nM, (5.7 ± 3.1) μM, (185 ± 74) nM, and (16 ± 6.9) nM,[24] respectively [the IC_{50} values of synthetic dcSTX (**2**)[4g] and TTX, used as controls, were (89 ± 36) nM and (5.0 ± 1.6) nM, respectively]. Thus, **7** was twice as potent as **2** on its Na_VCh-inhibitory activity. In the case of **8**, the inhibitory activity was markedly decreased, and it was approximately one hundred times less potent than **2**. SEE (**9**) showed about half weaker inhibitory activity than **2**. Interestingly, **25** showed the same level of inhibitory activity as **2**, although the hydrated form of the ketone at C12 in STXs is suggested to be important for their Na_VCh-inhibitory activity.[25] Further structure–activity relationship studies are under way to examine the inhibitory activity of other STX derivatives having a C–C bond at the C11 position.

Table 2: Na_VCh-inhibitory activity of **7** and its derivatives **8**, **9**, and **25** in a cell-based assay with Neuro-2a cells.

Compound	IC_{50} (mean ± SD)	*n*
SEA (**7**)	47 ± 12 nM	3
dcSEA (**8**)	5.7 ± 3.1 μM	3
SEE (**9**)	185 ± 74 nM	4
11-benzylidenSTX (**25**)	16 ± 6.9 nM[24]	5
dcSTX (**2**)[4g]	89 ± 36 nM	3
TTX	5.0 ± 1.6 nM	6

In conclusion, a total synthesis of **7** has been achieved from the silyl enol ether **19** in 35% overall yield. The synthesis features a Mukaiyama aldol condensation reaction to construct the C–C bond at the C11 position of STX. The derivatives (**8**, **9**, and **25**) were similarly synthesized. In a cell-based assay, **7** showed approximately twice more potent Na_VCh-inhibitory activity than **2**, but **8** showed about one hundred times less potent activity, in marked contrast to the relationship between **1** and **2**.[26] Interestingly, the inhibitory activity of **25** was at a similar level as that of **2**, and the mode of interaction of **25** with Na_VCh is quite intriguing.

Acknowledgments

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[14] The stereochemistry of olefin in **16a** was determined by NOESY (see the Supporting Information), and according to **16a**, the stereochemistry for **16b**–**f** was determined.

[15] Metal catalysts (Pd/C, $Pd(OH)_2$/C, PtO_2, $[RhCl(PPh_3)_3]$, $[Ir(cod)py(PCy_3)]PF_6$, and Raney Ni) were tried first under hydrogenation conditions. In the case of Pd/C and $Pd(OH)_2$/C, the desired product was not obtained and Boc-deprotected products were generated. In the case of the next three catalysts, the reaction did not proceed at all, and the starting material **15** was recovered quantitatively. Raney Ni in MeOH under hydrogenation conditions gave the desired **17** in 70% yield, but reproducibility was poor in large-scale reaction. In the case of single-electron reduction using activated Mg in dry MeOH,[16] **17** was obtained in 30% yield.

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[17] The ^{1}H NMR spectrum indicated the presence of only one diastereoisomer, but the stereochemistry at C11 in **17** was difficult to determine.

[18] Standard reaction conditions (DDQ and CAN) did not give **18** and caused removal of the Boc groups in **17**.

[19] For the synthesis of silyl enol ether **19**, see the Supporting Information.

[20] In the case of Bu_4NF, the substrate **21** decomposed immediately. When HF·Py was tried, the reaction proceeded extremely slowly.

[21] Intermediates **20**, **21**, **22**, and **23** were quite unstable under regular silica gel column purification conditions. After extensive investigation, we found that rapid purification on neutral silica gel gave acceptable results. Since C11 in **22** and **23** epimerized easily, the products were obtained as diastereomeric mixtures. Data for the major diastereomers are presented in Supporting Information (for the data collection process, **22** and **23** were purified by preparative thin layer chromatography. During this process, the minor diastereomer decomposed, and we collected only the major diastereomer).

[22] 11-BenzylidenSTX (**25**) exists as a keto-form at C12 (from ^{13}C NMR in D_2O), and is different from the other member of STX derivatives.

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[24] In the case of **25**, the IC_{50} values of **2** and TTX, which were used as controls, were (18 ± 2.1) nM ($n = 3$) and (5.0 ± 2.8) nM ($n = 4$), respectively.

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